A STUDY ON SCREENING PHYTOCHEMICAL ANALYSIS AND PHARMACOLOGICAL ACTIVITIES OF SOME MEDICINAL PLANT IN BUNDELKHAND REGION

A
Thesis
Submitted towards the Requirement for Award the Degree of

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In
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Under the Faculty of Science

By
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(Enrollment No. 220101077)

Under the Supervision
of
Dr. Manish Kumar
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Date…….. **Pooja Sahu**

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LISTOF ABBREVIATIONS

ABBREVIATIONS	INDICATION
TFC	Total Flavonoid Content
ROS	Reactive oxygen species
WHO	World health organization
TMP	Traditional medicinal plants
CVD	Cardio vascular diseases
TIA	Terpenoid Indole Alkaloids
DOS	Diversity oriented synthesis
HPLC	High-performance liquid chromatography
BHA	Butylated hydroxyl anisone
HCL	Hydrochloric acid
TPC	Total phenolic compounds
TFC	Total flavonoids Compound
GAC	Gallic acid equivalents
SN	Solanum nigrum
MFC	Murraya functional complex
TLC	Thin-layer chromatography
UPLC	Ultra-performance liquid chromatography
UV	Ultraviolet
NMR	Nuclear magnetic resonance
MS	Mass spectrometry
UPLC-ESI-MS	UPLC–electrospray ionization mass spectrometry
HR-FAB-MS	High-resolution fast atom bombardment mass spectrome
HMBC	Heteronuclear multiple-bond correlation
HMQC	Heteronuclear multiple-quantum coherence
CG-MS	Gas chromatography–mass spectrometry
TEMP	Total flavonoids extracted from Murraya paniculata

ABSTRACT

Medicinal plants are recognized as important reservoirs of bioactive compounds with antioxidant and antimicrobial properties. The present study was carried out comparatively to evaluate the extractive yield, presence of phytochemicals, antioxidant potentiality and antimicrobial activity of ***Murraya paniculata, Catharanthus roseus,*** and ***Solanum nigrum*** using different plant parts, namely leaves, fruits, and stems. To evaluate the influence of extraction solvents, methanolic, acetone, and aqueous extracts were prepared and thoroughly analysed.

Extractive yields were estimated to assess solvent efficiency, followed by qualitative phytochemical screening of secondary metabolites. Quantitative analysis of total phenolic and total flavonoid contents was carried out to evaluate the responsible antioxidant-related phytoconstituents. Antioxidant activity was assessed using DPPH and ABTS radical scavenging assays, and IC_{50} values were calculated to compare scavenging efficiency. Antimicrobial activity of the plant extracts was evaluated against selected bacterial and fungal strains using standard in vitro methods, and the efficacy was expressed in terms of zones of inhibition.

The results stated that extractive yield, phytochemical profile, antioxidant activity, and antimicrobial efficacy varied significantly with plant part and solvent nature. Leaf extracts consistently showed higher phenolic and flavonoid contents and showed stronger antioxidant and antimicrobial activity compared to fruits and stems. Methanolic extracts exhibited superior activity in both antioxidant and antimicrobial assays, followed by acetone and aqueous extracts. Among the studied plants, *Murraya paniculata* showed the most pronounced bioactivity, followed by *Catharanthus roseus* and *Solanum nigrum*.

The findings establish a strong relationship between phytochemical content and biological activity, highlighting the importance of solvent selection and plant-part specificity. The study in sighted the findings supporting the antioxidant and antimicrobial potential of these medicinal plants and offers a foundation for future research aimed at isolating bioactive compounds for therapeutic and nutraceutical applications.

Keywords - ***Murraya paniculata, Catharanthus roseus, Solanum nigrum***

CHAPTER 1

INTRODUCTION

The developments of modern medicine in the last century is the consequence of multiple, relentless struggle against environmental and inherent threats against human beings. The ailments associated with such detrimental factors had driven the attempts of researchers and physicians toward the new discovery of medicines, which are frequently promoted. Emergence of antibiotic-resistant bacteria and proliferation of invasive malignant tumours are two of the most perplexing problems of current scenarios that scientists are continuously trying to cure **(Jhanji, *et al.*, 2019; Siegel, *et al.*, 2019).** The gene for antibiotic resistance in bacteria was recognized to be present also in an earliest strain **(D'Costa, *et al.*, 2011),** far before the search of antibiotics, however resources were increasingly pushed towards alternative means of cure **(Al Salhi, *et al.*, 2019; Deepika, *et al.*, 2019).** Similarly, painful chemotherapeutic drugs are the only option for the main treatment, which administered in cancer patients, even if the side effects are well-known and thoroughly documented **(Shukla & Mehta, 2015).** Therefore such difficulties are encountered along with the development of therapeutics; scientists focussed their attention towards the phototherapy **(Chagas & Alisaraie, 2019).** Synthesis of natural products with effective antibacterial **(Ganeshpurkar & Saluja, 2017),** antifungal **(Parvu *et al.*, 2013)**, antiphrastic **(Meira *et al.*, 2015)**, and antitumor **(Mirzaei *et al.*,2016)** activities within the Plants are the foundation of modern medicine.

Natural Compounds which isolated from the plant are a significant of source of synthetic as well as traditional herbal medication and presently being used as the crucial health care system **(Tolambiya & Mathur, 2016; Retna & Ethalsha, 2013).** Medicinal plants are used conventionally to cure many diseases and transmissible disease. Herbal plants also comprised of own fewer side effects and another advantage is its cost effectiveness **(Ranjan & Kumar, 2020).** Herbs are the best remedies for treating dermatitis, psoriasis, sores, ringworm, abscesses, eczema, epilepsy, malaria and to produce heart tonics.

Herbal based treatments; presence of diverse phytoconstituents makes the herbal treatments invaluable since ancient times for preventing and treating incurable

diseases, including cancer, and become essential to global healthcare system. Medicinal plants are identified as safe and effective, which can be of natural substitute to synthetic drugs, with minimal side effects and promising medical results.

Medicinal plants are playing vital roles in the everyday life of peoples dwelling in developing countries of Asia and Africa, including Ethiopia. Medicinal plants are used as potential substitutes for modern medical treatments, which are not adequately available to all people and also limits the health and security of local people. Consequently, these plants play crucial roles in daily life and are severely connected to diverse social, cultural, and economic events associated with life, aging, illness, and death. Medicinal plants are being used to treat and diagnose illnesses and severe infections. From ancient times to present era, significance of plants have been increased as a potential sources of effective and safe medicines **(Russell-Smith *et al*. 2006).**

The world health organization (WHO) states that traditional medicine are indispensable, based on the entire combination of knowledge and practices that can be formally explained or used in the prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing. Approximately 75-90% of the population belongs to rural area in the world (except western countries) depend on ancient traditional medicinal system for their health related issues. The traditional medical systems are more culturally acceptable and meet psychologically comparatively to modern medical system. So the expensive drug doesn't matter because not only people below poverty line but also others are adopting.

Presently ethno medicinal practices are focusing on the development of safe and effective treatments. In Ethiopia a traditional health care system is prevalent from ancient times, but awareness about traditional medicinal plants (TMP) was limited comparatively to the multi-ethnic, cultural, and flora diversity of country, Also, large parts of Ethiopia conventionally using medicinal plants to treat health problems for livestock and humans infections. **(Redda *et al*., 2014; Regassa 2013; Abera 2014; Tamene 2020; Mulatu 2020).**

The healing properties of medicinal plant species lie in present phytochemical components that are responsible for pharmacological action on infections in the

human body **(Naseem 2014).** Phytochemicals may be divide into two groups on the basis of their metabolic potentiality within the plant, the first group of Phytochemicals has mainly sugars, amino acids, chlorophyll and proteins, and bio metabolites secondary constituents constituents includes phenolic compounds, alkaloids, flavonoids, saponins, tannins, and many more **(Krishnaiah 2007).**

Who estimated 32 % of global death were caused by cardio vascular diseases (CVDs), particularly atherosclerosis, which were thought to the leading cause of mortality worldwide. Coronary heart diseases are the only cause of **32.4%** of deaths in Egypt, adding the country among those with the highest CVD-related mortality rates worldwide **(WHO, 2023).** However statins, fibrates, and calcium channel as blockers are the conventional pharmacological interventions have been proven effective in management of CVDs, however their use left undesirable side effects, including liver dysfunction, gastrointestinal issues, a growing risk of rhabdomyolysis including muscle pain **(Shah *et al.*, 2022).**

Medicinal plant having great importance towards the treatment of incurable diseases worldwide. Medicinally important plant **Vinca rosea (*Catharanthus roseus*)** is a perennial herbaceous plant that belongs to the family Apocynaceae. *Catharanthus roseus* basically native to Madagascar. This plant is now widely grown in tropical and subtropical regions worldwide, including African country Egypt. This plant contains glossy green, opposite leaves and funnel-shaped white, pink, or purple coloured flowers. *Catharanthus roseus* grows in well-drained soils and is used as ornamentally and also contains significant medicinal properties **(Chaturvedi *et al.*, 2022).** *Catharanthus roseus* leaves contains various chemical constituents, including alkaloids, phenolic, fatty acids and flavonoids. Potentialities as anticancer compounds such as vincristine and vinblastine alkaloids are supported by recent studies. More over the *Catharanthus roseus* plant's leaves the present alkaloids like vindoline, vindolicine, and vindolinine are also found to be effective in treatment of diabetes and conditions developed due to oxidative stress **(Malhotra *et al.*, 2024).** Studies also refer to the presence of phenolic compounds such as flavonol glycosides and a caffeoylquinic acid in the leaves also imparts antioxidant properties. These antioxidant compounds together contribute to the diverse pharmacological activities of the plant and makes it medicinally important **(Mendonce *et al.*, 2025).** From this

standpoint present bioactive compounds unveil a wide array of biological activities, mainly as potential hope of cancer treatment. Alkaloids, Vincristine and vinblastine, significantly inhibit cancer cell proliferation and are integral components of chemotherapy regimens for treating cancers such as lymphoma and leukaemia **(Mendonce *et al*., 2025).**

Moreover the plant *Catharanthus roseus* bioactive compounds shows anti-inflammatory effects by modulating inflammatory pathways, imparting significant therapeutic relief caused by persistent inflammation. **(Mendonce *et al*., 2025).**

As traditional medicine, *Catharanthus roseus* has been widely used for its therapeutic importance. The Plants leaves variably used to manage the several diseases including infections, hypertension, and diabetes **(Sharma *et al*., 2024).** The bioactive compounds, especially alkaloids vincristine and vinblastine playing are being applied in chemotherapy regimens, are considered as significant in cancer treatment **(Goswami *et al*., 2024).**

Antioxidant and anti-inflammatory properties of *Catharanthus roseus* plant contributes to overall health and well-being of humans **(Hashim *et al*., 2024).** Regardless of these known benefits, further research is needed to discover its therapeutic potentiality. The further studies should be focus on plant extracts standardization, bioavailability studies, formulation development and clinical trials to confirm therapeutic efficacy. **(Khan *et al*., 2024).**

The new challenges has been raised due to ever-growing phenomenon of resistance to microorganisms, these are heading towards the search for new and effective antibacterial and antifungal agents as modern medicine and agriculture **(Prestinaci *et al*., 2015; Maillard *et al*., 2018; Partridge *et al*., 2018 and Khan *et al*., 2021)**.

Bioactive compounds are persistent and widely acceptable presently in the era of vast technological growth despite the widespread presence of new and advanced synthetic care preparations and plant fortified products. The plants and microorganisms are emerging as essential source of various natural compounds. Thus bioactive compounds with commonly used antibiotics or pesticides may reduce the risk of acquiring resistance by microorganisms when both are used together and may also permit the reduced dose of chemotherapeutic and plant

protection products without reducing their efficacy. Plant extracts and essential oils has become the basis of many process as preservative in raw and processed food, in pharmaceuticals and as alternative medicine and natural therapies due to antimicrobial. **(Betoni *et al*., 2006 and Barah *et al*., 2013).** Furthermore, numerous secondary metabolites are synthesized within the plant is responsible for antimicrobial, antioxidant and antitumor activity **(Bakkali *et al*.,2008; Silva *et al*., 2010 and Szandruk-Bender *et al*., 2020);** flavonoids demonstrated a strong chemo preventive effect **(Sporn and Liby 2020).** Chemoprevention termed as the prevention or delay of a specific disease like tumours using the chemicals or biological drugs. Earlier studies have recommended the use of plant-derived compounds in the diet to minimize the risk of cancer. Presently, chemoprevention is widely used to reduce the chances of colorectal cancer development. Thus, Plant derived phytochemicals and their derivatives are a rich source of bioactive compounds with anticancer property. Moreover about phytochemicals, a considerable group, flavonoids are further categorized into anthocyanins, flavones, flavonols, flavanones, flavanols, isoflavones or chalcones and occur in various forms: as glycoside derivatives or as free aglycones represent due to their differences in structure. These bioactive compounds are naturally present in fruits, vegetables, cereal grains, tea and wines the presence makes the human diet more significant **(Szandruk-Bender *et al*., 2020).** Phytochemicals, flavonoids contains significant antioxidant, anti angiogenic and antimicrobial agent specific for various biological functions including **(Sporn and Liby *et al*., 2020)** chemo preventive activity **(Merwid-Ląd *et al*., 2021).**

Furthermore, epidemiological studies reveal that flavonoid exhibit anticancer effects so, the higher dietary consumption of flavonoids also affects the life expectancy **(Kikuchi *et al*., 2019; Grosso *et al*., 2017; Godos *et al*., 2018 and Sak 2017).** Thus, scientists have been moved towards the effect of natural plant compounds, including flavonoids, on various types of neoplastic cells including human cervical cancer, lung cancer, and breast cancer **(Imran *et al*., 2019; Zanoaga *et al*., 2019 and Yao, 2017).** As plant based essential oils constituents, phenolic compounds, saponosides and alkaloids, the Secondary metabolites are being remained as object in vivo as well as in vitro research **(Moga *et al*., 2016).**

Solanum nigrum leaves belong to the family Solanaceae Juss., is commonly known as black nightshade or simply blackberry nightshade. *Solanum nigrum* plant comes from the region of Eurasia and was later spread in different countries Americas, South Africa and Australasia. *Solanum nigrum* is a common agricultural weed with medicinal properties is spread throughout the world **(Subramani *et al.*, 2017 and Edmonds *et al.*, 1997).** A no of bioactive compounds were identified in *Solanum nigrum* leaves are alkaloids (solanine, soladulcine, solasodine, chaconine), steroid, saponins, resins and essential oils with constituents, among others, including dillapiol, α-cadinol, p-cymene, α-phellandrene, β-pinene and brown, green and blue dyes:. However, presence of high solanine content in black nightshade is made a poisonous plant despite all those beneficial features **(Mohy-ud-dint *et al.*, 2010; Dhellot *et al.*, 2006 and Al-Qirim *et al.*, 2008).**

Moreover, excess consumption of Nightshade may lead respiratory, circulatory and gastrointestinal disorders ***(*diarrhea, nausea or vomiting).** Nevertheless, presence of medicinal properties black nightshade was still used to treat wounds, ulcers and diarrhea and remedy toothache and nosebleeds as traditional medicines due to easily availability and also cost effective. Additionally, some people also believed that black nightshade is an effective medicament for some skin problems called psoriasis, haemorrhoids and deep skin infections (abscesses) and directly use it. Although there is no more information is available about the mode of action of *Solanum nigrum* as medicine **(Dukes, 1985)** except the effects of *Solanum nigrum leaves* extracts on a broad spectrum of pathogens, including human, animal and plant pathogens as well as cancer cells and cell cultures **(Raju *et al.*, 2012; Gabrani *et al.*, 2012, Tai *et al.*, 2012, Campisi *et al.*, 2019 and Nawaz *et al.*, 2021).**

This study aimed to investigate the chemical composition of Solanum nigrum leaves. aerial parts and root extracts to evaluate their biological properties (e.g., antibacterial, antifungal, chemo preventative and/or ant oxidative).The selection of lines used was dictated by the composition of the extracts, the possibilities of their use in chemoprevention and earlier studies by other authors. It should be noted that the plant extracts studied differ from those studied by other authors. Therefore, the results presented in this paper should

be considered as the beginning of research on compounds obtained from *Solanum nigrum leaves* **(applsci-12-06845)**

Natural biological processes and external factors like radiation, pollution, medication and cigarette smoke generates Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)**(Tanab *et al.*, 2022)**. These two classes of reactive species break down carbohydrates, lipids, proteins, and nucleic acids **(Bhattacharyya *et al.*, 2014**). Although these reactive species namely reactive oxygen species (ROS) and reactive nitrogen species are advantageous at low to moderate concentrations, however at higher quantities they are harmful and lead to oxidative stress **(Singh *et al.*, 2009).** Furthermore, high levels of free radical generation and low endogenous antioxidant defence in the human body the increased the Oxidative stress many fold. Thus oxidative stress leads to cellular damage, ageing, cardiovascular disease, diabetes, cancer and neurological illness **(Adebiyi *et al.*, 2017).**

Therefore, this oxidative stress is managed by exogenous dietary antioxidants which can neutralize or stop this oxidative stress-induced disorder. Various bioactive compounds are available as plants materials or found in functional meals are being evaluated for their potentiality to treat these oxidative stress generated including cancer **(Maruca *et al.*, 2019).** Antioxidants can be divides into two categories: synthetic antioxidants and natural antioxidants. Some of synthetic antioxidants are butylated hydroxyl toluene, butylated hydroxyl anisole, Propyl gallate and tributyl hydroquinone that harm humans' livers and cause cancer **(Alam *et al.*, 2019).** Now food and pharmaceutical industries has focused on natural antioxidants that have beneficial medical, nutritional, and safe properties instead synthetic antioxidants. The natural antioxidants from plant sources are rich in phenolic, flavonoids, vitamin C, carotenoids, tannins, and proanthocyanins and act as defence barriers of body against generated free radicals **(Baba & Malik, 2015).** Therefore, study on discoveries of nutritional and bioactive components in edible wild plants is still continued to find out important bioactive compounds, which can reduce oxidative stress **(Sharifi-Rad *et al.*, 2020).** So, Compounds from plant origin are being considered as the potential substitutes by production cells due to cost effectiveness and less side effects **(Krishnaiah *et al.*, 2011).** Abundance and effectiveness of existing phytochemicals viz. alkaloids, tannins, flavonoids, and phenolic compounds are

synthesized within the plants makes them as potential barriers against severe ailments **(Yu *et al*., 2021).**

Solanum nigrum leaves are an herbaceous plant also a great source of phytochemicals, which is widely distributed throughout the world **(Emmanuela *et al*., 2024)**. In English this plant is known as black nightshade, while Makoi, Kachcipandu, Munatakali, Piludi, and Kamuni are the Hindi, Gujrati, Telugu, Tamil, and Marathi names of the plant (**Guo *et al*., 2024).** S. nigrum has been propagated in Americas, Australasia, and South Africa from its native place Eurasia. This herb is known for its medicinal properties and included in medicinal flora **(Dai *et al*., 2024).** This plant is being used as folk medicine since a log period for the treatment & prevention of various ailments. This plant contains analgesic properties and also used as treatment of tracheitis, aerodontalgia, mastitis cancer and liver damage **(***Wang et al., 2024***).** This plant contains the remarkable medicinal properties but accessibility to the population is being minimized due to deforestation, herbicides, expansion in agricultural areas, industrialization **(***Wang et al., 2024***).** *Solanum nigrum* contains numerous bioactive compounds namely steroidal glycosides, steroidal alkaloids, steroidal oligoglycosides, such as solamargine, solasonine, solavilline, and solanine, steroidal saponins and glycoprotein, polyphenolic compounds - gallic acid, protocatechuic acid, catechin, caffeic acid, epicatechin, rutin, and naringenin, which possess antioxidant, anticancer, HIV and anti-diabetic properties as the previous studies reported due to presence of reverse transcriptase inhibitors **(***Pradhan & Huidrom et al., 2024)***.** Many studies also reported a correlation between biological activities and therapeutic properties of plants **(Aouadi** *et al.,2024)* and it is also relates the biological qualities of the plant may be affect by the availability of nutrients, texture and vitality of soil from which plant uptake. However, no study has been done which states the effect of soil texture types on the phytochemical and antioxidant properties of *Solanum nigrum.*

Presently Plants have been essential part of human life for millennia, used as vital and primary resources of food as well as clothing, and shelter. Plant parts are essential components of traditional medicine but presently are commercial utilizing various ailments. Other uses of plant materials are also as dietary supplements, in food preservation, crafts, cosmetics, essential oils, natural flavouring agents, and scented products. The plant originated materials are not limited to health and well-being but

also offer significant economic benefits, particularly in rural areas through cultivation **(Hamilton, 2004).** World Health Organization states that, 80% of the population in developing countries depends on Ayurvedic medicines as primary remedies.

The *Murraya* is a prime medicinal plant. Historical manuscripts from the first to fourth centuries AD in Tamil literature and later in Kannada literature states its use as a vegetable flavouring ingredient. This basically originated from tropical trees of the Rutaceae family, which includes about 150 genera and 1600 species, curry leaves, known as Kari Patta in India, were named "Murraya" in name of discoverer, a botany professor at the University of Gottingen, John Adam Murraya **(Ayyanar, 2013).** The distinctive flavour and aesthetic appeal makes this plant so specific In India and SriLanka, particularly in the southern regions, their use extending to Reunion Island, South Africa, and Malaysia also. Curry leaves is basically native to India, Bangladesh, and SriLanka, curry leaves are now became a profitable agricultural export product **(Hamilton, 2018).** Height of the small aromatic tree is up to 6 meters tall, features bright, glossy black leaves with a strong aroma, white flowers arranged in small clusters, and purplish-black, berry-like ripe fruits are also arranged in same manner. Leaves as well branches can be in both forms fresh or dried, which gives a spicy aroma and possess therapeutic properties on warming.

In developing countries, these medicinal plants, Curry leaves are also enhancing the economic growth as well as cultural development other than healthcare and skincare. Approximately 80% of the global population relies on traditional medicine as the World Health Organization states, which produced the substantial $19.4 billion global revenue for herbal remedies in 2010. Traditional plant medicine have been proved as safe guards for human beings, which leads the economically expansion of medicinal plant market with an increase of 20 % in India and significant in China, Germany and Thailand.

Murraya koenigii leaves, commonly known as "curry leaves," is widespread in tropical and subtropical regions. Curry leaves and *Murraya paniculata* are found in India among the 14 global species in the Murraya genus; Curry leaves possess the medicinal attributes, which makes this plant significant traditional medicinal plant. Curry patta is also known as “krishnanimba,” in Ayurvedic medicine. Various parts of the curry patta such as leaves, roots, bark, and fruit, are recognized for their biological activities **(Ahluwalia, 2013).**

The leaves, slightly bitter and pungent, are used as anthelmintics, analgesics, digestives, and appetizers in Indian cuisine. They are also utilized to treat piles, inflammation, cuts, dysentery, bruises, and edema, while the roots and bark possess purgative and stimulating properties. Recent studies have highlighted the essential oil extracted from Curry leaves, showcasing antioxidative, hepatoprotective, antimicrobial, antifungal, anti-inflammatory, and nephroprotective activities in animal models. These medicinal properties are attributed to chemical constituents such as carbazole alkaloids and other metabolites like terpenoids, flavonoids, phenolics, carbohydrates, carotenoids, vitamins, and nicotinic acid **(Gahlawat, 2014).**

Despite the growing interest in *Murraya koenigii's* use in traditional medicines and home remedies, limited research has evaluated its pharmacological and medicinal efficacy comprehensively. This review aims to discuss the traditional medicinal use of Curry leaves and their bioactive compounds, highlighting their pharmacological effects. By systematically categorizing, comparing, and summarizing phytochemical screenings, identifications, and pharmacological activities, this review seeks to provide a clear overview of the therapeutic potential of and guide future research endeavours.

The evolution of drug-resistant microorganisms is partly due to overuse and misuse in people and animals, and the fact that antimicrobial agents are often given without professional oversight. One of the strategies for combating antimicrobial resistance is the development of new drugs, however, the WHO states that declining private investment and lack of innovation in the development of new antibiotics are undermining efforts to combat drug-resistant infections **(World Health Organization, 2020).** The public is therefore becoming increasingly aware of problems with overuse, misuse and side-effects of antibiotics.

One of the potential sources of new antimicrobial agents is plants. This is because plants have been the main source for drugs and other medicines since ancient times. (4) About 50% of all pharmaceutical products distributed in the United States have plant origin, even though very few are used as antimicrobials **(Cowan, *et al.*, 1999).**

Several studies indicate that *Murraya koenigii* has both antioxidant and antibacterial activity against a variety of microorganisms. Methanol extract of *Murraya koenigii* has been reported as demonstrating significant immunomodulatory effect against

ovalbumin and protection against cyclophosphamide induced myelosuppression **(Ghasemzadeh *et al*., 2014).** Another study on breast cancer cell lines reports that aqueous extract of *Murraya koenigii* reduced tumour size and lung metastasis of 4T1 cells in vitro **(Yeap *et al*., 2015).**

Previous phytochemical investigations of the *Murraya* functional complex (MFC), including *Murraya paniculata* and *Murraya exotica*, reveal that these plants contain diverse bioactive constituents such as coumarins, flavonoids, alkaloids, and volatile oils. Alkaloids are especially concentrated in the roots. Pharmacological studies further confirm that MFC exhibits anti-inflammatory, analgesic, antibacterial, antioxidant, and insecticidal effects **(Lu *et al*., 2021a).** However, despite numerous individual studies, a comprehensive systematic review of MFC is still lacking. Thus, summarizing its phytochemistry, pharmacological properties, mechanisms, pharmacokinetics, and toxicology is essential to support its standardized application in the Chinese Pharmacopoeia.

The present study was carried out on the extraction of three parts: Leaves, stem and fruits collected from three different plants (***Murraya paniculata, Solanum nigrum*** **and** ***Catharanthus roseus***) using three solvents methanol, acetone and Double distilled water for the evaluation of phytochemical screening, antioxidant and antimicrobial activity. The aim of the present study is screening of phytochemical analysis and Pharmacological study of some medicinal plant in Bundelkhand region, my study is based on the given objects which are as follows:

1.1 Objectives of the study

1. To collection of medicinal plants from different locations of Bundelkhand region.
2. To preparation of Plant extracts in three different solvents.
3. To study the qualitative screening of present Phytochemicals.
4. To study the antioxidant activity of plant extracts.
5. To Study antibacterial and antifungal activity for different Plant extracts.

Extreme climatic conditions affect the defensive activities as a survival mechanism against excessive oxidative stress in plants which are induced to enhance secondary bio metabolite synthesis with against adverse environmental conditions.

CHAPTER -2

REVIEW OF LITERATURE

Catharanthus roseus is one of the most important plants as described in Ayurveda. This plant is known as Sadabahar is grown in every home throughout India as ornamental plant. This plant comprises a no of significant bioactive compounds to cure various critical diseases as described in Ayurveda. These incurable diseases have necessitated its continued exploration and consumption. The presence of bioactive compounds also has antimicrobial activity. Therefore, this study is carried out to evaluate the antimicrobial property and phytochemical components of *Catharanthus roseus* plant's leaves extract. Moreover about the study phytochemical screening of different extracts namely methanol extracts of *Catharanthus roseus* plant's leaves was done. Antimicrobial activity of Methanol extract against *Salmonella*, *Staphyloccus aureus* and *Escherichia coli* was carried out. The 100 µg/µl methanol extract showed good activity against *Salmonella* and *Staphylococcus aureus* comparing to *Escherichia coli organism.*

2.1 Taxonomy

Table 2.1 Taxonomy

	Murraya paniculata	***Solanum nigrum***	***Catharanthus roseus***
Kingdom	Plantae	Plantae	Plantae
Phylum	Charophyta	Tracheophyta Spermatophyta	Tracheophyta Magnoliophyta
Class	Equisetopsida	Magnoliopsida (Dicotyledons)	Magnoliopsida (Dicotyledons)
Order	Sapindales	Solanales	Gentianales
Family	Rutaceae	Solanaceae	Apocynaceae
Genus	Murraya	Solanum	Catharanthus
Species	Paniculata	nigrum	roseus
Binomial name	*Murraya Paniculata*	*Solanum nigrum*	*Catharanthus roseus*
Botanical Name	*Murraya Paniculata (L.)Jack*	*Solanum nigrum L.*	*Catharanthus roseus (L.) G.Don*

Catharanthus roseus was previously known as *Vinca rosea*. *Catharanthus roseus* is a large family which is comprised of about 1500 species grow mainly in tropical region Solanum In rain forest regions, many of these species are large trees with buttress roots, on the other hand some plants are smaller, evergreen or deciduous trees, shrubs or climbers are grown in other warm regions of the world, and one or two grow in temperate regions (*Vinca rosea).* The plants posses' milky latex sap, which is often of economically important because of its medicinal use, or for the production of rubber. Sometimes this sap is found toxic.

Catharanthus roseus plant's leaves are simple, undivided and are either opposite or in rings around the stem. There is a calyx with five parts, either these parts may be separate or joined to form a tube. The flowers are in clusters and are often large and showy. Floral structure consists of usually five petals joined into a tube at the base and five stamens joined together. The seed capsule has two parts and may be either inside the flower or not. Seeds vary in size and may be small with a hairy tuft **(Nerium)** or large and woody **(Allamanda).** Members of this family usually have simple leaves with milky sap, five part calyx, clusters of flowers, five large petals joined at the base and five stamen Solanum Most parts of many members of this plant's family are poisonous Solanum Each plant of this family (**Apocyanaceae)** possesses different medicinal behaviour **(Varuna, Khan, & Sharma, 2010).**

Phytochemicals compounds present in plant are of significant importance. Medicinal plants contain a variety of phytochemicals and have been traditionally used for treating a wide range of disease Solanum. These compounds are considered to be naturally safe and efficacious with fewer side effects **(Gaonkar, & Hullatti, 2020).***Catharanthus roseus* is pharmacologically significant plant. The present review is going to elucidate the pharmacological importance of *Catharanthus roseus* through evaluation of present bio metabolite Solanum.

Catharanthus roseus mainly comprised of carbohydrates, alkaloids, glycosides, flavonoids, tannin, saponins, proteins, amino acids, fats and oils are the main phytoconstituents present in the plant **(Prajapati, Purohit, Sharma, & Kumar, 2002).** More than 100 monos Terpenoid Indole Alkaloids (TIA) are produced in different parts of the plant **(Barnett, Cullinan, Gerzon, Hoying, Jones, Newlon, Poore, Robinson, Sweeney, Todd, Dyke, & Nelson, 1978).** Phytochemical vindoline is the chief component (up to 0.5%) produces in *Catheranthus roseu* Solanum Other

significant compounds are vincaleukoblastine (vinblastine), 22-oxovincaleukoblastine (vineristine), reserpine, vincamine, alstonine, leurocristine, ajmalicine, vinine, vinomine, vinoxine, vintsine, leurosine are produced in different part of *Catharanthus roseus* **(Heijden Jacobs, Snoijer, Hallard, &Verpoorte 2004).** Alkaloids present in different organs can be summarized below. In addition to vinca alkaloids present in *Catharanthus roseus* plant are large molecule with similar structure, containing an indole nucleus (Catharanthine portion) connected to a dihydroindole nucleus (vindoline portion) by a carbon-carbon rin**g (Kameswrarao, Giri, Kesavalu, & Apparao, 2015).**Periwinkle Plant produces biometabolites vincristine and vinblastine, semi synthetic vindesine and vinorelbineare vinca alkaloidsare found anticancerous **(Sutrisna, 2015).**

Catharanthus roseus is an important source of alkaloids, carbohydrates, flavonoids, triterpenoids, tannins, coumarin, quinone and phenolic compounds are produced within the plant **(Uniyal, Bala, Mathur, & Kulkarni, 2001).**

Catharanthus roseus leaves synthesize alkaloids and carbohydrates in large quantity. *Catharanthus* leaves have shown antibacterial and anti-diabetic effects and also found capable to decrease the blood sugar **(Singh, Vats, Suri, Shyam, Kumari, & Ranganathan, 2001).** In addition to leaf, *Catharanthus roseus* flowers contain sufficient quantity of tannins, tri terpenoids and alkaloids which having a good healing property especially for diabetic wound **(Zhoe, Shao, &, Tang 2009**). *Catharanthus roseus* contains an antioxidant property which is because of presence of polyphenols in leave Solanum the stem and root of *Catharanthus roseus* contain considerable amount of coumarin, quinones responsible for antibacterial activities (**Ferrers, Pereira, Valentao, Andrade, Seabra, & Sottomayor, 2008**). Alkaloid alstonine is used in the synthesis of drugs namely vincristine and vinblastine which is obtained from the root bark of *Catharanthus roseus* **(Bhadra, Vani, & Shanks, 1993).** in addition to alkaloids, a wide range of phenolic compounds are also produced by *Catharanthus roseus*, these are mainly C6C1 compounds such as 2,3-dihydoxybenzoic acid, phenylpropanoids such as cinnamic acid derivatives, flavonoids and anthocyanins **(Harborne and William 2009).** During biosynthesis, the regulatory pathway of these bio metabolites in *Catharanthus roseus* has been reviewed and found the biosynthesis of an important intermediate, chorismate in plant

metabolism, both types of compounds compete with the indole alkaloid during this process **(Hall & Yeoman, 1986).**

All parts of the Catharanthus roseus are medicinally significant. The leaves and stem parts of the plant are the rich source of dimeric alkaloids, vinacristine and vinblastine, that are crucial cancer drugs, even as roots are also repository of significant bio metabolites ajmalicine and serpentine as antihypertensive drugs **(Berrier, Jacquesy, Jouannetaud & Renoux, 1987)**.The leaves are used as traditional herbal medicaments worldwide including India, West Indies as well as Nigeria to control and cure diabetes **(Pearce, 1990).** The leaves are supposed to contain approximately 150 valuable alkaloids among other pharmacologically bio active compound Solanum these antihyperglycemic compounds have been reported in laboratory animals **(Ibrahim, Mehjabeen & Lakshmi, 2011).** Juices of Fresh leaf from *Catharanthus roseus* have been found to reduce blood glucose level in normal and alloxan diabetic rabbits **(Singh, Vats, Suri, Shyam, Kumari, & Ranganathan, 2001).** Leaves and twigs of *Catharanthus roseus* also have impact on hypoglycaemic activity in streptozotocin induced diabetic rat Solanum.

The control and management of diabetes using herbal drugs have proven to be more advantageous over synthetic medicines due to their ease of access, reduced cost, lesser complications, and lower side effect Solanum Herbal medicines work by means of different mechanisms aiming at dropping insulin resistance, rising insulin secretion, protecting pancreatic beta cells, and in this manner lowering circulating blood glucose levels **(Jeeva & Anlin Sheebha, 2014).**

Ayurveda is playing a significant role as natural remedies from ancient times in Indian subcontinent. Currently the demand for Traditional medicine system in India is growing. The commercial drug producers are now focusing on the medicinal potential of plants and natural product Solanum.

In India a vast diversity persists among plant grown in temperate as well as tropical area under the different climatic condition. A wide variety of plants having therapeutic importance can be found all over the world. Many weeds in our environment are unknown to us, comprises highly effective medicinal characteristics that can cure a variety of significant health *issues* **(Parihar *et al.,* 2021; Chaudhary *et al.,* 2021).**From ancient times, India has long been known as a potential source of natural remedies among ancient cultures ***(*Parihar *et al.,* 2021).**

In present scenario, exhaustive usages of chemically synthesized antibiotics have badly affected the human immune system by generating drug resistance pathogenecity. This problem has led the extensive search of natural remedies to withstand such condition Solanum the growing demand for novel drugs that can overcome the problem of drug resistance contribute to a revival of interest in plant based compounds for drug discovery. Nature endows medicinal plants for our health and survival. ***(*Prajapati *et al.*, 2002)** In present time, the use of herbal has become even more important, while pharmaceuticals are making synthetic drugs based on natural constituents from medicinal plant. A great part of herbal compounds constitutes the main part in drug discovery to produce synthetic molecular analog. This specifies the importance of study of phytochemicals in Diversity Oriented Synthesis (DOS) of natural product like pharma compound Solanum **(Stessy *et al.*, 2016)**

Medicinal plants grow worldwide, which are intended for medicinal purpose. Among those *Catharanthus roseus* is a globally significant plant which found in tropical area Solanum Ayurveda states the use of plant as traditional medicine system in India that comprises the therapeutic importance of plants and natural product Solanum some plants of family Apocynaceae are medicinally important including *Catharanthus roseus* leave Solanum

Catharanthus roseus is a perennial tropical medicinal plant **(Malar Retna *et al.*, 2013).** Family Apocynaceae comprises eight species of *Catharanthus*, out of which, seven species are endemic to Madagascar and *Catharanthus coriaceous, Catharanthus lanceus, Catharanthus longifolius, Catharanthus ovalis*, and *Catharanthus roseus* widely spread in India **(Chandrashekhar *et al.*, 2021).** Furthermore, about *Catharanthus roseus* plant, this produces more than 130 Terpenoids Indole Alkaloids (TIAs). Some terpenoids indole alkaloids are very useful pharmaceutical and medicinal plants due to their strong and important pharmacological activities **(Van der Heijden *et al.*, 2004; Almagro *et al.*, 2015).**

Catharanthus roseus is a significant curative plant **(Das *et al.*, 2017).** This dicotyledonous angiospermic plant integrates two therapeutically important compounds terpene indole alkaloids: vinblastine and vincristine, which are the major constituents of the plant and fight against a variety of diseases **(Zhu *et al.*, 2015).** *Catharanthus* is a Greek word that means "unadulterated blossom." It has many

synonyms as Madagascar periwinkle, bright eyes, Cape periwinkle, graveyard plant, old maid, pink periwinkle, and rose periwinkle myrtle. In India this is frequently referred to as "Nayantara" or "Sadabahar" **(Rai *et al.,* 2021; Dubey *et al.,* 2020).**

Catharanthus roseus is a well-recognized herbal medicinal plant **(Sultana *et al.,* 2024; Jamal *et al.,* 2024).** *Catharanthus roseus* is widely distributed in South Africa and Australia and grows well in the United States and Southern Europe. This is an ornamental plant, widely distributed throughout India. Different parts of *Catharanthus roseus* are used in folklore herbal medicines to treat many types of infectious disease Solanum Vinblastine and Vincristine, these two Vinca alkaloids, anticancer chemicals, are abundant in *Catharanthus* roseus leaves **(Arulvendhan *et al.,* 2024).**

Previous researches reveal that *Catharanthus roseus* contains more than 130 alkaloid Solanum The presence of active components vincristine and vinblastine make this plant specific and are widely used in the treatment of various cancers, such as breast cancer, lung cancer, and melanoma **(Leveque *et al.,* 2007; Ferreres *et al.,* 2008).** *Catharanthus roseus* leaves contain 70 different bioactive compounds including ajmalicine, reserpine, and serpentine. Alkaloids are the main therapeutic compound of *Catharanthus roseus* and are significantly used in the remedy of severe complications like diabetes, high blood pressure, dysmenorrhea, asthma, constipation, and cancer **(Pham *et al.,* 2020).** Two major derivatives terpene indole alkaloids, namely vincristine and vinblastine are produced in the plants **(Bhambhani *et al.,* 2021)** along with large amounts of volatile and phenolic compounds such as cafe oil, quinic acid, and flavone glycosideSolanum These compounds confer antioxidant activity against reactive oxygen species and play an important role in the plant's defence system **(Prabha *et al.,* 2020).** Plants compounds which impart antioxidant activity are mainly used for hypertension, diabetes, blood cancer, malaria, non-small-lung cancer, Hodgkin’s lymphoma, and improving memory. These plant synthesized compounds also has antimicrobial, antioxidant, anti-diarrheal, and hypolipidimic activity, as well as wound-healing activity **(Allamsetty *et al.,* 2020**). The current work is focused on the phytochemicals and antimicrobial activity of methanol extract of *Vinca rosea.*

Plants are regarded as significant tools in the drug formulation Solanum Anciently plants are being used as traditional medicines and also considered as the base of

Homeopathy, Ayurveda. In present time, several research studies establish the plants as potential remedies against severe diseases with low side effects comparatively to synthetic drug Solanum A large population approximately 85% peoples in developing countries depends on traditional medicines obtained from plant resource Solanum

Therefore, medicinally important plants contain significant amount of various bioactive metabolites in different chemical composition **(Karthikeyan *et al.,* 2009).** Bioactive metabolites present in Medicinal plants the key raw materials for pharmaceuticals formulations **(Augusti *et al.,* 2006).**Currently focus towards the phytochemicals research has been intensified and results the findings of new anti-infective compounds from higher plants which are physiologically significant **(Duraipandiyan *et al.,* 2006).**

The investigations of new effective bioactive compounds comprised of antimicrobial as well as antifungal activity is the most promising effort in present time against the developing resistant to pathogen against synthetic *antibiotics* **(Singh *et al.,* 2019; Yadav *et al.,* ., 2021; Nath *et al.,* 2010 and Maurya *et al.,* 2018).**

Bioactive compounds investigations and familiarity to present technically progressive population worldwide, is significant relative to extensively usage of commercially synthetic preparation Solanum Sources of Natural compounds from microorganism as well as the plant world are limitless Solanum The prevalent antibiotics as well as pesticides are used with natural plant's derived bioactive compounds in a combination, which reduces the risk of resistance development in pathogens without affecting the effectiveness of plant produced product Solanum Plants extract and essential oils from plants impart antimicrobial activity have significant application in pharmaceuticals, as alternatives of medicine and natural therapies, in food processing industries **(Shankar *et al.,* 2016).**

Furthermore, Plant synthesizes a variety of Secondary bio metabolite compounds which are significant for antimicrobial, antioxidant and antitumor activity **(Mishra *et al.,* 2019 & Rana *et al.,* 2021);** Flavonoids, a major Bioactive compounds exhibits a strong chemo preventive effect **(Kumar *et al.,* 2021).**

Moreover the epidemiological investigations state that partly consumption of flavonoids rich diets have effect on life expectancy **(Kikuchi *et al.,* 2019 and Grosso *et al.,* 2017),** due presence of anticancer activity **(Godos *et al.,* 2018 and Sak *et al.,* 2017).** Therefore, scientists are focussing on the significant medicinal activities of

natural bio compounds synthesized by a plant which includes the effect of flavonoids against various kind of lung cancer, breast cancer, cervical, tumourogenic cells as well as neoblastic cells **(Imran *et al.*, 2019; Zanoaga *et al.*, 2019 and Yao *et al.*, 2017).** Secondary metabolites such as essential oils constituents, phenolic compounds, saponosides and alkaloids remain the object of much in vivo as well as in vitro research **(Moga *et al.*, 2016, Górniak *et al.*, 2019; Djeussi *et al.*, 2013 and Subramani et al., 2017).**

Plant *Solanum nigrum* belongs to the family Solanaceae. *Solanum nigrum* is known with others name as Makoi or black nightshade. Traditionally *Solanum nigrum* has been broadly used in healing of various illnesses such as fever, pain and inflammation. The plant *Solanum nigrum* is also comprised of antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic properties **(Ayesha *et al.*, 2010).** *Solanum nigrum* fruits berries have been traditionally used in the cure of stomach ulcers as folk medicine **(Singh *et al.*, 2011).**

Solanum nigrum is grown as locally agricultural weed worldwide **(Subramani,*et al.*,2017 ; Edmonds *et al.*,1997).** Investigated bioactive compounds in *Solanum nigrum* are alkaloids **(solanine, soladulcine, chaconine, solasodine),** steroid, resins and essential oils with constituents, among others, including dillapiol, α-cadinol, p-cymene, α-phellandrene, β-pinene and dyes: brown, green as well as blue and saponins,. Even so, presence of solaine content in higher amount makes the black nightshade as a poisonous plant regardless of all those remarkable features **(Dhellot *et al.*, 2006; Al-Qirim *et al.*, 2008).**

Solanum nigrum is an annual herbaceous, semiclimber plant which comes under the family Solanaceae. *Solamun nigrum* is known as Black nightshade in English, whereas, Kachcipandu, Munatakali, Piludi, Kamuni and Makoi are the Gujrati, Telugu, Tamil, Marathi and Hindi, names for the plant **(Guo *et al.*, 2024).**

Solanum nigrum plant Average length of this plant is usually 10 to 15 cm, with a semi-climbing, green, smooth hebacious stem with uniformly oval shaped & slightly clogged opposite leave Solanum This plant is grown in the wet woods, waste land, quagmire, old field, ditches road side, in wet cultivated land and river bund.7500 medicinal plants & above are listed, still discovery of new one is continued **(Rana *et al.*, 2021).** Presently, available medicines for diabetes include insulin and colourful oral anti-diabetic agents similar as sulfonylureas, glinides and biguanides

(Mishra and Shankar, 2004). Synthetic medicines consist of numerous serious side effects; thus, the search for further effective and safer hypoglycaemic agents is one of the most significant areas of discussion. In diabetes, hyperglycaemic condition leads the synthesis of Reactive Oxygen Species (ROS), affecting lipid peroxidation and membrane damage. Formation of free radicals plays a critical role not only in the development of diabetes mellitus but also secondary complication, eye, blood vessel, nerve damage and kidney **(Rana *et al.*, 2021).** Antioxidants are the potential molecules by blocking the peroxidation chain response, which prevents the death of ß- cells, professionals and people caused with diabetes Solanum so, severity of this disease, let us transform our commitments into conduct to make healthier communities with better access to quality diabetes education, diabetes drugs, and care **(Kumar *et al.*, 2021).**

Plant origin secondary metabolites and their semi-synthetic derivatives playing a vital role in anticancer drug treatment. Well-known dicot plant, *Solanum nigrum* an annual branched herb, with very small flowers, five petals in the leaves, small fruits, is fall into the family of Solanaceae. This common plant mainly grow in waste lands as well as cultivated lands world-wide, and phytochemicals are abundantly found in the plant which make this plant is a reservoir of which have ***(*Lee *et al.*, 2006).**

Compound Solanine was mainly synthesized in the Solanaceae family mainly potato plant **(*Solanum tuberosum* leaves)** and nightshade plant **(*Solanum nigrum* leaves) (Kodamatani *et al.*, 2005; Friedman *et al.*, 2006; Shindo *et al.*, 2004 & Zywicki *et al.*, 2005).** The green peel and the sprouts of the potato tuber are much rich in solanine **(Korpan *et al.*, 2004; Glossman Mitnik *et al.*,2007; Stobiecki *et al.*, 2003).** Other than solanine, *Solanum nigrum* contains many steroid alkaloids such as, Saponin, solasonine, solanine and Solamargine etCatharanthus , which used for antitumor cure and such alkaloids also found with the inhibitory effect on tumour growth in animal models and breast cancer tumour cells **(Wang *et al.*, 2005; Son *et al.*, 2003).**

Another tumourogenic effect is show when oral administration of *Solanum nigrum* derived polysaccharides against cervical cancer of tumour bearing mice. These contents present in plant, increases bax gene and decrease the Bcl - 2 and mutant p-53 expression. This results apoptosis in tumour cells **(Jian *et al.*, 2009).** Saponins and other alkaloids have antimicrobial and antioxidant activity as reported in *Solanum nigrum* **(Muto *et al.*, 2006).** Glycoproteins derrivbed from *Solanum nigrum* is a dose

dependent radical scavenger against many free radical of DPPH, OH, Superoxide anion and also induce apoptosis in HT-29 cells (**Heo *et al.*, 2004; Heo *et al.*, 2004; Adebayo *et al.*, 2008).**

In a study an active compound active compound *solanine* was isolated & purified by using acid extraction along with alkaline precipitation methods from leaves of Solanum nigrum is found to be good sources of Steroidal glycol-alkaloid *Solanum* their structures were characterized by using various spectroscopic data of FTIR, HPLC, NMR, MS as well as UV. Anti- oxidative effects of *solanine* were tested for DPPH, superoxide anion radical and the hydroxyl radical besides antibacterial activity were analysed against pathogens *Escherichia coli,* Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa and its cytotoxicity effects were tested using Hep-2 and AGS cell line **(Rajalakshmi *et al.*, 2017).**

In this study, in vitro antibacterial, antifungal, anticancer and antioxidant activities of methanol leaf and root extracts from *Solanum nigrum* leaves to determined phytochemical Solanum The Present chemical of *Solanum nigrum* leaves extract was analysed through liquid chromatography coupled with mass spectrometry (LC-MS/MS). Antibacterial activities were studied using Disc diffusion and micro dilution assays furthermore antifungal activity assessed by using the poisoned food technique. In vitro activity on the cell culture model was assessed by MTT assay, viability measurement and by determination of cellular ROS by DCFDA assay. In these study minimal inhibitory concentrations for extracts from aerial parts ranged from 125 to 500 µg/ml. In a dose dependent study the growth inhibition coefficient was ranged from 17–56%. The depended on the dose. in a dose dependent study of antifungal effect of *Solanum nigrum* extracts on the tested filamentous fungi, an inhibitory effect of 50–56% on fungi was observed against Chaetomium globosum and Alternaria alternate. Both extracts also reduced a cellular vitality of, A549, MCF7 and HeLa and THP1cancer line Solanum Furthermore, as an effect of extract declined the number of viable cells and a reduction in oxygen radicals in the cells incubated with the extract of aerial part Solanum The achieved results indicate the opportunity of using *Solanum nigrum* extracts from the aerial part as antimicrobial aspect Solanum Both extracts possess the chemo preventive properties as inhibiting the growth of cancer cells and reducing the level of free radicals as shown in the study. The studied extracts

can be used in agriculture, veterinary usages and medicine as their broad biological activity **(Ge˛barowska, *et al.*, 2022).**

2.2 PHARMACOLOGICAL ACTIVITIES

Previous Several studies have reported the pharmacological activities of various solvent extracts and isolated bioactive compounds from *Solanum nigrum* using. Isolated bioactive compounds from plant Solanum nigrum have exhibited many pharmacological properties including antitumor, antioxidant, anti-inflammatory, immunomodulatory, antihypertensive, antimicrobial, and antiviral activities **(Yang *et al.*, 2016; Veerapagu *et al.*, 2018; Tian *et al.*, 2019; Guo *et al.*, 2020; Sivaraj *et al.*, 2020).**

2.2.1 Antioxidant Activity

Free radicals accumulations in the body are the main cause of aging and tissue damage. Continued generation of free radicals lead the Oxidative stress is beginning of many human diseases, such as atherosclerosis, ischemic reperfusion injury, inflammation, cancer, neurodegenerative diseases and aging. Antioxidants protect the body's vitality and cell from the oxidizing and damaging effects of free radical Solanum many studies have been focussed on in vitro and in vivo assessment of the antioxidant activity of the extracted bioactive compounds from Solanum nigrum. Studied fruit extract of *Solanum nigrum* for DPPH_ radical, superoxide radical, ABTS radical cation, phosphomolybdenum reduction, and Fe3+ reducing power assays assessment. The highest DPPH_ radical scavenging activity was noticed for 73.16% at 120 µg/ml (IC50 = 81.02 µg/ml), the maximum superoxide radical scavenging activity was 60.06% at 120 µg/ml (IC50 = 57.82 µg/ml), the maximum ABTS radical cation scavenging activity was 67.70% at 60 µg/ml (IC50 = 35.56 µg/ml), the maximum phosphomolybdenum reduction was 96.77% at 120 µg/ml (RC50 = 21.25 µg/ml), and the maximum Fe3+ reduction was 72.10% at 120 µg/ml (RC50 = 63.74 µg/ml). These outcome of the study suggested that flavone and oleic acid, may be responsible for the antioxidant activity of the fruit extract of *Solanum nigrum* **(Sivaraj *et al.*, 2020).** Leaf extract of *Solanum nigrum* supposed to increase intracellular GSH levels and decrease`OS levels as determined in primary rat astroglial cell cultures, which is suggested that the leaf extract of *Solanum nigrum* could restore the oxidative status by using glutamate as a stressor, prevent the increase in the glutamate uptake, and inhibit the excytotoxicity of glutamate **(Campisi *et al.*,**

2019). In a study, hydroxyl radical scavenging assay, 60% ethanol crude extract of *Solanum nigrum* had DPPH scavenging rates of 68.45% and 49.12% **(Teng *et al.*, 2014).** lunasin peptide purified from *Solanum nigrum* when used in a dose dependent manner it blocks the Fenton reaction between Fe2+ and H2O2 by chelating Fe2+, inhibits oxidative DNA damage ,lunasin peptide may play an important therapeutic role in the chemoprevention for oxidative carcinogenesiSolanum **(Jeong *et al.*, 2010).**

In a study, a treatment of glucose oxidase or xanthine oxidase alone or in combination with 0.01, 0.1, 1, 10, and 20 μg/ml of glycoprotein was given for 4 h for control cell line and NIH 3T3 cell lines, the viabilities of the NIH 3T3 cells were obtained 40.4%, 47.3%, 53.0%, 68.2%, and 85.3%, respectively, compared with 33.3% of the control group (glucose oxidase). The viabilities were 75.1%, 79.3%, 83.2%, 86.0%, and 95.1%, respectively, compared with 71.2% of the control group (xanthine oxidase). Viabilities of against control group (xanthine oxidase) were found increased **(Heo and Lim, et al., 2004)**.Finally, isolated and purified compounds from *Solanum nigrum* found effective antioxidants for the further studies antibacterial and Larvicidal Activity.

In present scenario, multidrug resistance in bacterial and fungal are posing great threats to human health and creating a global challenge that need an effective solution. Various Pharmacological studies have carried out for the determination of antifungal activities using different parts of *Solanum nigrum* extracted in four solvents (ethanol, chloroform, petroleum ether, and distilled water) against five fungal strains viz. Aspergillus's Niger, A. flavors, Saccharomyces cervisae, Alternaria alternate, and Fusarium oxysprum. The MIC was ranged from 250-1,000 μg/ml, and the measured zone of inhibition was in the range of 9.3 mm of stem extract in distilled water against A. flavus and 32.42 mm of fruit extract in chloroform against A. niger **(Mazher *et al.*, 2017).** The maximum inhibition zone was given by ethanol extract of *Solanum nigrum* were 16.88, 11.33, and 19.25 mm for *Staphylococcus aureus, Escherichia coli,* and *Aeromona sobria* at a concentration of 200 mg/ml, respectively **(Ge, 2019).** Solasodine-3-O-β-D-glucopyranoside isolated from *Solanum nigrum* showed an effective fungicidal activity against both azole-sensitive and azole- resistant *Candida albicans* strains with the MIC value of 32 mg/ml, which may be recognized as the essential part of the glucosyl moiety. Subsequent pharmacological studies states the mechanism of the activity of solasodine-3-O-β-D-glucopyranoside alkalized the

intracellular vacuole and elicited vacuole permeability to contribute to cell death in *Candida albicans* **(Chang *et al.*, 2017).**Findings of another study also stated that solasodine-3-O-β- D-glucopyranoside could reduce the virulence of *Candida albicans* through inhibiting adhesion and yeast-to-hyphal morphological transformation by preventing the biofilm formation of *Candida albicans* at concentrations of 16 mg/L or above. Addition of 1 mM cAMP to down regulation of adhesion-related genes such as ALS3, EAP1, and HWP1 prevents the growth rate of solasodine-3-O-β-D-glucopyranoside-treated biofilm from 27.11% to 77.15%. In addition to solasodine-3-O-β-D-glucopyranoside regulates the Ras-cAMP-PKA signaling pathway by reducing the intracellular cAMP content which inhibits the activity of *Candida albicans* **(Li *et al.*, 2015).**

In study against green synthesis of silver Nano particle (AgNPs) in graded concentrations (2.5, 5, and 10 ppm) exposed, to fight against mosquito larvae of Candida *quinquefasciatus* and An. Stephensi, the obtained IC50 and IC90 values of dry leaves, fresh leaves, and berry extracts of *Solanum nigrum* for An. Stephensi were 1.33, 1.59, and 1.56 ppm and 3.97, 7.31, and 4.76 ppm, respectively. The IC50 and IC90 values of dry leaf, fresh leaf and berry extracts of *Solanum nigrum* for *Candida quinquefasciatus* were 1.26, 1.33, and 2.44 ppm and 14.38, 38.04, and 13.42 ppm, respectively **(Rawani *et al.*, 2013).**

Furthermore, conducted a slide-dip and leaf-dip assays in a glasshouse experiments the a caricidal activity of crude extracts of *Solanum nigrum* for Tetranychus cinnabarinus, reveals the LC50 and LC90 values of 3.50, 3.99, 4.70, 12.15, 13.99, and 14.92 g/L after 1, 3, and 7 days of treatment, respectively. **(Chen and Dai, 2016).**

In a study, the methanol leaf extract was found effect vive which gave highestlarvicidal activity (mortality rate of 90%) against the early fourth instar larvae of *Candida quinquefasciatus* at 1,000 ppm as the study of **(Rahuman *et al.*, 2009).** In another findings stated that when green peach aphid were exposed to leaf extract from *Solanum nigrum* for 24, 48, and 72 h after exposure mortality rates were 28.54%, 56.8%, and 57.42% **(Madanat *et al.*, 2016).** Moreover, the isolated active compounds solamargine and solasonine from *Solanum nigrum* were found to effective against Plasmodium yoelii 17XL in mice, by supressing the parasitemia were 64.89% and 57.47%, respectively against Plasmodium yoelii 17XL as well as

parasitemia **(Chen *et al.*, 2010).** However, there is a lack of research on the mechanism underlying the insecticidal effect.

Ethno medicinal study of plant *Solanum anguivi* stated by local traditional practitioners that traditionally it reduces the diabetes risk and chronic inflammatory diseases atherosclerosis Solanum Carried out a study on is qualitative phytochemical assessment, estimation of total flavonoid and phenolic contents, antioxidant capacity and antibacterial activities of the fruits extracts of the plant. Qualitative phytochemical screening of the crude extracts obtained from the fruits of the plant indicated the presence of alkaloids, flavonoids, phenols, glycosides, steroids, terpenoids, saponins and tannin Solanum This study states the highest total phenolic and total flavonoid content were obtained in the ethanol extract of the fruits, followed by dichloromethane and n-hexane extract. Estimated total phenolic content (in gallic acid equivalents, GAE) ranged from 113.3 to 202.72 mg GAE/gm & total flavonoid content (in catechin equivalent, CE) varied from 61.72 to 142.64mg (CE)/g. Moreover the study, it revealed that all fruits extracts of *Solanum anguivi* exhibited antioxidant activity by DPPH RSA (% Inhibition) of the fruits extract varied from 35.11% to 80.13%. A linear correlation with RSA also gave by total phenolic and Flavonoid content. Antibacterial activity against gram-positive and gram-negative bacteria varied from 12.5 to 16.75 mm as results revealed. Antimicrobial activity of the extracts of the plant exercised stronger bactericidal effect on gram-positive bacteria than on gram-negative bacteria as the results exhibited. **(Misganaw *et al.*, 2021)**

Medicinal plants are traditionally recognized for containing diverse bioactive molecules with strong antioxidant properties, and many of them are used in managing long-term illnesses despite limited scientific evidence supporting their effectiveness Solanum In this study, the antioxidant activity of two leaf extracts of *Solanum nigrum* (SN) a Solanaceae plant widely consumed as a leafy vegetable-was investigated. Two types of extracts were prepared: a methanol–water extract (80:20) termed SN1, and a purely aqueous extract termed SN2. The total polyphenol content, as well as the levels of individual phenolic acids and flavonoid compounds, was quantified. To determine whether these extracts could counteract oxidative imbalance caused by glutamate, primary rat astrocyte cultures were examined for changes in glutathione content, intracellular reactive oxygen species, and cell viability following treatment with SN1

and SN2. Both extracts showed strong radical-scavenging ability in a cell-free assay and were effective in restoring redox balance in astrocytes exposed to glutamate. Furthermore, they prevented excessive glutamate uptake and reduced glutamate-induced excitotoxicity, thereby protecting the cells from damage. These findings demonstrate that both extracts possess significant antioxidant and neuro protective effect Solanum.

World Flora Online currently recognizes 21 valid species within the genus Murraya. Among them, *Murraya paniculata* leaves (Jack)and *Murraya exotica* leaves (Rutaceae) are the most commonly utilized species and are officially included in the Chinese Pharmacopoeia ***(*Chinese Pharmacopoeia Commission, 2020).**

The traditional Chinese medicinal material known as Murrayae Folium Cacumen (MFC) is prepared from the dried foliage and tender twigs of *Murraya paniculata* and *Murraya exotica* **(Lu *et al.*, 2021a).** In traditional medical practice, MFC is valued for its ability to strengthen qi, relieve various types of pain, stimulate blood circulation, and disperse blood stasi Solanum It is frequently prescribed for conditions such as abdominal pain, rheumatic disorders, arthralgia, dental pain, inflammatory swellings, and snakebite-related symptoms **(Chinese Pharmacopoeia Commission, 2020).**

MFC also serves as a primary component in Sanjiu Weitai granules, a widely used patented Chinese herbal formulation intended for gastrointestinal complaints **(Lu *et al.*, 2021b)**. Historical medical literature also documents its inclusion in formulas such as Baihu Dan, recorded in the Jingyue Complete Book, which is used for treating swelling of the face, head, limbs, and eye Solanum Similarly, in the Compilation of Chinese Medicine, it is listed as an ingredient in Zhitong Jing, where it supports blood circulation and pain relief.

The leaves of *Murraya paniculata* are widely used as a culinary spice across India, SoutheastnAsia, Pakistan, and Malaysia. In Malaysian cuisine, the foliage is commonly added to soups and various fish and meat preparations, and it has even been incorporated into spicy chicken dishes served by several commercial fast-food chains **(Ng *et al.*, 2012; Saqib *et al.*, 2015).** Historically, *Murraya paniculata* has held an important position in traditional medicine systems of China, India, and Indonesia. According to the Chinese Pharmacopoeia, the plant exhibits analgesic activity and shows potential in managing microbial infections and inflammatory disorders **(Zhang *et al.*, 2011).**

Different parts of the plant are employed for diverse therapeutic uses: powdered stem bark is traditionally applied as an antidote for snakebites, while the roots are used to alleviate general body pain. The leaves, known for their astringent and irritant qualities, are used in Indonesian folk practices to manage diarrhoea and dysentery **(Saqib *et al.,* 2015).** Additional traditional applications include treatment of coughs, hysteria, rheumatism, toothaches, stomach discomfort, and gout **(Gautam *et al.,* 2012a)**. The plant also finds use in remedies for bites, wound healing (using crushed leaves), alcohol-related fluid retention, abortion induction, and venereal diseases **(Kinoshita & Firman, 1996a; Rahman *et al.,* 2010).** Essential oils rich in terpenoids derived from its flowers are valued in the cosmetic and fragrance industries **(Rout *et al.,* 2007; Rehman *et al.,* 2013).**

Murraya exotica, another species commonly grown as an ornamental shrub in tropical and subtropical regions, are recognized for its glossy foliage and fragrant white blossoms **(Sharma & Arora, 2015).** Ethnomedicinally, it is used to relieve pain, abdominal discomfort, and rheumatic conditionSolanum The leaves are particularly rich in coumarins, which possess antioxidant, anti-cancer, anti-mycobacterial, antifungal, antiviral, and anti-inflammatory activitieSolanum Its roots contain numerous coumarins and alkaloids, including paniculidines **(He *et al.,* 2017).**

Previous phytochemical investigations of the Murraya functional complex (MFC), including *Murraya paniculata* and *Murraya exotica*, reveal that these plants contain diverse bioactive constituents such as coumarins, flavonoids, alkaloids, and volatile oil Solanum Alkaloids are especially concentrated in the root Solanum Pharmacological studies further confirm that MFC exhibits anti-inflammatory, analgesic, antibacterial, antioxidant, and insecticidal effects **(Lu *et al.,* 2021a).** However, despite numerous individual studies, a comprehensive systematic review of MFC is still lacking. Thus, summarizing its phytochemistry, pharmacological properties, mechanisms, pharmacokinetics, and toxicology is essential to support its standardized application in the Chinese Pharmacopoeia.

Thus far, a total of 316 chemical constituents have been reported from the Murraya functional complex (MFC). These include flavonoids, coumarins, alkaloids, sterols, phenylpropanoids, organic acids, spirocyclopentenones, along with approximately 404 volatile oil component Solanum These compounds have been characterized through a range of analytical techniques, such as thin-layer chromatography (TLC),

high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (UPLC), ultraviolet (UV) spectroscopy, nuclear magnetic resonance (NMR), mass spectrometry (MS), combined NMR–MS analysis, UPLC-electrospray ionization mass spectrometry (UPLC-ESI-MS), high-resolution fast atom bombardment mass spectrometry (HR-FAB-MS), heteronuclear multiple-bond correlation (HMBC), heteronuclear multiple-quantum coherence (HMQC), and gas chromatography-mass spectrometry (GC-MS) **(Saikia *et al.*, 2021).**

Among these constituents, flavonoids are the dominant class in *Murraya paniculata*, whereas coumarins are most abundant in *Murraya exotica*. Alkaloids are concentrated primarily in the roots, with only minimal presence in stems and leaves **(Lu *et al.*, 2021a).**

The composition of mature leaves includes 63.2% moisture, 1.15% total nitrogen, 6.15% fat, 18.92% total sugars, 14.6% starch, 6.8% crude fibre, and 13.06% total ash. In addition, they contain 1.35% acid-insoluble ash, 1.82% alcohol-soluble extractives, 27.33% cold-water extractives at 20°C, and as much as 33.45% hot-water-soluble extractives ***(Selvamani & Balamurugan, 2015).*** Among these constituents, the most noteworthy groups are the carotenoids, essential oils, and various carbazole alkaloid Solanum.

Overall, the phytochemical profile of *Murraya* can be broadly classified into a total of 93 flavonoids have been identified in the Murraya functional complex (MFC). Among these, 72 are polymethoxylated flavonoidSolanum from total flavonoids, 90 compounds were obtained from *Murraya paniculata* and 24 from *Murraya exotica*, indicating that *Murraya paniculata* is the primary source of flavonoid constituents **(Lu *et al.*, 2021a).** Additionally, 21 flavonoids were common to both *Murraya paniculata* and *Murraya exotica* **(Negi *et al.*, 2015; Sangkaew *et al.*, 2020).** Structurally, flavonoids in MFC can be grouped into six major categories: flavones, flavonols, flavanones, flavan-3-ols, chalcones, and isoflavones, with flavones representing the predominant structural clasSolanum

In total, 170 coumarins have been reported in MFC. These coumarins, 89 compounds were isolated from Murraya paniculata, 130 from M. exotica, and 49 were shared by both species **(Liu *et al.*, 2018; Wang X. *et al.*, 2019; Wang Y. *et al.*, 2019).** Based on structural features, coumarins are categorized into six types: simple coumarins,

prenylated coumarins, pyranocoumarins, furanocoumarins, dimeric coumarins, and benzocoumarins .

Furthermore, 29 alkaloids have been identified in MFC Seventeen alkaloids were isolated from Murraya paniculata and twelve from *Murraya exotica*, with no alkaloids found to be common between the two specie Solanum These alkaloids can be divided into six structural classes: quinoline, indazole, indole, carbazole, organic amine alkaloids, and other miscellaneous alkaloid Solanum.

Research on the antimicrobial and antibacterial properties of the *Murraya* functional complex has largely concentrated on *Murraya paniculata*, while *Murraya exotica* have received comparatively little attention. Several compounds isolated from the methyl acetate leaf extract of *Murraya paniculata*-including murrangatin, murrangatin-2′-acetate,murranganonsenecionate, micropubescin, and 3′, 4′, 5′, 7-tetramethoxyflavone exhibit notable activity against Porphyromonas gingivaliSolanum Among these, compounds and display stronger antibacterial effects than the crude extract, suggesting that coumarins are key contributors to the plant's antimicrobial action **(Rodanant *et al.*, 2015).**

Extracts of *Murraya paniculata* tested against various human pathogenic bacterial strains have demonstrated broad-spectrum antibacterial potential. Ethanol and hydro alcoholic extracts show mild to moderate inhibitory effects, whereas the methanol leaf extract exhibits pronounced activity against both Gram-positive and Gram-negative bacteria. The antibacterial action is thought to be associated with the high levels of phenolic and flavonoid constituents **(Gautam *et al.*, 2012a).** In another study, the ethanol leaves extract showed broad inhibitory activity against Staphylococcus aureus, with a MIC_{50} value of 406 μg/mL **(Panda *et al.*, 2020).**

Volatile oils from *Murraya paniculata* have also demonstrated strong antibacterial potential. (**Saikia *et al.*,. 2021)** reported significant inhibition of Mycobacterium smegmatis and Pseudomonas aeruginosa, both with MIC_{50} values of 4 μg/mL. Analysis of these oils using GC-MS identified 29 constituents, most of which were sesquiterpene hydrocarbons (approximately 80%), with caryophyllene being the predominant compound (20.93%). **(Silva *et al.*,. 2020)** further showed that these oils were active against Mycobacterium kansasii (MIC_{50} = 250 μg/mL) and moderately effective against Mycobacterium tuberculosis, demonstrating both anti-streptococcal and anti-mycobacterial properties (MIC_{50} = 500 μg/mL).

Hydro distillation followed by GC-FID and GC-MS analysis of *Murraya paniculata* leaf volatile oils identified 18 compound Solanum In vitro antifungal tests revealed a strong inhibitory effect-91.2% suppression of Sclerotinia sclerotiorum mycelial growth. Another GC-MS investigation of fresh leaves obtained through hydro distillation identified 13 compounds; with the extract consisting of 99.3% sesquiterpenes and 0.3% monoterpene Solanum the major constituents included β-caryophyllene, α-zingiberene, and α-caryophyllene. **(Selestino Neta *et al.*,. 2017)** also observed that both the volatile oils and β-caryophyllene (320) displayed moderate antibacterial activity (MIC_{50} < 1.0 mg/mL).

Essential oil obtained from the leaves of *Murraya koenigii* has been reported to possess strong antibacterial potential. It effectively inhibits the growth of Pasteurella multocida, Bacillus subtilis, Staphylococcus aureus, Corynebacterium pyogenes, and Proteus vulgariSolanum Even when diluted to 1:500, the oil continued to suppress the first three bacterial species **(Pate *et al.*, 2023).**

Furthermore, fractionation of the acetone leaf extract yields three prominent carbazole alkaloids-mahanimbine, murrayanol, and mahanine. These constituents have shown antibacterial, mosquitocidal, and topoisomerase I and II inhibitory activities, highlighting their multifunctional biological significance **(Dubey *et al.*, 2022).**

With respect to antioxidant potential **(Tachibana *et al.*, 2001)** reported that leaf-derived compounds such as mahanimbine and koenigine demonstrate significant antioxidant activity. Their antioxidant capacity was evaluated using the β-carotene-linoleic acid assay. Among the different extracts examined, the oleoresin of Murraya koenigii displayed the strongest effect, showing 83.2% activity at a concentration of 100 ppm. In comparison, the methanol and aqueous extracts produced activities of 16.7% and 11.3%, respectively. For reference, the synthetic antioxidant butylhydroxyanisole exhibited 90.2% activity at the same concentration **(Rao *et al.*, 2007).**

Ethanol leaves extracts also showed considerable free radical-scavenging capability, as demonstrated by DPPH and nitric oxide (NO) assays **(Sindhu & Arora, 2012).** In the DPPH radical-scavenging test, the ethanol extract produced a high inhibition level of 63.54%, further supporting its strong antioxidant potential **(Sumalatha, & Nithya, 2014).**

Several in vitro investigations have demonstrated that the 50% ethanolic extract of *Murraya paniculata* leaves exhibits strong antioxidant potential **(Gautam *et al.*, 2012b).** Using GC-MS**,(Rodríguez *et al.*,. 2012)** identified 18 constituents in the volatile oils of *Murraya paniculata* leaves obtained through hydro distillation, with β-caryophyllene being the predominant component. These volatile oils also displayed significant antioxidant activity.

Studies on *Murraya exotica* indicate that the ethyl acetate fraction of the leaves-rich in flavonoids and polyphenolic compounds-shows the highest antioxidant capacity among the various sequentially extracted fractions **(Kaur *et al.*, 2016).** Additionally, methanol extracts of *Murraya exotica* have been reported to contain abundant polyphenols and flavonoids, which contribute to their notable antioxidant activity **(Khatun *et al.*, 2014).**

Total flavonoids extracted from *Murraya paniculata* (TFMP) have been shown to significantly reduce kidney injury in diabetic rat model Solanum Their protective actions in diabetic nephropathy appear to be linked to improvements in glucose and lipid metabolism, reductions in oxidative stress, and modulation of inflammatory cytokines **(Zou *et al.*, 2014). In a later study, Zou *et al.*, (2021)** reported that TFMP also helped prevent the development of diabetic cardiomyopathy in type 2 diabetic rat Solanum This cardio protective effect was associated with enhanced expression of NF-E2-related factor 2 and heme oxygenase-1, leading to attenuation of oxidative stress, inflammation, and apoptosis Solanum.

Leaf extracts of *Murraya paniculata* have additionally demonstrated hypoglycemic properties in alloxan-induced diabetic rats, lowering blood glucose and improving complications such as hypercholesterolemia and hypertriglyceridemia. These extracts also minimized tissue damage associated with diabetic condition *Solanum* Their glucose-lowering activity is comparable to standard ant diabetic drugs like glibenclamide and metformin and is thought to act through inhibition of ATP-sensitive potassium channels **(Menezes *et al.*, 2017).**

Research on MFC has revealed that it contains an extensive range of phytochemicals, with over 720 compounds identified to date, including 404 constituents belonging to volatile oil *Solanum* Extracts prepared from MFC, along with their isolated molecules, have been reported to possess a broad range of biological activities Solanum These include anti-inflammatory, antibacterial, antimicrobial, antitumor,

antioxidant, anti-diabetic, anti-Alzheimer, and analgesic effect Solanum Among the various chemical groups, flavonoids, coumarins, and volatile oils are considered the principal bioactive constituents responsible for most pharmacological action Solanum

Although *Murraya paniculata* and *Murraya exotica* are closely related, their chemical profiles differ. Flavonoids are the major constituents in *Murraya paniculata*, whereas coumarins dominate in *Murraya exotica*. In *Murraya paniculata*, flavonoids-particularly polymethoxyflavones-are associated with strong anti-inflammatory, antibacterial, antimicrobial, antitumor, and chondroprotective potential. Conversely, in Murraya exotica, prenylated coumarins exhibit diverse biological properties, such as anti-inflammatory, antibacterial, antimicrobial, antitumor, possible anti-Alzheimer, chondroprotective, anti-implantation, estrogen-like, and antihypertensive activitieSolanum Investigations into the volatile oil fraction of MFC have primarily emphasized their antibacterial, antimicrobial, and potential anti-Alzheimer effect *Solanum.*

Despite substantial progress, several scientific gaps remain. Although MFC demonstrates strong pharmacological potential, the specific plant parts, extract fractions, and active molecules responsible for these effects are not yet fully understood, indicating the need for deeper investigation. Additionally, existing studies have mostly concentrated on polymethoxyflavones and coumarins, while comprehensive quality-control measures for MFC have been largely overlooked. Therefore, establishing a standardized and reliable quality-control framework for MFC is an important priority for future research to ensure consistency and reliability in the use of MFC, further quality-focused research is essential. Although numerous studies have examined the pharmacological properties of *Murraya paniculata* and Murraya exotica, only two comparative pharmacodynamic investigations have been conducted, and both reported no significant statistical differences between the two specie Solanum Most available studies rely heavily on in vitro experiments, while in vivo investigations remain limited, and only two reports have explored potential clinical application Solanum This lack of comprehensive in vivo and clinical data restricts the broader therapeutic development of MFC.

Given these limitations, future research should prioritize in vivo pharmacological studies, clarify the links between efficacy and underlying mechanisms, and explore the structure-activity relationships of key compound Solanum Although the antitumor

potential of MFC has been described-primarily in terms of cytotoxicity-no validated in vivo tumour models have been established to date. Therefore, animal models and clinical tumour studies are urgently needed to further substantiate these finding *Solanum.*

Additionally, the mechanistic basis for many traditional uses of MFC-such as the treatment of stomach pain, rheumatism, arthralgia, and toothache, and swelling-remains poorly defined. Except for the protective effects on gastric lesions, few mechanistic explanations have been systematically reported. Volatile oils, which represent one of the most abundant components and contribute significantly to the plant's insecticidal activity, also show considerable chemical variability depending on geographic origin. This variability further underscores the need for standardized quality specifications for MFC.

The broader issue of "heterogeneous equivalence"-where multiple botanical sources are used interchangeably-remains a concern in many traditional Chinese medicinal material Solanum. This creates challenges in terms of ensuring consistent efficacy and establishing robust quality-control systems **(Lu *et al.*, 2021b).** To address this, it is necessary to evaluate whether *Murraya paniculata* and *Murraya exotica* can be justifiably used as equivalent sources of MFC. Our comprehensive review of the phytochemistry, pharmacology, pharmacodynamics, pharmacokinetics, and toxicity of both species provides an essential foundation for their rational application and offers direction for future scientific inquiry.

CHAPTER – 3

MATERIALS & METHODS

3.1 MATERIALS

3.1.1 Chemical and Media used

- 1, 1-diphenly-2-picrylhydrazyl (DPPH): Himedia Laboratories, Mumbai
- 10% NH4OH: Himedia Laboratories, Mumbai,
- 1-octanol- antifoaming solution: Himedia Laboratories, Mumbai,
- 2, 6-di-tert-butyl-4-methyphenol (BHT): Himedia Laboratories, Mumbai,
- 2-deoxy-2-ribose: Himedia Laboratories, Mumbai,
- Absolute alcohol - Jiangsu Huaxi International Co. Ltd., China,
- Acetic acid: Qualigens fine chemicals, Mumbai,
- Acetic anhydride: Himedia Laboratories, Mumbai,
- Aluminum nitrate: Himedia Laboratories, Mumbai,
- Ammonia solution: Himedia Laboratories, Mumbai,
- Benzene: Himedia Laboratories, Mumbai,
- Bovine serum albumin: Himedia Laboratories, Mumbai,
- Butylated hydroxyl anisone (BHA): Himedia Laboratories, Mumbai,
- Calcium carbonate: Qualigens fine chemicals, Mumbai,
- Calcium sulphate: Qualigens fine chemicals, Mumbai,
- Chloroform: Qualigens fine chemicals, Mumbai,
- Diethyl-ether: Himedia Laboratories, Mumbai,
- Dinitrisalicylic acid : Himedia Laboratories, Mumbai,
- Dragendorff's reagent: Himedia Laboratories, Mumbai,
- EDTA- Himedia Himedia Laboratories, Mumbai,
- Ferric chloride: Himedia Laboratories, Mumbai,
- Folin–Ciocalteu reagents: Himedia Laboratories, Mumbai,
- Formaldehyde: Qualigens fine chemicals, Mumbai,
- Gallic acid: Himedia Laboratories, Mumbai,
- Glucose: Himedia Laboratories, Mumbai,
- Hydrochloric acid (HCL): Qualigens fine chemicals, Mumbai,
- Hydrogen peroxide: Himedia Laboratories, Mumbai,

- Hydroxyl amine: Himedia Laboratories, Mumbai,
- $KMnO_4$ solution. : Himedia Laboratories, Mumbai,
- L- ascorbic acid: Himedia Laboratories, Mumbai,
- Malt Dextrose Agar: Himedia Laboratories, Mumbai,
- Methanol: Qualigens fine chemicals, Mumbai,
- Mueller Hinton agar: Himedia Laboratories, Mumbai,
- Nitroblue tetrazolium reagent: Himedia Laboratories, Mumbai,
- Olive oil: Himedia Laboratories, Mumbai,
- Petroleum spirit: Himedia Laboratories, Mumbai,
- Potassium acetate: Himedia Laboratories, Mumbai,
- Potato dextrose agar media: Himedia Laboratories, Mumbai,
- Quercetin : Himedia Laboratories, Mumbai,
- Sodium carbonate: Himedia Laboratories, Mumbai,
- Sodium hydroxide: Himedia Laboratories, Mumbai,
- Sulfuric acid (H_2So_4): Ranchem Pvt. Ltd., New Delhi,
- TCA: Himedia Laboratories, Mumbai,
- Thiobarbituric acid: Himedia Laboratories, Mumbai,

3.1.2 Instruments Used

- Autoclave
- BOD Incubator
- Flame photometer
- Hot Air Oven
- Incubator
- Laminar Air Flow
- Micro Pipettes
- Muffle furnace
- Soxhelet apparatus
- UV -Vis Spectrophotometer
- Water bath

3.2 METHODS

The present study was carried out on the extraction of three parts: Leaves, stem and fruits collected from three different plants (***Murraya paniculata, Solanum nigrum*** **and** ***Catharanthus roseus***) using three solvents methanol, acetone and Double distilled water for the evaluation of phytochemical screening, antioxidant and antimicrobial activity.

Table 3.1 Classification

	Murraya paniculata	*Solanum nigrum*	*Catharanthus roseus*
Kingdom	Plantae	Plantae	Plantae
Phylum	Charophyta	Tracheophyta Spermatophyta	Tracheophyta Magnoliophyta
Class	Equisetopsida	Magnoliopsida (Dicotyledons)	Magnoliopsida (Dicotyledons)
Order	Sapindales	Solanales	Gentianales
Family	Rutaceae	Solanaceae	Apocynaceae
Genus	Murraya	***Solanum***	***Catharanthus***
Species	*Paniculata*	***Solanum nigrum***	***Catharanthus roseus*** *leaves*

All these plants parts were collected dried and extracted in different solvents for the study of antioxidant potentiality, qualitative phytochemical screening and antibacterial and antifungal activity of plant. The study was divided into seven sections:

A. Collection of plant material leaves, stem and fruits from different areas of Bundelkhand University campus and also were grown in different location in Jhansi district (UP)and near about Jhansi area and Govt. Science college and jiwaji university campus Gwalior District.
B. Preparation of plant extracts in aqueous, acetone and alcoholic solvents.
C. Study the qualitative analysis of phytochemicals in plant extracts.
D. Study the evaluation of antioxidant activity of plant extracts.
E. Study of Plant extracts for its antibacterial activities.
F. Study of Plant extracts for its antifungal activities.

Murraya paniculata





Catharanthus roseus

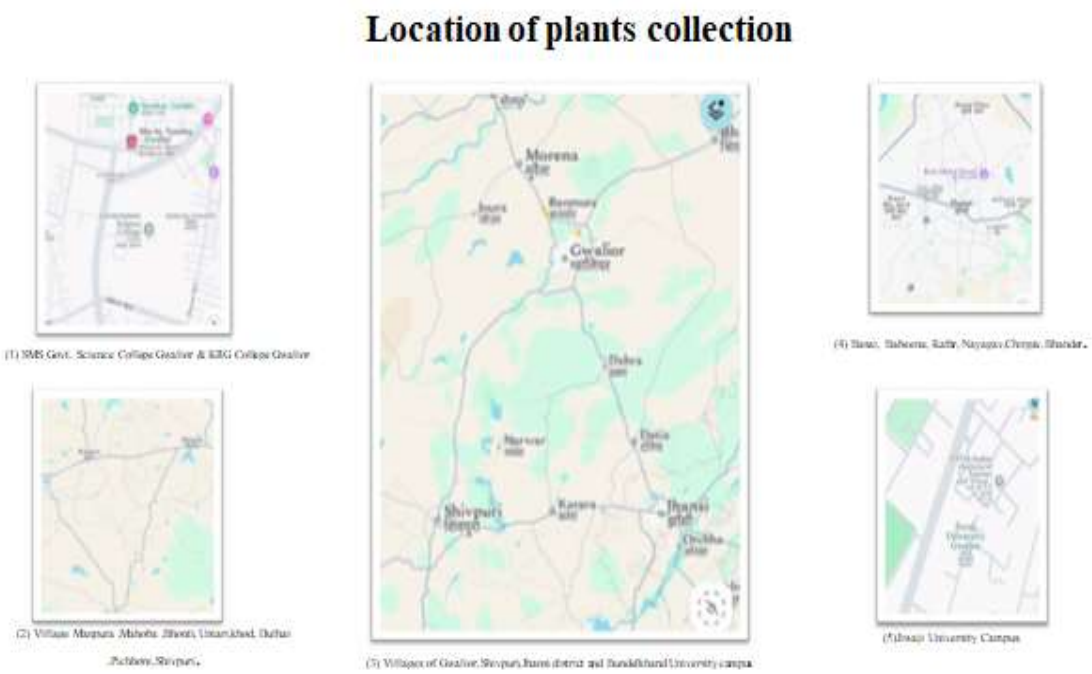




Solanum nigrum







3.2.1 Collection of Plant materials:

The plant parts: young leaves, stems and fruits were collected from fully mature plants *Catharanthus roseus, Solanum nigrum and Murrya paniculatum* from University campus, were grown in different colleges ,houses, nursery, forest and roadside in Jhansi, Shivpuri and Gwalior District. Plant materials were washed separately under running tap water, followed by rinse using sterilized distilled water. Excess of water was removed from the plant material using filter paper before they were used for extraction. Separated Plant leaves were shed dried. Dried leaves were grinded and extracted with methanol, Acetone and double distilled water using Soxhelet apparatus.

3.2.2 Qualitative analysis of phytochemicals present in edible Plant:

Phytochemical screening included the following parameters which were described below with detailed methodology **(Adebayo and Ishola, 2009).**

3.2.2.1 Test for Tannins:

For this test, 1.0 g of the dried Plant extracts ed sample from each cultivated species were taken in test tube added 20 ml of water then boiled and filtered. A few drops of 0.1% ferric chloride was added in filtrate .A brownish green or a blue-black coloration developed.

3.2.2.2 Test for Phlobatannins:

0.5 g of aqueous extract of dried Plant extracts ed sample was boiled with 1% aqueous HCl, deposition of red precipitate was taken as positive test for the presence of phlobatannins.

3.2.2.3 Test for Saponin:

About 2 g of the Plant extracts was taken in a 20 ml of distilled water filled test tube and boiled upon a waterbath and then filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. 3 drops of olive oil was mixed in froth and shaken forcefully, and then emulsion formation indicated the presence of saponins.

3.2.2.0 Test for Flavonoids:

Aqueous filtrate of Plant extracts was taken and 5 ml of diluted ammonia solution was added. Later addition of concentrated sulphuric acid formed yellow colour which indicated presence of flavonoids.

3.2.2.1 Test for Steroids:

Crude extract of Plant was mixed with chloroform and few drops of conc. H_2SO_4, shaken well and allowed to stand for some time. Formation of red colour at the lower layer indicated the presence of steroids.

3.2.2.2 Test for glycosides:

2 ml of glacial acetic acid with one drop of ferric chloride solution was added to 5ml of the Plant extract. Addition of 1ml of concentrated sulphuric acid in reaction mixture formed a brown ring at the interface indicated the presence of cardiac glycosides.

3.2.2.3 Test for Anthroquinones:

0.5gm of the Plant extracts was boiled with 10 ml of sulphuric acid and filtered. 5 ml of chloroform was added in filtrate and shaken. The layer of chloroform was separated out and taken in another test tube and 1ml of diluted ammonia was added. Colour change indicated the presence of Anthraquinines.

3.2.3 Antioxidant activity of Plant extracts:

3.2.3.1 Determination of total phenolic compounds (TPC):

Total phenolic compound in Plant extracts was determined by using Folin-Ciocalteau reagent according to the method of (***Harborne 1989)*** with some modifications. 150 µl of each sample (1000 µg/ml) was taken and, added with 1200 µl of distilled water and 450 µl of aqueous sodium carbonate solution. About 100µl of Folin-Ciocalteu reagent was added to the reaction mixture and agitated. This reaction mixture was allowed to stand for 90 minutes and the absorbance was measured at 760 nm using UV/visible spectrophotometer (UV Vis spectrophotometer Make-Systronics, Model no- 2701). Concentration of total phenolic compounds was calculated based on standard curve of Gallic acid (200-1000 µg/ml) and the results were expressed as mg of Gallic acid equivalent (GAE) per gram of the extracts.

3.2.3.2 Determination of Total Flavonoids Compound (TFC):

The presence of total Flavonoid in above Plant samples was determined following the method of **(Park *et al.*, 1997)**, 1 ml of Plant extracts (1000 µg /ml) was taken in 4.3 ml of distilled water, 0.1 ml of 10% aluminium nitrate and 0.1 ml of 1 M aqueous potassium acetate were added to this reaction mixture and incubated for 40 min at room temperature. After incubation, the absorbance was taken at 415 nm. Total flavonoid concentration will be calculated using quercetin (200 µg /ml to 1000 µg/ml) as standard.

3.2.3.3 Scavenging effect on 1, 1-Diphenly-2- picrylhydrazyl (DPPH)

The Scavenging effect of Plant extracts on 1, 1-Diphenly-2- picrylhydrazyl are calculated by using the method of Burits and Bucar, 2000.The hydrogen atom or electron donating capability of the Plant extracts was measured from the discoloration of the purple-coloured methanolic solution of 1, 1- Diphenly-2-picrylhydrazyl (DPPH). This Spectrophotometric assay used the stable radical DPPH as a reagent.

A range of concentration from 100 µg to 500 µg/ml of methanol and aqueous extract of Plant and 0.004 % methanol solution of DPPH were prepared.1000 µl extract was taken from every concentration and add 4.0 ml of DPPH.A blank was also prepared by adding 1000 µl methanol and 4.0 ml of DPPH. After an incubation period of 20-30 min Absorbance was taken at λ = 517 nm (UV Vis spectrophotometer Make-

systronics, Model no: 2701). Inhibition of free radical by DPPH in per cent (%) was calculated by the given formula.

$$\% \text{ Inhibition} = \frac{(A_{blank} - A_{sample})}{(A_{blank})} \times 100$$

Where "A_{blank}" is taken as the absorbance of the control (Solvent) and "A_{sample}" is the absorbance of the test sample. Extract concentration provided 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration. Tests were carried out in triplicate.

3.2.3.3 ABTS Cation Radical Scavenging Assay:

ABTS radical scavenging activity was performed as described by Re R, **Pellegrini (1999)** with a slight modification.7mM ABTS in14.7mM ammonium peroxo-disulphate was prepared in 5 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted and its absorbance absorbance was taken at 734nm. Absorbance must be 0.70±0.02 .Various concentration 100, 200. 300. 400 and 500 µg/ml of the sample solution and quercetin standard were prepared by dissolving in ethanol (20µl) and finally added to 980µl of ABTS radical solution and the mixture was incubated in darkness for 10 min. The decrease in absorbanance and reading will be taken at 734 nm.

$$\% \text{ Inhibition} = \frac{(A_{blank} - A_{sample})}{(A_{blank})} \times 100$$

Where "A_{blank}" is the absorbance of the control (blank) and "A_{sample}" is the absorbance of test samples. All the tests were performed in triplicate and the graph was plotted with the mean values.

3.2.4 Antimicrobial activity

The antimicrobial activity of Plant was determined by Agar well diffusion method of **Akpata and Akinrimisi (1977).**

3.2.4.1 Reagents for the Agar well Diffusion Test

A. Mueller-Hinton Agar Medium

Commercially available dehydrated Mueller-Hinton agar media was used in the present antimicrobial study.

B. Preparation of Mueller-Hinton Agar

Mueller-Hinton agar was prepared according to the manufacturer's directions. After autoclaving, it was allowed to cool down to 45 to 50°C in a water bath. Freshly prepared and cooled medium was poured into pre sterilized flat-bottomed glass petri dishes of 100 mm diameter, on an untitled, horizontal surface to give a uniform depth of approximately 4 mm. Each plate with a diameter of 100 mm contains 25 to 30 ml of media.

C. Preparation of antibiotic stock solutions

Antibiotics, used in the present study were streptomycin and fluconazole. Accurately weighed Powder was dissolved in the DMSO solvent in a concentration 20, 40, 60, 80 and100mg/ml, and stored in pre sterilized vials. The stock was stored at 4ºC.

3.2.4.2 Inoculum Preparation

Bacterial strain of *Escherichia coli* (MTCC-1610), *Staphylococcus aureus* (MTCC-3160) and *Salmonella* (MTCC-3425) were grown on nutrient agar. A single colony of bacteria was picked up by loop and transferred to a tube containing 4 to 5 ml of Nutrient broth medium. The inoculated tubes were incubated at 37°C±2°C until turbidity of 0.5 McFarland standard was attained (usually 2 to 6 hours). *Candida albicans* (MTCC-3958) was also selected from 24 hr old potato dextrose agar culture plate.

a. Inoculation of Plates:

After incubation of inoculated tubes, turbidity of the each inoculums broth was adjusted by mixing with fresh nutrient broth if necessary. A sterile cotton swab was dipped into the adjusted broth and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. Spread the swab on the entire surface of Muller Hinton agar surface. This procedure was repeated by spreading two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums. Finally, the edges of the agar petri dishes were swabbed.

b. Well formation:

Inoculated petri dishes were aseptically punched with flame sterilized cork borer under laminar air flow (Make: Multitech). Standard size of wells was kept about 6.0 mm in diameter and 4.0 mm in depth.

Methanol and aqueous extracts were prepared in a range of concentration 20, 40, 60, 80 and 100 mg/ml or µg/µl by dissolving in a solvent DMSO and introduced into the wells in the plates with help of micro pipette. Negative control wells were made by introducing 100µl of the extracting solvent (DMSO, Distilled water and Methanol) directly. Antibiotic streptomycin (Brand name Ambistryn-s 1.0 g) was taken as positive control against bacteria *Staphylococcus aureus*, *Salmonella, Escherichia coli* and antifungal fluconazole (Tablet Fluka 150 mg) for two fungi and *Candida albicans* on the agar petri dishes. Petri dishes were kept in laminar flow for 30 minutes for pre-diffusion of extract and then incubated at 37ºC for 24 hours. Resulting zone of Inhibition was measured using a Hi media zone scale.

Biostatistics Methods

The experimental data generated in the present investigation were analysed using **standard bio statistical approaches** to ensure data reliability, consistency, and scientific robustness. All experimental determinations were performed in **triplicate (n = 3)**, and the results were expressed as **mean ± standard deviation (SD)**. The mean values were used to describe the average response of the samples, while the standard deviation provided an estimate of variability among replicate measurements, thereby indicating the precision of the experimental procedures

To evaluate differences among various **plant parts, extraction solvents, and tested concentrations**, appropriate inferential statistical methods were applied. **One-way analysis of variance (ANOVA)** was used wherever applicable to determine whether observed variations between group means were statistically significant. A probability level of $\mathbf{p < 0.05}$ was considered indicative of statistical significance.

Graphical representation of data in the form of bar diagrams and line graphs was employed to enhance visual interpretation and comparative analysis. These graphical tools aided in clearly illustrating solvent-dependent, plant-part-specific, and concentration-dependent trends observed throughout the study.

CHAPTER – 4

RESULT AND DISCUSSION

4.1 Extractive Value of Different Solvents

The comparative extractive values of different plant parts using Methanol, aqueous, and acetone solvents are illustrated in Figure 4.1 The graphical representation clearly demonstrates substantial variation in extractive yield depending on solvent polarity, plant species, and plant part.

Table 4.1 Extractive value of different plants parts using different solvents

Extractive value (mg/g)				
Plant's Name	**Plants Part**	**Solvents**		
		Methanol	**Aqueous**	**Acetone**
Murraya paniculata	Leaves	600.29± 7.78	550.57±3.53	450.38±2.4
	Fruits	30.88±5.1	30.9±5.0	30.1±7.5
	Stem	40.2±4.5	65.3±3.5	31.1±4.3
Catharanthus roseus	Leaves	590.85±3.2	610.7±5.8	455.65±5.5
	Fruits	21.1±6.5	22.2±7.1	19.9±2.3
	Stem	10.89±4.4	12.1±2.5	12.0±1.9
Solanum nigrum	Leaves	630.38±2.5	670.55±1.8	470.19±6.2
	Fruits	91.5±7.8	112.8±7.2	80.4±2.1
	Stem	30.8±6.2	33.9±6.6	28.5±1.9
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)				

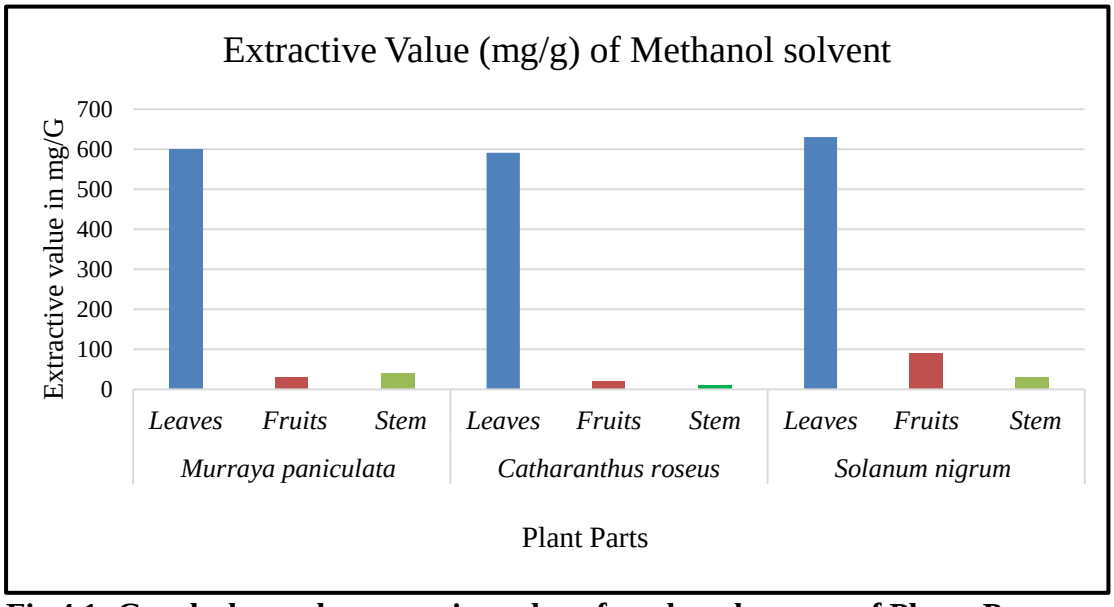


Fig.4.1: Graph shows the extractive value of methanol extract of Plants Parts.

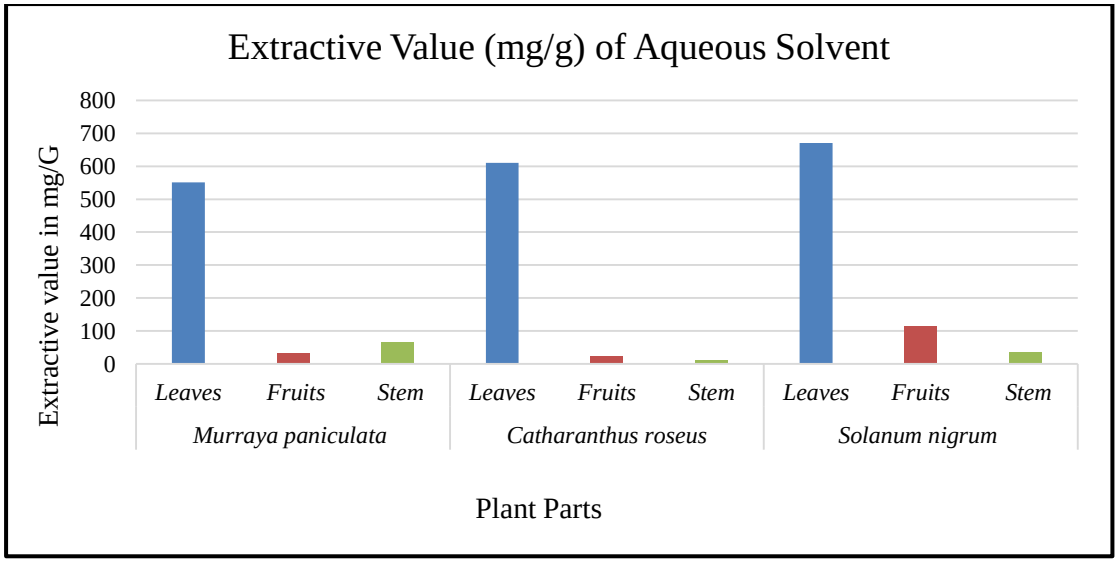


Fig.4.2: Graph showing the extractive value of aqueous extract of Plants Parts.

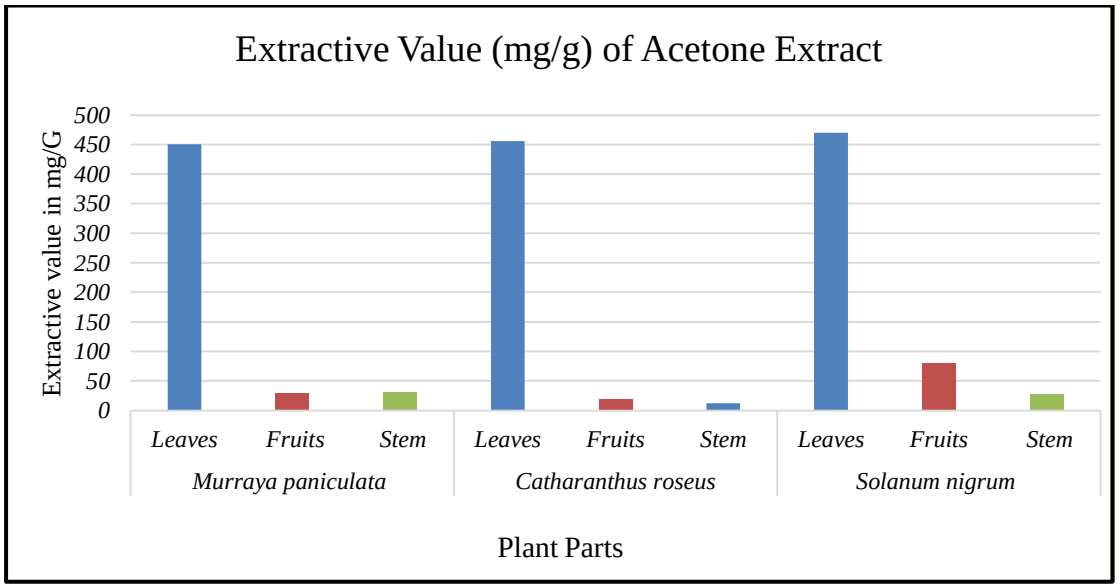


Fig.4.3: Graph shows the extractive value of acetone extract of Plants Parts.

Leaves of all three plant species exhibited markedly higher extractive values compared to Fruits and stems, irrespective of the solvent used. Among solvents, methanol and aqueous extracts consistently produced higher extractive yields than acetone.

4.2 Solvent Influence on Extractive Value

As shown in **Figure 4.1 and 4.2,** methanol and water were the most efficient solvents for extracting phytoconstituents from all studied plants. Methanol extracts of leaves yielded particularly high extractive values, such as in ***Solanum nigrum*** (630.38 mg/g) and ***Murraya paniculata*** (600.29 mg/g). This efficiency is attributed to methanol's ability to dissolve a wide spectrum of polar and moderately polar

compounds, including phenolic, flavonoids, alkaloids, and glycosides. Similar solvent-dependent extraction efficiency has been reported by **(Ahmed *et al.*, 2021)** and **(Altemimi *et al.*, 2020).**

As shown in table 4.1, Aqueous extracts also exhibited high extractive values, especially in leaves samples of ***Solanum nigrum*** (670.55 mg/g) and ***Catharanthus roseus*** (610.7 mg/g). Water is particularly effective in extracting hydrophilic constituents such as sugars, tannins, and certain phenolic acids, which explains the elevated aqueous extractive values. **(Kumar *et al.*, 2020)** reported comparable findings for medicinal plants commonly used in traditional formulations.

In contrast, acetone extracts consistently showed lower extractive values across all plant parts and species as given in the table 4.1 and Fig. 4.3. Although acetone is effective for extracting certain low-molecular-weight secondary metabolites, its limited ability to solubilize highly polar phytochemicals may account for the reduced extractive yield observed in the present study, as also noted by **(Bhuyan *et al.*,2022).**

4.3 Qualitative phytochemical screening

Qualitative phytochemical screening of Methanol, aqueous, and acetone extracts of leaves, fruits, and stem parts of ***Murraya paniculata*, *Catharanthus roseus*,** and ***Solanum nigrum*** revealed clear solvent- and tissue-dependent variations. Overall, **Methanol extracts exhibited the widest range of phytochemicals,** followed by aqueous extracts, while acetone extracts showed comparatively limited detection.

Across all three species**, leaves extracts consistently contained higher levels of alkaloids, flavonoids, phenolic, and tannins** than fruits and stems, indicating that leaves are the primary sites of secondary metabolite accumulation. This trend is consistent with the metabolic role of leaves in synthesizing defence related compounds and antioxidants **(Harborne, 1998).**

Among the studied plants as shown in table 4.2, ***Catharanthus roseus*** Methanol leaves extract showed **strong presence (+++) of alkaloids, flavonoids, and phenolic,** supporting earlier reports that identify this species as a rich source of in dole alkaloids and phenolic antioxidants **(Kokate, 2019).** In contrast, acetone leaves extracts of ***Catharanthus roseus*** showed absence of most phytochemicals, highlighting the strong influence of solvent polarity on extraction efficiency.

Table- 4.2: Qualitative analysis of phytochemicals

Plant Name	Plant Part	Extracts	Type of Phytochemicals							
			A	**F**	**Ph**	**Tn**	**S**	**G**	**T**	**St**
Murraya paniculata	Leaves	Methanol	++	+++	+++	++	+	++	++	+
		Acetone	++	++	++	+	-	+	++	+
		Aqueous	+	++	++	++	++	++	-	-
	Fruits	Methanol	+	++	++	+	-	+	+	-
		Acetone	+	+	+	-	-	-	-	-
		Aqueous	-	+	+	+	-	-	-	-
	Stem	Methanol	+	++	++	+	-	+	+	-
		Acetone	+	+	+	-	-	-	+	-
		Aqueous	-	+	+	+	+	-	-	-
Catharanthus roseus	Leaves	Methanol	+++	+++	+++	++	+	++	++	+
		Acetone	-	-	-	-	-	-	-	-
		Aqueous	++	++	++	++	++	+	-	-
	Fruits	Methanol	++	++	++	+	+	+	+	+
		Acetone	+	+	+	+	+	+	+	+
		Aqueous	+	+	+	+	-	-	-	-
	Stem	Methanol	++	++	++	+	-	+	+	-
		Acetone	+	+	+	-	-	-	+	-
		Aqueous	+	+	+	+	+	+	-	-
Solanum nigrum	Leaves	Methanol	++	++	++	++	+	++	+	+
		Acetone	+	+	+	+	+	+	+	-
		Aqueous	+	+	+	++	+	+	-	-
	Fruits	Methanol	+	+	+	+	-	+	-	-
		Acetone	-	-	-	-	-	-	-	-
		Aqueous	-	-	-	-	-	-	-	-
	Stem	Methanol	+	+	++	+	+	+	+	-
		Acetone	+	-	+	-	-	-	-	-
		Aqueous	-	-	+	+	-	-	-	-

Present, (-): Absent, A= Alkaloids, S= Saponin, F= Flavonoids, St= Steroids, Ph= Phenols, Tn= Tanin, T=Terpenoids G= Glycosides.

According to table ***4.2, Murraya paniculata*** showed a balanced phytochemical profile, with Methanol leaves extracts rich in flavonoids, phenolic, tannins, and glycosides, while aqueous extracts were particularly effective in extracting tannins and saponins. Similar solvent-specific extraction patterns have been supported by **(Altemimi *et al.* 2020).**

In ***Solanum nigrum***, leaves again demonstrated superior phytochemical richness compared to fruits and stems, with Methanol extracts showing moderate to strong presence of phenolic and flavonoids. Fruit extracts, particularly in aqueous and acetone solvents, showed minimal phytochemical detection, suggesting limited accumulation of secondary metabolites in reproductive tissues **(Kumar *et al.*, 2020).**

These findings underline the importance of **solvent selection and plant-part choice** in phytochemical investigations and support the preferential use of **Methanol leaves extracts** for further quantitative and bioactivity studies **(Bhuyan *et al.*, 2022).**

4.4 Evaluation of antioxidant activity of Plant's Parts extracts

4.4.1 Total Phenolic Content (TPC)

The total phenolic content (TPC) of Methanol extracts obtained from leaves, fruits, and stem of ***Murraya paniculata, Catharanthus roseus,*** and ***Solanum nigrum*** is presented in the given table and expressed as mg Gallic acid equivalents per gram of extract (mg GAE/g). The results indicate marked variations in phenolic concentration among plant species as well as among different plant parts.

Across all three plants, as shown in below given tables and figures, **leaves consistently exhibited the highest phenolic content,** followed by fruits and stem. In ***Murraya paniculata,*** leaves extracts recorded the maximum TPC (32.1 ± 0.03 mg GAE/g), while fruits (30.1 ± 0.3 mg GAE/g) and stem (27.7 ± 0.2 mg GAE/g) showed comparatively lower but still substantial phenolic levels. This suggests that ***Murraya paniculata*** is a particularly rich source of phenolic compounds, especially in its foliar tissues.

A similar pattern was observed in ***Catharanthus roseus,*** where leaves demonstrated significantly higher TPC (28.4 ± 0.4 mg GAE/g) than fruits (23.8 ± 0.3 mg GAE/g) and stems (20.0 ± 0.5 mg GAE/g). In ***Solanum nigrum,*** although overall phenolic levels were lower compared to ***Murraya paniculata,*** leaves still exhibited the highest TPC (27.7 ± 0.1 mg GAE/g), followed by fruits (18.8 ± 0.5 mg GAE/g) and stem (16.0 ± 0.8 mg GAE/g).

For ***Solanum nigrum,*** leaves again demonstrated the highest TPC (27.7 ± 0.1 mg GAE/g), while fruits (18.8 ± 0.5 mg GAE/g) and stem (16.0 ± 0.8 mg GAE/g) exhibited lower phenolic levels. Comparable trends were observed by **(Kumar *et al.***

2020), who reported that phenolic compounds are preferentially accumulated in photo synthetically active tissues.

The observed trend (**Leaves > Fruits > Stem**) can be attributed to the physiological role of leaves as the primary site of biosynthesis and accumulation of secondary metabolites, including phenolic compounds. Phenolics play a crucial role in plant defence against oxidative stress, ultraviolet radiation, pathogens, and herbivores, which explains their higher concentration in photo synthetically active tissues. Structural tissues such as stems generally accumulate lower levels of phenolic, consistent with the present findings.

The higher TPC values obtained using methanol as the extraction solvent highlight its effectiveness in solubilizing a wide range of phenolic compounds due to its polarity. Previous studies have reported that Methanol extracts often yield higher phenolic content compared to aqueous or less polar organic solvents, supporting the reliability of the present results.

Table 4.3 Total phenolic content of Methanol extract from different plant's parts.

Plant	**Total phenolic content of Methanol extract (GAE mg eq. /g)**	
Murraya paniculata	Leaves	32.1±0.03
	Fruits	30.1±0.3
	Stem	27.7±0.2
Catharanthus roseus	Leaves	28.4±0.4
	Fruits	23.8±0.3
	Stem	20.0±0.5
Solanum nigrum	Leaves	27.7±0.1
	Fruits	18.8±0.5
	Stem	16.0±0.8
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)		

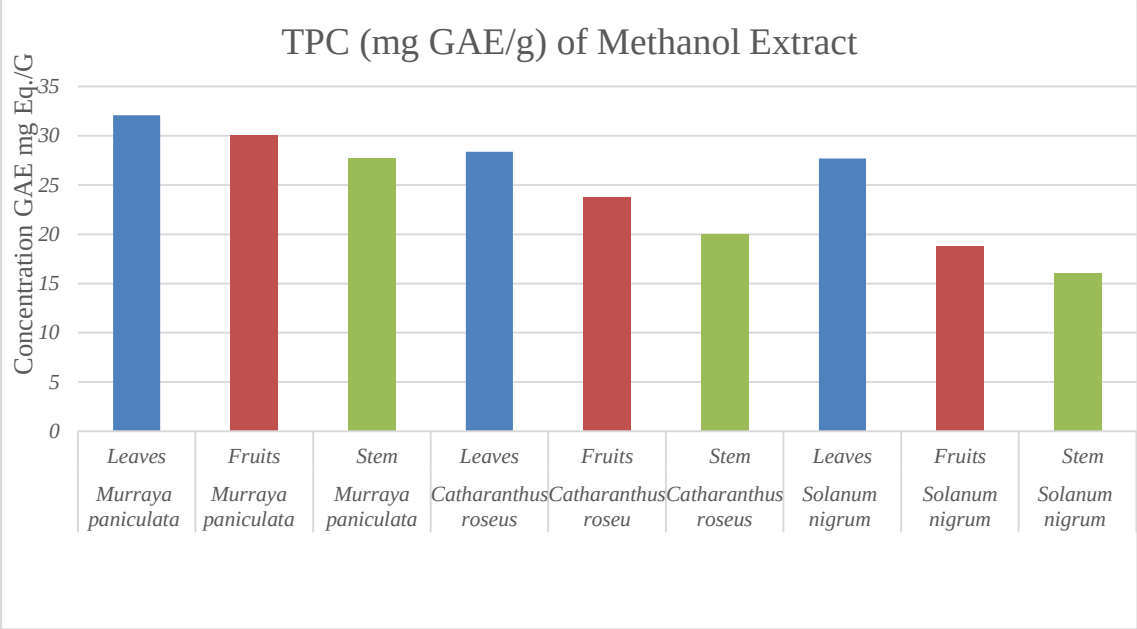


Fig.4.4 Total Phenolic Content of Methanol extracts from different plant parts of three Plants.

Comparatively, ***Murraya paniculata*** leaves exhibited superior phenolic content among the studied species, indicating stronger antioxidant potential. This observation aligns with earlier reports that associate *Murraya paniculata* with abundant bioactive Phenolics such as flavonoids, phenolic acids, and carbazole alkaloids, which contribute to its medicinal and nutraceutical importance. ***Catharanthus roseus*** and ***Solanum nigrum*** also demonstrated appreciable phenolic levels, reinforcing their traditional use in herbal medicine and their relevance as natural antioxidant sources.

The use of methanol as an extraction solvent significantly influenced phenolic recovery. Methanol is recognized as an efficient solvent for extracting a broad range of phenolic compounds due to its polarity and ability to disrupt plant cell walls **(Altemimi *et al.*, 2020). (Ahmed *et al.*, 2021)** also reported that Methanol extracts generally yield higher total phenolic content compared to aqueous and less polar solvents.

Comparative analysis among species revealed that ***Murraya paniculata*** exhibited superior phenolic content, particularly in leaves extracts, indicating stronger antioxidant potential. ***Catharanthus roseus*** and ***Solanum nigrum*** also demonstrated considerable phenolic levels, supporting their traditional medicinal use and relevance as natural antioxidant sources **(Kumar *et al.*, 2020; Bhuyan *et al.*, 2022).**

The total phenolic content (TPC) of acetone extracts obtained from leaves, fruits, and stems of ***Murraya paniculata***, ***Catharanthus roseus***, and ***Solanum nigrum*** was quantified and expressed as mg Gallic acid equivalents per gram of extract (mg GAE/g). The results demonstrate noticeable variation in phenolic concentration across plant species as well as among different plant parts.

Table 4.4: Total phenolic content of Acetone extract from different plant's parts.

Plant	**Total phenolic content of Acetone extract (GAE mg eq. /g)**	
Murraya paniculata	Leaves	26.4±0.03
	Fruits	24.1±0.3
	Stem	20.7±0.2
Catharanthus roseus	Leaves	24.4±0.4
	Fruits	22.8±0.3
	Stem	21.0±0.5
Solanum nigrum	Leaves	21.7±0.1
	Fruits	16.8±0.5
	Stem	12.0±0.8
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)		

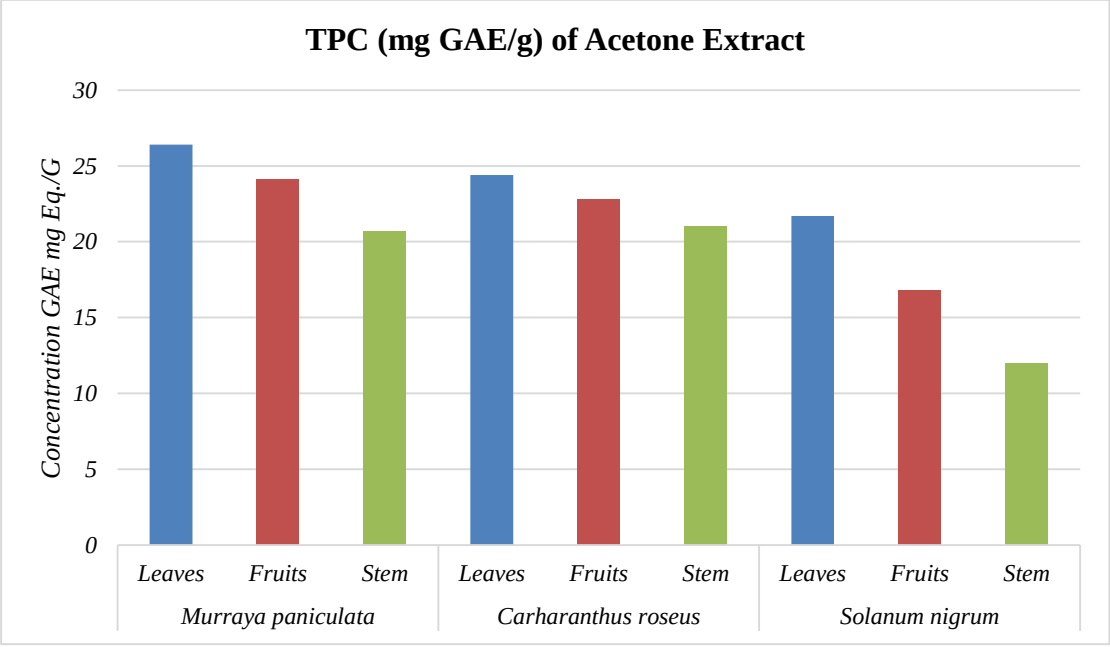


Fig.4.5 Total Phenolic Content of Acetone extracts from different plant parts of three Plants.

In ***Murraya paniculata***, the acetone leaves extract exhibited the highest TPC (26.4 ± 0.03 mg GAE/g), followed by fruits (24.1 ± 0.3 mg GAE/g) and stems (20.7 ± 0.2

mg GAE/g). This pattern indicates preferential accumulation of phenolic compounds in foliar tissues, even when extracted with a moderately polar solvent such as acetone. Similar observations were reported by **Ghosh *et al.* (2023),** who highlighted leaves as major reservoirs of phenolic constituents in ***Murraya paniculata*.**

For ***Catharanthus roseus*,** leaves again showed the maximum phenolic content (24.4 ± 0.4 mg GAE/g), while fruits (22.8 ± 0.3 mg GAE/g) and stems (21.0 ± 0.5 mg GAE/g) recorded slightly lower values. The relatively narrow variation between fruits and stems suggests a more uniform distribution of phenolics in this species compared to the others. According to **Bhuyan *et al.* (2022),** such inter-organ variation is species-dependent and influenced by metabolic allocation and tissue function.

Table 4.4 stated that, In ***Solanum nigrum*,** acetone leaves extracts contained the highest TPC (21.7 ± 0.1 mg GAE/g), followed by fruits (16.8 ± 0.5 mg GAE/g) and stems (12.0 ± 0.8 mg GAE/g) also shown in fig. 4.5. The comparatively lower phenolic content observed in ***Solanum nigrum*** stems reflects limited accumulation of secondary metabolites in structural tissues, as also reported by **Kumar *et al.* (2020).**

This trend corroborates the established understanding that leaves serve as primary sites for phenolic biosynthesis due to their exposure to environmental stressors such as ultraviolet radiation and oxidative stress. Phenolic compounds act as protective antioxidants and defensive metabolites, leading to their enhanced concentration in leaves tissues **(Sharma *et al.*, 2024).**

Although acetone is effective for extracting certain low-molecular-weight phenolic, methanol generally exhibits superior efficiency for a broader spectrum of phenolic compounds **(Altemimi *et al.*, 2020; Ahmed *et al.*, 2021).** Nevertheless, the appreciable phenolic yield obtained using acetone confirms its suitability as an alternative solvent for antioxidant-related studies.

The total phenolic content (TPC) of aqueous extracts prepared from leaves, fruits, and stems of ***Murraya paniculata*, *Catharanthus roseus*,** and ***Solanum nigrum*** was determined and expressed as mg Gallic acid equivalents per gram of extract (mg GAE/g). The obtained results reveal distinct variations in phenolic concentration among plant species and different plant parts when extracted using water as the solvent.

Table 4.5: Total phenolic content of aqueous extract from different plant's parts.

Plant	Total phenolic content of Aqueous extract (GAE mg eq. /g)	
Murraya paniculata	Leaves	25.4±0.03
	Fruits	22.1±0.3
	Stem	19.7±0.2
Catharanthus roseus	Leaves	21.4±0.4
	Fruits	17.8±0.3
	Stem	14.0±0.5
Solanum nigrum	Leaves	18.7±0.1
	Fruits	12.8±0.5
	Stem	10.0±0.8
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)		

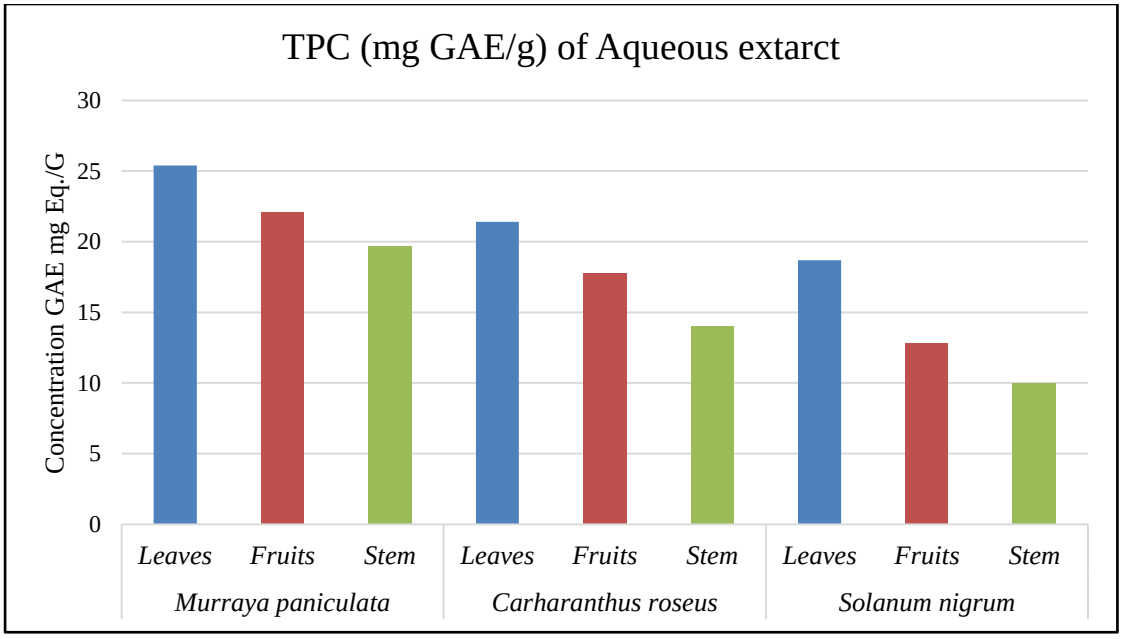


Fig.4.6 Total Phenolic Content of Aqueous extracts from different plant parts of three Plants.

In ***Murraya paniculata***, the highest TPC was recorded in leaves extracts (25.4 ± 0.03 mg GAE/g), followed by fruits (22.1 ± 0.3 mg GAE/g) and stems (19.7 ± 0.2 mg GAE/g) as shown in table 4.5 and fig. 4.6. Although aqueous extraction yielded lower phenolic values compared to Methanol and acetone extracts, the appreciable phenolic content observed in ***Murraya paniculata*** leaves highlights the presence of water-soluble phenolic compounds. Similar findings have been reported by **Ghosh *et al.* (2023),** who emphasized the abundance of hydrophilic phenolics in the leaves of ***Murraya paniculata*.**

Table 4.5 and fig. 4.6 also showed that ***Catharanthus roseus***, aqueous leaves extracts exhibited the highest TPC (21.4 ± 0.4 mg GAE/g), while fruits (17.8 ± 0.3 mg GAE/g) and stems (14.0 ± 0.5 mg GAE/g) showed comparatively lower phenolic concentrations. The reduced phenolic recovery in aqueous extracts may be attributed to the limited solubility of certain flavonoids and phenolic acids in water. According to **Bhuyan *et al.* (2022),** solvent polarity strongly influences phenolic extraction efficiency, with water extracting primarily polar phenolic fractions.

Similarly, ***Solanum nigrum***, leaves again demonstrated the highest phenolic content (18.7 ± 0.1 mg GAE/g), followed by fruits (12.8 ± 0.5 mg GAE/g) and stems (10.0 ± 0.8 mg GAE/g) as the table 4.5 and fig. 4.6 stated. The relatively low TPC values observed in aqueous extracts of ***Solanum nigrum*** indicate that a significant proportion of its phenolic constituents may be moderately polar or non-polar in nature, which are better extracted using organic solvents. Comparable observations were reported by **Kumar *et al.* (2020)** in studies evaluating solvent-dependent phenolic extraction.

This trend reinforces the role of leaves as the primary site of phenolic biosynthesis and accumulation. Leaves are directly exposed to environmental stressors such as ultraviolet radiation and oxidative conditions, leading to enhanced production of phenolic antioxidants that function in plant defence and stress mitigation **(Sharma *et al.*, 2024).**

When compared with Methanol and acetone extracts reported earlier, aqueous extracts consistently exhibited lower total phenolic content. This observation aligns with previous studies indicating that organic solvents are generally more efficient than water in extracting a broad spectrum of phenolic compounds **(Altemimi *et al.*, 2020; Ahmed *et al.*, 2021).** Nevertheless, the detectable phenolic content in aqueous extracts remains significant from a pharmacological and nutraceutical perspective, particularly considering the safety and traditional relevance of water-based preparations.

Among the studied species, ***Murraya paniculata*** maintained the highest phenolic content even in aqueous extracts, further confirming its potential as a rich natural source of antioxidant compounds. ***Catharanthus roseus*** and ***Solanum nigrum***

also demonstrated measurable phenolic levels, supporting their continued use in traditional medicine systems.

4.4.2 Total Flavonoid Content (TFC)

Flavonoids are a prominent class of polyphenolic compounds widely recognized for their **antioxidant, anti-inflammatory, antimicrobial, and anticancer properties**. In the present study, the use of **quercetin as a reference standard** provides a more specific estimation of flavonoid concentration, as quercetin represents a major flavonol commonly distributed in medicinal plants **(Patel & Shah, 2020).**

4.4.2.1 Total Flavonoid Content (TFC) of Methanol Extracts

The total flavonoid content (TFC) of Methanol extracts obtained from leaves, fruits, and stem of ***Murraya paniculata, Catharanthus roseus,*** and ***Solanum nigrum*** was quantified and expressed as QE mg equivalents per gram of extract (QE mg /g). Results are presented as mean ± standard deviation.

Among all plant parts analysed, **leaves extracts exhibited the highest flavonoid content**, followed by fruits and stems in all three plant species. ***Murraya paniculata*** leaves showed the maximum TPC value (22.1 ± 0.2 mg GAE/g), whereas the lowest Flavonoid content was recorded in the stem extract of ***Catharanthus roseus*** (10.0 ± 0.5 mg GAE/g).

Table 4.6: Total flavonoid content of Methanol extract from different plant's parts.

Plant	**Total Flavonoids content of Methanol extract (QE mg eq. /g)**	
Murraya paniculata	Leaves	22.1±0.2
	Fruits	18.1±0.5
	Stems	13.7±1.2
Catharanthus roseus	Leaves	19.4±0.6
	Fruits	13.8±0.3
	Stems	10.0±0.5
Solanum nigrum	Leaves	17.7±01.1
	Fruit	15.8±0.5
	Stem	12.0±0.8
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)		

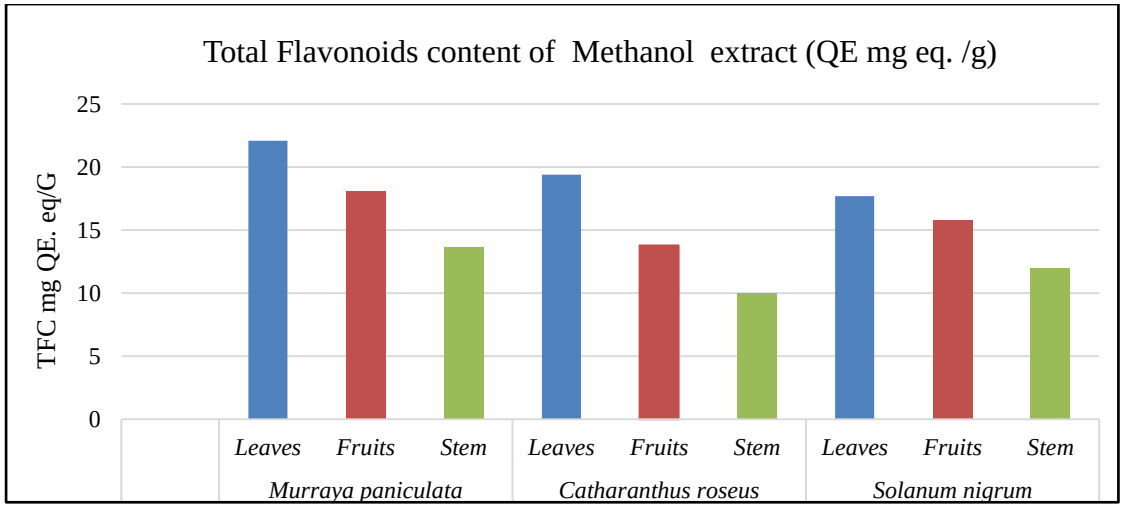


Fig.4.7 Total Flavonoid Content of Methanol extracts from different plant parts of three Plants.

4.4.2.2 Total Flavonoid Content (TFC) of Acetone Extracts

The total flavonoid content of acetone extracts from leaves, fruits, and stems of ***Murraya paniculata***, ***Catharanthus roseus***, and ***Solanum nigrum*** was quantified using **quercetin as the calibration standard** and expressed as mg QE/g of extract (mean ± SD).

As the table 4.7 and fig. 4.8 showed that Leaves extracts of all studied plants exhibited **significantly higher flavonoid content** compared to fruits and stems. Among the samples analysed, ***Murraya paniculata*** leaves showed the highest flavonoid concentration (16.8 ± 0.1 mg QE/g), whereas the lowest value was recorded in the stem extract of ***Catharanthus roseus*** (6.0 ± 0.3 mg QE/g).

Table 4.7: Total flavonoid content of Acetone extract from different plant's parts.

Plant	**Total Flavonoid content of Acetone extract (QE mg eq. /g)**	
Murraya paniculata	Leaves	16.8±0.1.2
	Fruits	12.1±0.8
	Stem	10.7±1.1
Catharanthus roseus	Leaves	15.4±0.2
	Fruits	8.8±0.6
	Stem	6.0±0.3
Solanum nigrum	Leaves	13.7±01.2
	Fruits	10.2±0.4
	Stem	08.0±0.3
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)		

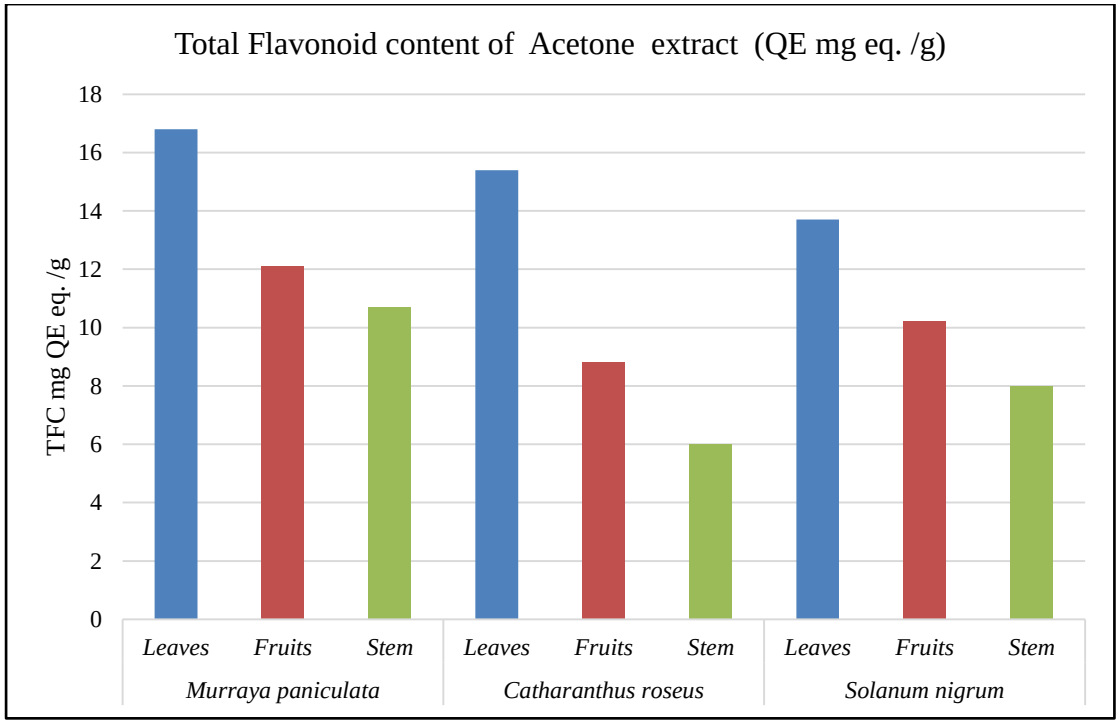


Fig.4.8 Total Flavonoid Content of Acetone extracts from different plant parts of three Plants.

4.4.2.3 Total Flavonoid Content (TFC) of Aqueous Extracts

In all three plant species, **leaves extracts showed the highest flavonoid content,** followed by fruits and stems. ***Murraya paniculata*** leaves exhibited the maximum TFC (15.9 ± 1.3 mg QE/g), whereas the lowest value was recorded in the stem extract of ***Catharanthus roseus*** (6.1 ± 0.7 mg QE/g).

Table 4.8: Total flavonoid content of Aqueous extract from different plant's parts.

Plant	**Total Flavonoids content of Aqueous extract (QE mg eq. /g)**	
Murraya paniculata	Leaves	15.9±0.1.3
	Fruits	10.1±0.5
	Stem	9.8±1.4
Catharanthus roseus	Leaves	13.4±0.2
	Fruits	8.1±0.8
	Stem	6.1±0.7
Solanum nigrum	Leaves	11.7±01.3
	Fruits	9.2±0.9
	Stem	7.20±0.8
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)		

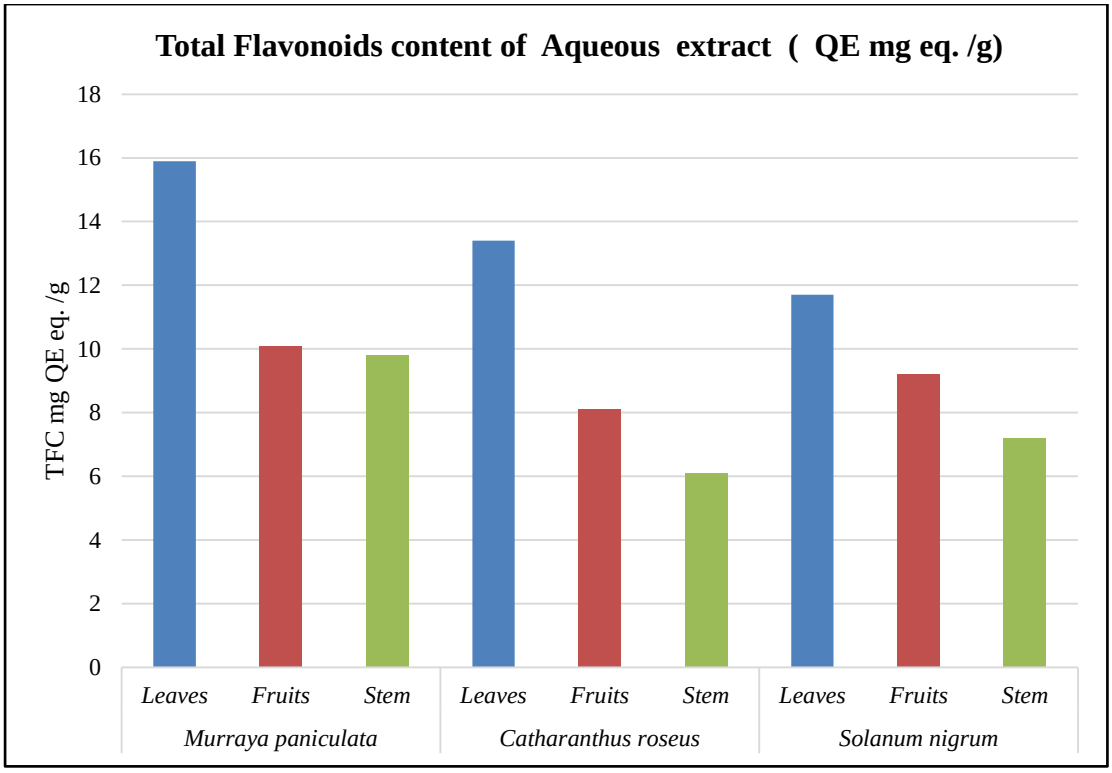


Fig.4.9 Total Flavonoid Content of Aqueous extracts from different plant parts of three Plants.

The aqueous extracts showed overall lower flavonoid content compared to organic solvents such as acetone or methanol, which is consistent with previous reports indicating limited solubility of certain flavonoid aglycones in water **(Alam *et al.*, 2023).** Nevertheless, the appreciable flavonoid levels detected in aqueous leaves extracts highlight their relevance for traditional medicinal preparations, where water is commonly used as the extraction medium.

4.4.3 DPPH Radical Scavenging Activity of different extracts of *Leaves, Stem and fruits parts of three different plants*

The DPPH radical scavenging activity of Methanol, acetone, and aqueous extracts of ***Murraya paniculata*** leaves, fruits, and stems was evaluated at concentrations of **100–500 µg /ml.** Results were expressed as **percentage inhibition (mean ± SD)** and compared with quercetin as the standard antioxidant.

All extracts exhibited a **concentration-dependent increase in DPPH radical scavenging activity,** indicating progressive enhancement of antioxidant capacity with increasing extract concentration.

Table 4.9 DPPH Radical Scavenging Activity of Methanol extracts of plant parts of Murraya paniculata					
		DPPH_%Inhibition			
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Murraya paniculata	100	13.75±1.75	12.25±1.22	11.25±1.25	38.28±2.8
	200	28.5±1.85	24.5±1.24	17.5±1.58	46.56±1.56
	300	41.25±1.25	35.75±3.0	28.75±1.78	62.82±1.8
	400	61±1.6	54.8±1.54	35±2.2	75.64±1.75
	500	69.75±1.36	63.25±2.5	51.25±1.98	81.42±1.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

Methanol extracts showed strong antioxidant activity across all plant parts. At **100 µg /ml**, leaves, fruits, and stem extracts exhibited **13.75 ± 1.75%**, **12.25 ± 1.22%**, and **11.25 ± 1.25%** inhibition, respectively. A steady increase was observed with concentration, reaching maximum inhibition at **500 µg /ml**, where fruits extract showed the highest activity (**69.75 ± 1.36%**) followed by leaves,fruits (**63.25 ± 2.5%**) and stem(**51.25 ± 1.98%**) extracts.

The superior performance of Methanol extracts indicates efficient extraction of phenolic and flavonoid antioxidants, which has been widely reported for polar organic solvents **(Kumar &Pandey, 2020; Singh *et al.*, 2022).**

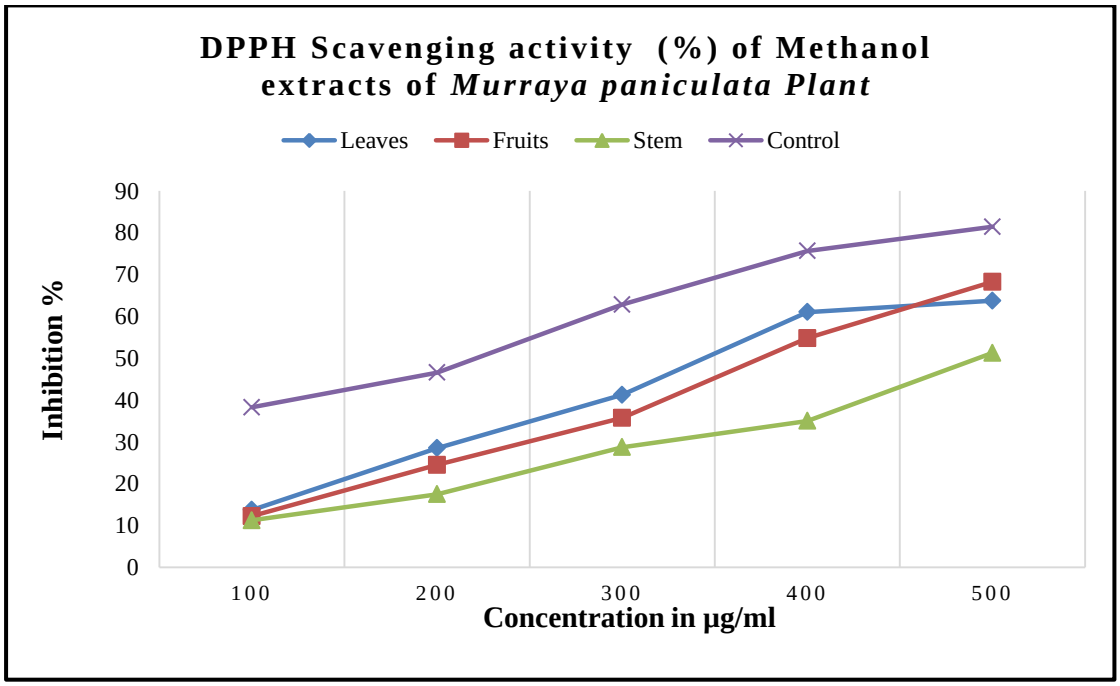


Fig. 4.10 DPPH Radical Scavenging Activity of Methanol extracts of *plant parts of Murraya paniculata.*

4.4.3.1 DPPH Radical Scavenging Activity of Acetone extracts of *plant parts*

Acetone extracts demonstrated moderate to strong antioxidant activity. At **100 µg /ml**, leaves, fruit, and stem extracts showed **12.80 ± 1.20%**, **10.56 ± 1.56%**, and **10.01 ± 1.10%** inhibition, respectively. At the highest concentration (**500 µg/mL**), leaves extract exhibited the highest inhibition (**68.75 ± 1.86%**), followed by fruits (**59.81 ± 1.98%**) and stem (**45.06 ± 1.45%**).

Table 4.10 DPPH Radical Scavenging Activity of Acetone extracts of different *parts of plant Murraya paniculata*

DPPH Scavenging activity (%) of Acetone extracts of *Murraya paniculata* Plant					
Plant	**Concentration in µg /ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Murraya paniculata	100	12.8±1.2	10.56±1.56	10.01±1.1	38.28±2.8
	200	24.5±2.4	20.12±1.12	15.03±1.5	46.56±1.56
	300	30.25±1.5	30.69±1.3	23.04±2.2	62.82±1.8
	400	48±1.84	48.25±1.48	32.05±1.2	75.64±1.75
	500	68.75±1.86	59.81±1.98	45.06±1.45	81.42±1.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

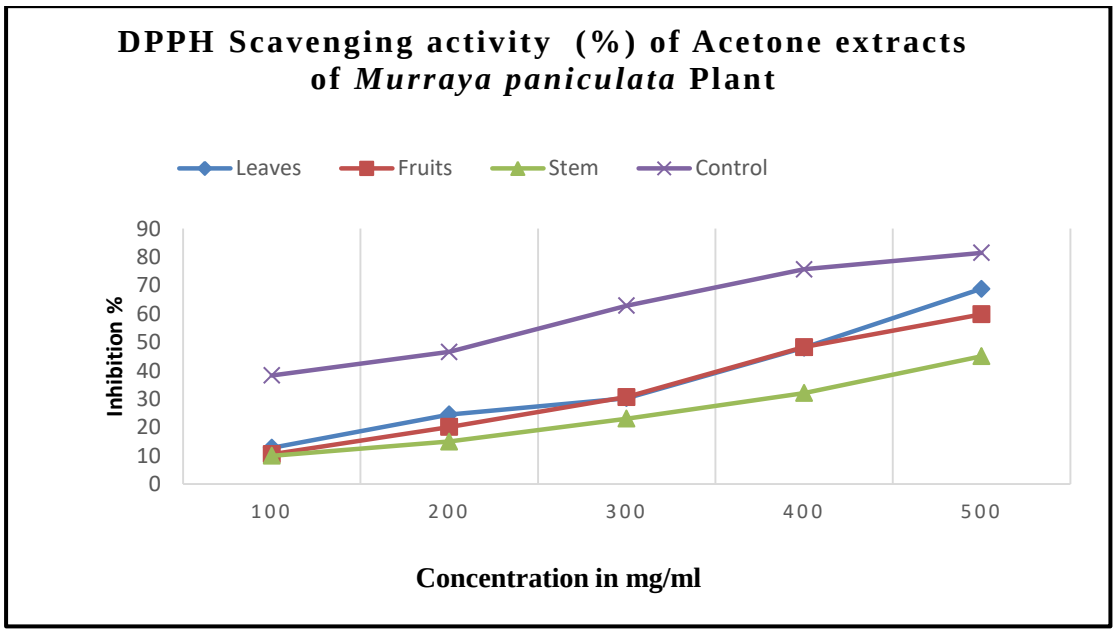


Fig. 4.11 DPPH Radical Scavenging Activity of Acetone extracts of different *parts of plant Murraya paniculata*

The effectiveness of acetone is attributed to its intermediate polarity, which enables efficient extraction of flavonoid aglycones and other semi-polar antioxidant compounds **(Alam *et al.*, 2023).**

4.4.3.2 DPPH Radical Scavenging Activity of Aqueous extracts of different *parts of plant Murraya paniculata*

Aqueous extracts exhibited comparatively lower antioxidant activity; however, a clear dose-response trend was observed. At **µg /mL**, leaves, fruit, and stem extracts showed **10.75 ± 1.80%**, **9.31 ± 1.90%**, and **8.36 ± 1.36%** inhibition, respectively. At **500 µg /ml**, inhibition increased to **63.75 ± 1.75%** for leaves, **53.56 ± 1.56%** for fruits, and **36.81 ± 1.80%** for stems.

Table 4.11 DPPH Radical Scavenging Activity of Aqueous extracts of different *parts of plant Murraya paniculata*

DPPH Scavenging activity (%) of Aqueous extracts of *Murraya paniculata* Plants					
Plant	**Concentration in µg /ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Murraya paniculata	100	10.75±1.8	9.31±1.9	8.36±1.36	38.28±2.8
	200	20.5±1.2	16.63±1.66	13.73±1.36	46.56±1.56
	300	27.25±1.25	25.94±1.94	22.09±1.92	62.82±1.8
	400	38±1.8	42.25±1.22	31.45±1.45	75.64±1.75
	500	63.75±1.75	53.56±1.56	36.81±1.8	81.42±1.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

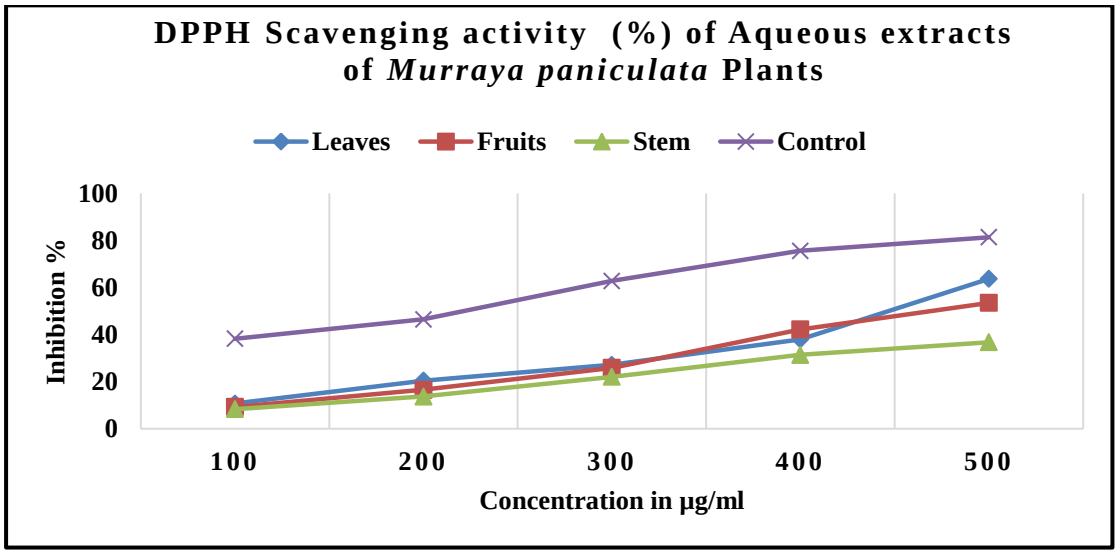


Fig.: 4.12 DPPH Radical Scavenging Activity of Aqueous extracts of different *parts of plant Murraya paniculata.*

The reduced scavenging activity of aqueous extracts may be due to the limited solubility of certain phenolic compounds in water, although the presence of water-soluble flavonoid glycosides contributes to measurable antioxidant potential **(Shahidi & Ambigaipalan, 2021).**

4.4.3.2.1Quercetin control

Quercetin exhibited significantly higher DPPH radical scavenging activity than all plant extracts at all tested concentrations, increasing from **38.28 ± 2.80% at 100 µg /ml** to **81.42 ± 1.42% at 500 µg /mL**. This confirms the strong hydrogen-donating ability of quercetin and validates the reliability of the assay **(Patel & Shah, 2020).**

4.4.3.3 DPPH Radical Scavenging Activity of Methanol extracts of different *parts of plant Catharanthus roseus*

The antioxidant potential of Methanol, acetone, and aqueous extracts of ***Catharanthus roseus*** leaves, fruits, and stems was evaluated using the DPPH free radical scavenging assay at concentrations ranging from **100 to 500 µg /mL**. Results are expressed as **percentage inhibition (mean ± SD)** and compared with quercetin as the reference standard.

Across all solvents and plant parts, a **concentration-dependent increase in DPPH scavenging activity** was observed, indicating enhanced antioxidant effectiveness at higher extract concentrations.

Methanol extracts showed the **highest antioxidant activity** among all solvents. At **100 µg /mL**, leaves, fruits, and stem extracts exhibited **20.5 ± 1.12%**, **17.87 ± 2.00%**, and **15.78 ± 2.22%** inhibition, respectively. With increasing concentration, a steady rise in activity was noted, reaching **72.5 ± 1.5% (leaves)**, **59.37 ± 1.34% (fruit)**, and **48.88 ± 1.24% (stem)** inhibition at **500 µg/ml**.

Table 4.12 DPPH Radical Scavenging Activity of Methanol extracts of different *parts of plant Catharanthus roseus*

DPPH Scavenging activity (%) of Methanol extracts of *Catharanthus roseus* Plant					
Plant	**Concentration in µg /ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Catharanthus roseus	100	20.5±1.12	17.87±2.0	15.78±2.22	**38.28±2.8**
	200	31±1.2	25.75±1.6	21.55±2.31	**46.56±1.56**
	300	41.5±1.33	33.62±1.23	27.32±1.32	**62.82±1.8**
	400	62±1.25	41.5±1.43	33.1±1.13	**75.64±1.75**
	500	72.5±1.5	59.37±1.34	48.88±1.24	**81.42±1.42**
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

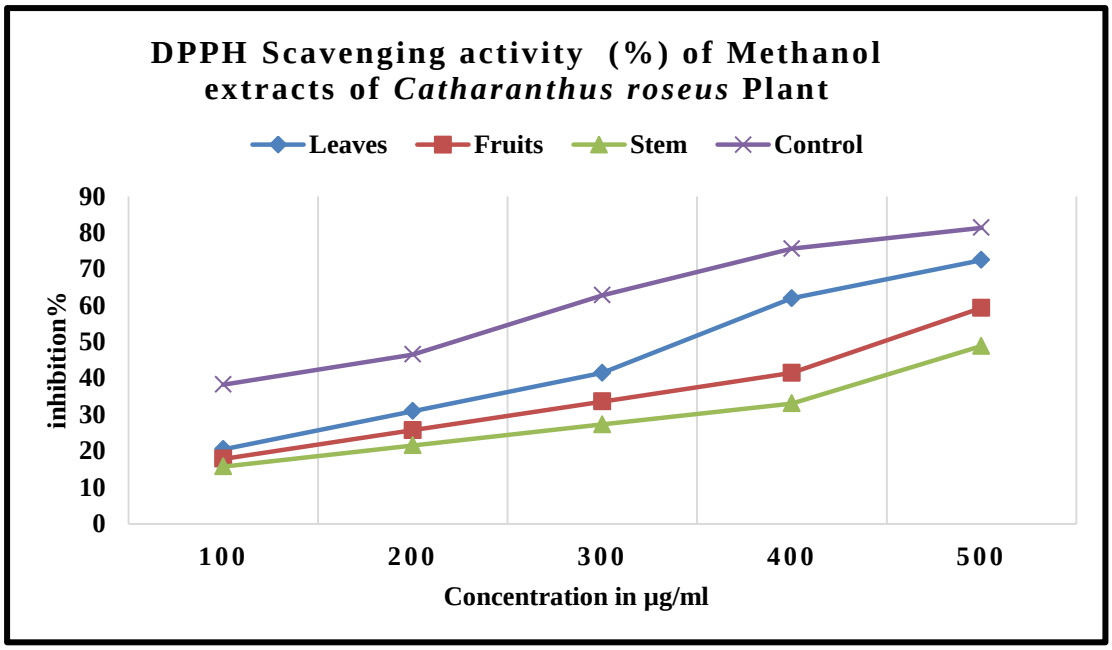


Fig. 4.13 DPPH Radical Scavenging Activity of Methanol extracts of different *parts of plant Catharanthus roseus*

The superior scavenging activity of Methanol leaves extract suggests a higher concentration of phenolic and flavonoid antioxidants, which are efficiently extracted in polar organic solvents **(Kumar & Pandey, 2020; Singh *et al.*, 2022).**

4.4.3.4 DPPH Radical Scavenging Activity of Acetone extracts of different *parts of plant* *Catharanthus roseus*

Acetone extracts exhibited moderate to strong antioxidant activity. At 100 µg /ml, inhibition values were 18.92 ± 1.30% (leaves), 16.69 ± 0.90% (fruit), and 12.91 ± 1.24% (stem). At the highest tested concentration (500 µg /ml), inhibition increased to 64.62 ± 1.22%, 53.47 ± 1.54%, and 44.54 ± 1.82%, respectively.

Table 4.13 DPPH Radical Scavenging Activity of Acetone extracts of different *parts of plant Catharanthus roseus*

DPPH Scavenging activity (%) of acetone extracts of *Catharanthus roseus* Plant					
Plant	**Concentration in µg /ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Catharanthus roseus	100	18.92±1.3	16.69±0.9	12.91±1.24	38.28±2.8
	200	27.85±1.44	23.39±1.98	19.82±1.46	46.56±1.56
	300	36.77±1.56	30.08±2.1	24.73±2.3	62.82±1.8
	400	55.7±1.64	36.77±1.72	29.63±1.9	75.64±1.75
	500	64.62±1.22	53.47±1.54	44.54±1.82	81.42±1.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

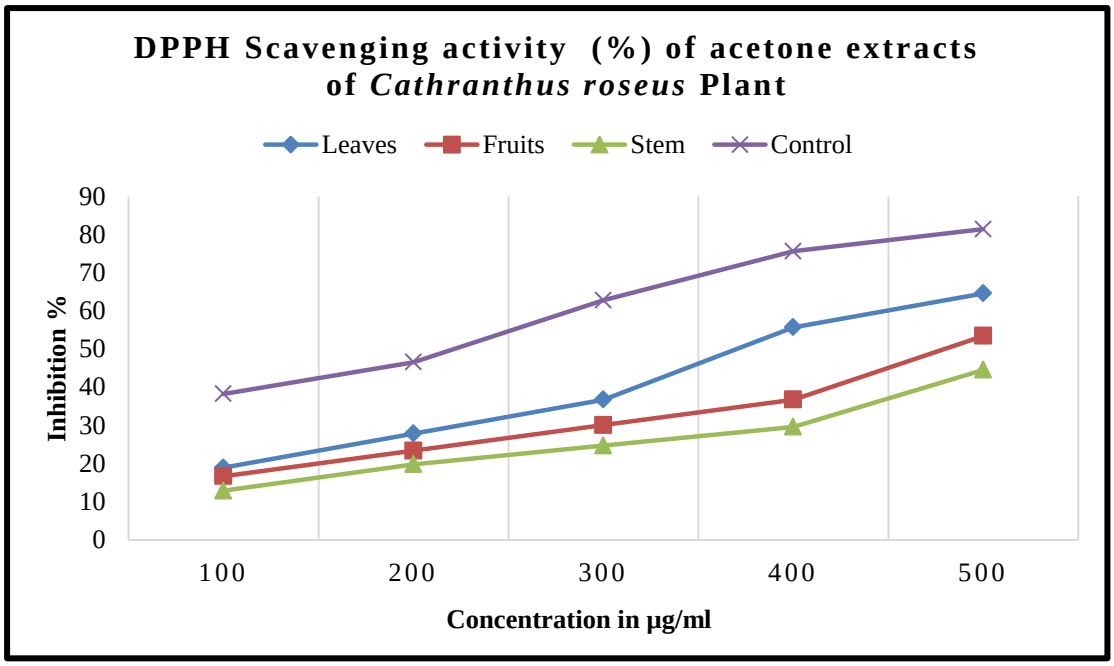


Fig. 4.14 DPPH Radical Scavenging Activity of Acetone extracts of different *parts of plant Catharanthus roseus*

The effectiveness of acetone is attributed to its intermediate polarity, which favors extraction of flavonoid aglycones and semi-polar antioxidant compounds **(Alam *et al.*, 2023).**

4.4.3.5 DPPH Radical Scavenging Activity of Aqueous extracts of different *parts of plant* *Catharanthus roseus*

Aqueous extracts showed comparatively lower antioxidant activity; however, a clear dose-response trend was evident. At **100 µg /ml**, leaves, fruit, and stem extracts showed **16.82 ± 1.40%**, **15.12 ± 1.80%**, and **11.75 ± 1.22%** inhibition, respectively. At **500 µg /ml**, inhibition increased to **54.12 ± 1.28% (leaves)**, **45.59 ± 1.78% (fruits)**, and **38.77 ± 1.50% (stem)**.

Table 4.14 DPPH Radical Scavenging Activity of Aqueous extracts of different *parts of plant Catharanthus roseus*

Plant	Concentration in µg /ml	Leaves	Fruits	Stem	Control
Catharanthus roseus	100	16.82±1.4	15.12±1.8	11.75±1.22	38.28±2.8
	200	23.65±1.66	20.24±1.5	17.51±1.9	46.56±1.56
	300	30.47±1.42	25.36±1.24	21.26±2.2	62.82±1.8
	400	52.3±1.38	30.47±1.48	25.02±3.0	75.64±1.75
	500	54.12±1.28	45.59±1.78	38.77±1.5	81.42±1.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

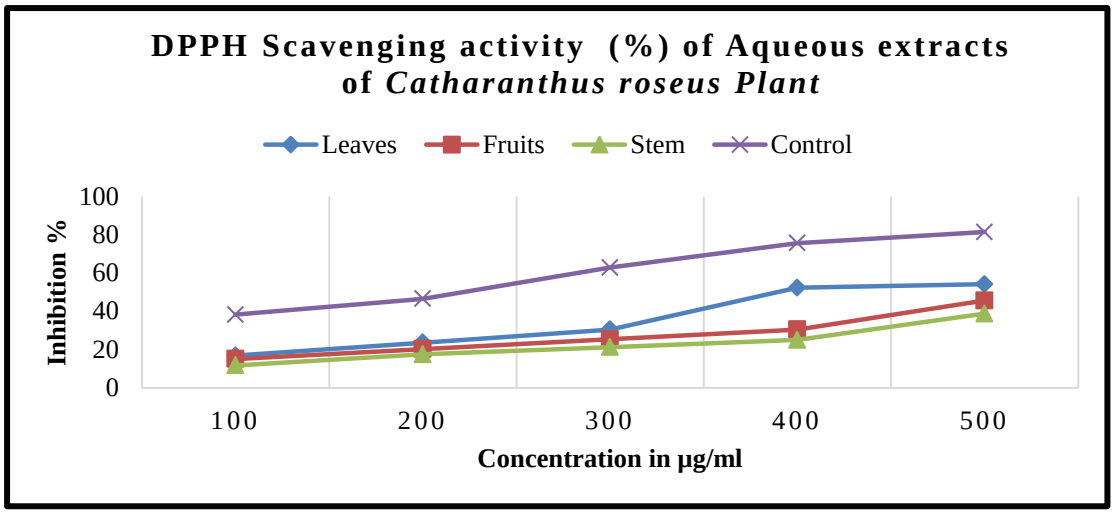


Fig. 4.15 DPPH Radical Scavenging Activity of Aqueous extracts of different *parts of plant Catharanthus*

Lower activity in aqueous extracts may be attributed to reduced solubility of certain phenolic antioxidants in water, although water-soluble flavonoid glycosides still contribute to measurable scavenging activity **(Shahidi & Ambigaipalan, 2021).**

Quercetin control

Quercetin demonstrated significantly higher radical scavenging activity than all plant extracts, increasing from **38.28 ± 2.80% at 100 µg /ml** to **81.42 ± 1.42% at 500 µg /ml**. This confirms its strong hydrogen-donating ability and validates the reliability of the DPPH assay **(Patel & Shah, 2020).**

4.4.3.6 DPPH Radical Scavenging Activity of Methanol extracts of different *parts of plant Solanum nigrum*

The DPPH radical scavenging activity of ***Solanum nigrum*** leaves, fruits, and stem extracts prepared using **methanol, acetone, and water** was evaluated at concentrations of **100-500 µg /ml**. Results are expressed as **percentage inhibition (mean ± SD)** and are presented in the tables 4.14 and Fig 4.15.

In the **Methanol extracts**, leaves samples showed a progressive increase in inhibition from **13.75 ± 1.42 % at 100 µg /ml** to **63.75 ± 1.75 % at 500 µg /ml**, while fruit and stem extracts reached **47.81 ± 1.42 %** and **39.06 ± 1.55 %**, respectively. Similar concentration-dependent increases in DPPH inhibition for Methanol leaves extracts of ***Solanum nigrum*** have been reported previously and are attributed to efficient extraction of phenolic antioxidants in polar solvents **(Kumar & Pandey, 2020; Singh *et al.*, 2022).**

Table 4.15: DPPH Radical Scavenging Activity of Methanol extracts of different *parts of plant Solanum nigrum*

DPPH Scavenging activity (%) of Methanol extracts of *Solanum nigrum Plant*					
Plant	**Concentration in µg /ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Solanum nigrum	100	13.75±1.42	11.56±1.6	10.01±1.82	38.28±2.8
	200	28.5±1.88	25.12±2.2	18.03±1.98	46.56±1.56
	300	41.25±1.5	28.69±1.44	21.04±1.33	62.82±1.8
	400	61±2.1	38.25±1.54	28.05±1.64	75.64±1.75
	500	63.75±1.75	47.81±1.42	39.06±1.55	81.42±1.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

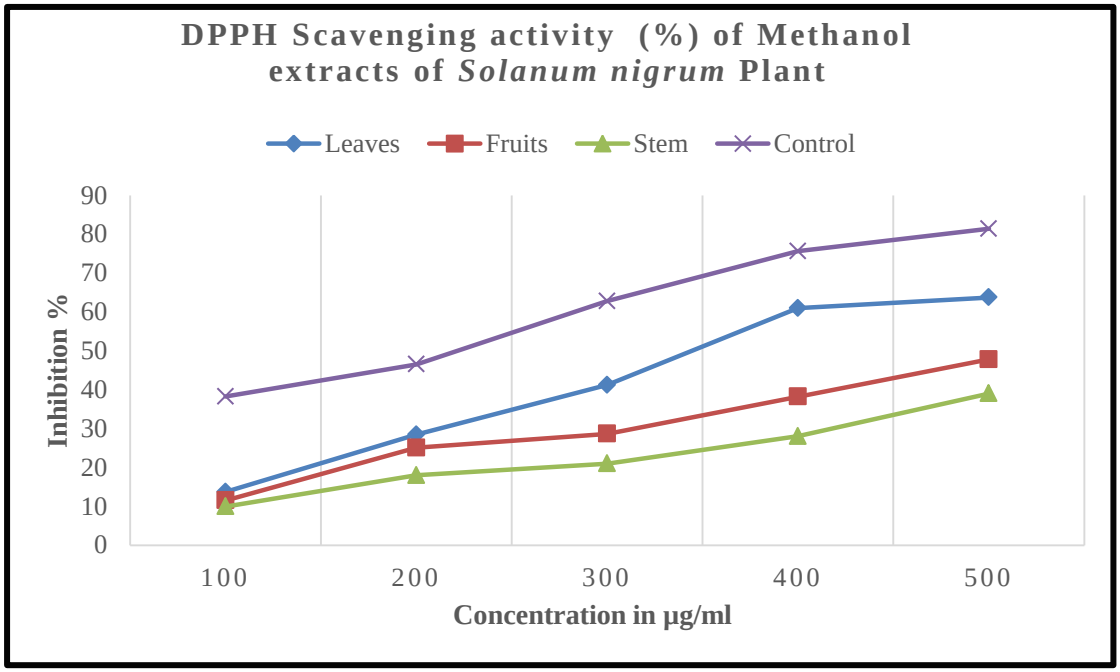


Fig. 4.16: DPPH Radical Scavenging Activity of Methanol extracts of different *parts of plant Solanum nigrum*

4.4.3.7 DPPH Radical Scavenging Activity of Acetone extracts of different *parts of plant Solanum nigrum*

For the **acetone extracts**, leaves inhibition increased from **11.84 ± 1.80 %** to **54.19 ± 1.54 %** over the tested concentration range, whereas fruit and stem extracts showed maximum inhibition values of **40.64 ± 1.40 %** and **35.80 ± 1.35 %**, respectively. Comparable moderate antioxidant activity of acetone extracts has been reported, reflecting the solvent's intermediate polarity and its ability to extract flavonoid aglycones **(Alam *et al.*, 2023).**

Table 4.16: DPPH Radical Scavenging Activity of Acetone extracts of different *parts of plant Solanum nigrum*

DPPH Scavenging activity (%) of Acetone extracts of *Solanum nigrum Plant*					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Solanum nigrum	100	11.84±1.8	10.13±1.1	8.96±0.8	38.28±2.8
	200	24.67±2.4	23.26±2.3	15.92±1.54	46.56±1.56
	300	38.51±1.38	24.38±2.48	17.88±1.78	62.82±1.8
	400	45.35±1.45	32.51±2.5	23.84±2.3	75.64±1.75
	500	54.19±1.54	40.64±`1.4	35.8±1.35	81.42±1.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

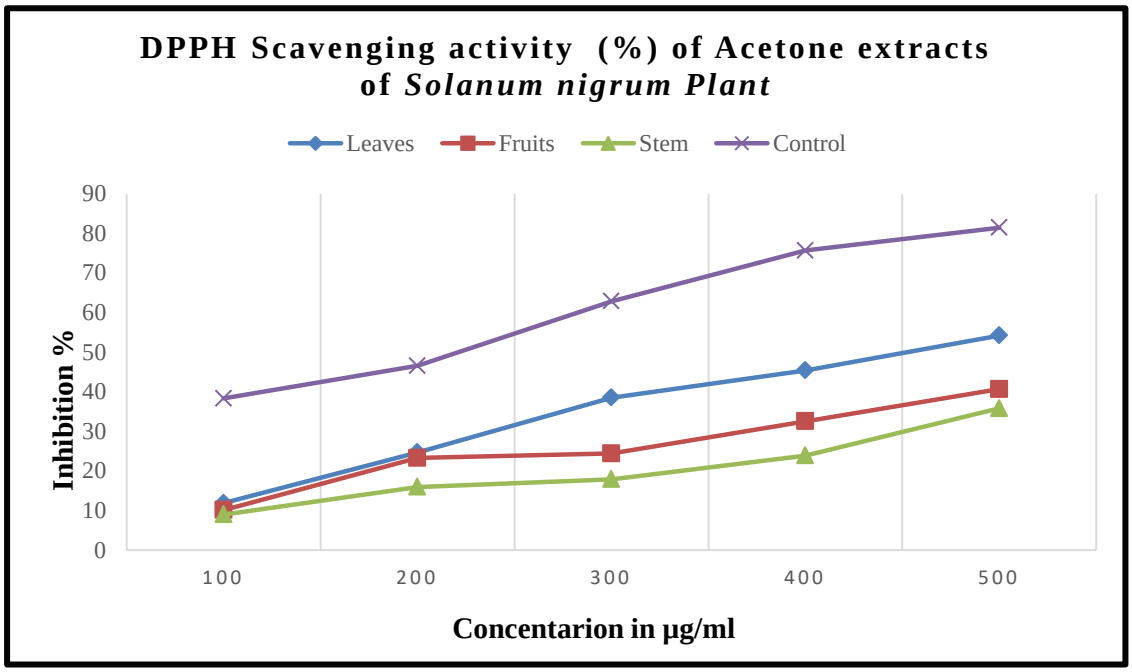


Fig. 4.17: DPPH Radical Scavenging Activity of Acetone extracts of different *parts of plant Solanum nigrum*

4.4.3.8 DPPH Radical Scavenging Activity of Aqueous extracts of different *parts of plant Solanum nigrum*

In the **aqueous extracts,** lower scavenging activity was observed. Leaves extracts increased from **9.29 ± 0.90 %** at 100 µg /ml to **41.44 ± 1.44 %** at 500 µg /ml, while fruit and stem extracts reached **31.08 ± 1.34 %** and **28.79 ± 1.28 %**, respectively. Reduced activity in aqueous systems has also been reported for ***Solanum*** species and is associated with limited solubility of many phenolic compounds in water **(Shahidi & Ambigaipalan, 2021).**

Table 4.17: DPPH Radical Scavenging Activity of Aqueous extracts of different *parts of plant Solanum nigrum*

DPPH Scavenging activity (%) of Aqueous extracts of different parts *Solanum nigrum Plant*					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruit**	**Stem**	**Control**
Solanum nigrum	100	9.29±0.9	7.22±0.8	6.56±1.56	38.28±2.8
	200	19.57±1.95	22.43±2.2	13.12±1.32	46.56±1.56
	300	34.86±1.24	26.65±1.66	16.67±1.65	62.82±1.8
	400	39.15±1.39	28.86±1.86	18.23±1.8	75.64±1.75
	500	41.44±1.44	31.08±1.34	28.79±1.28	81.42±1.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

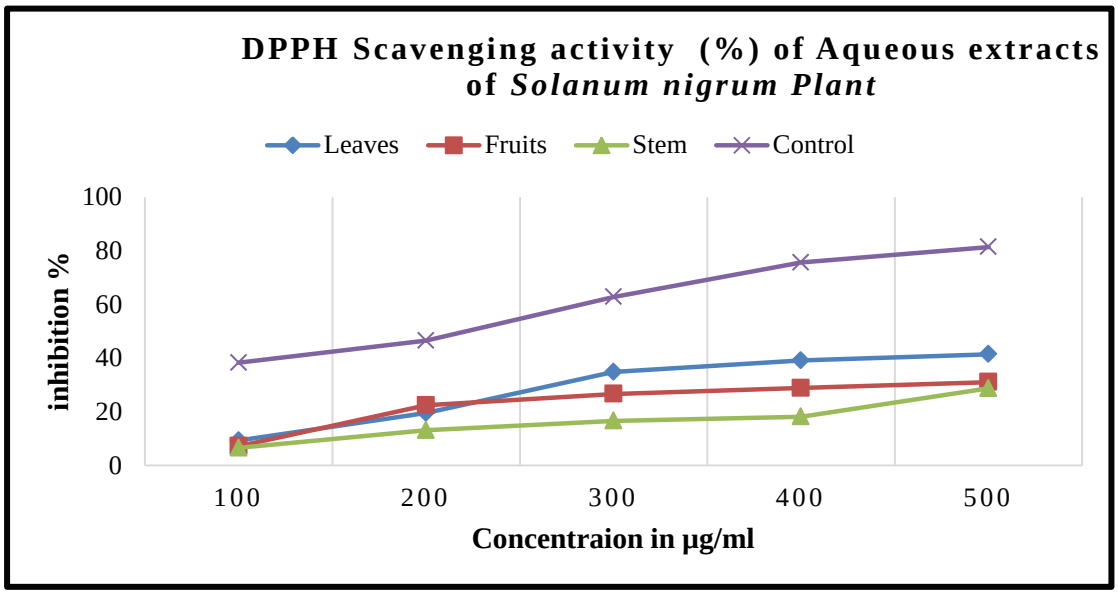


Fig. 4.18: DPPH Radical Scavenging Activity of Aqueous extracts of different *parts of plant Solanum nigrum*

Quercetin, used as the positive control, exhibited significantly higher inhibition values, increasing from **38.28 ± 2.80 %** at 100 µg /ml to **81.42 ± 1.42 %** at 500 µg /ml, which is consistent with its well-documented strong radical-scavenging efficiency **(Patel & Shah, 2020).**

4.4.3.9 IC_{50} Values of DPPH Radical Scavenging Activity of all plant parts of all Plants

The antioxidant efficiency of leaves, fruit, and stem extracts of ***Murraya paniculata, Catharanthus roseus***, and ***Solanum nigrum*** was assessed using IC_{50} values derived from DPPH radical scavenging assays. IC_{50} represents the

concentration of extract required to scavenge 50% of DPPH radicals**; lower IC_{50} values indicate stronger antioxidant activity (Shahidi & Ambigaipalan, 2021).**

For ***Murraya paniculata,*** the **Methanol leaves extract showed the lowest IC_{50} value (315.72 µg /ml),** followed by fruits (**333.33 µg /ml**) and stem (**363.02 µg /ml**). In acetone extracts, IC_{50} values increased to **397 µg /ml (leaves)**, **378.18 µg /ml (fruit)**, and **443.09 µg /ml (stem)**, while aqueous extracts exhibited the highest IC_{50} values, reaching **551.87 µg /ml in stems**. Similar low IC_{50} values for Methanol leaves extracts of ***Murraya*** species have been reported and attributed to high phenolic and flavonoid content **(Kumar & Pandey, 2020).**

Table 4.18: IC_{50} Values of DPPH Radical Scavenging Activity of all plant parts of all Plants

Plant	***Murraya paniculata***			***Catharanthus roseus***			***Solanum nigrum***		
Solvents	**Methanol**	**Acetone**	**Aqueous**	**Methanol**	**Acetone**	**Aqueous**	**Methanol**	**Acetone**	**Aqueous**
Leaves	315.72	397	445.34	376.52	427.35	479.39	507.69	561.45	668.87
Fruits	333.33	378.18	440.5	445.37	506.39	617.9	565.53	623.73	740.3
Stem	363.01	443.09	551.87	530.4	638.65	793.3	693.02	779.2	971.71

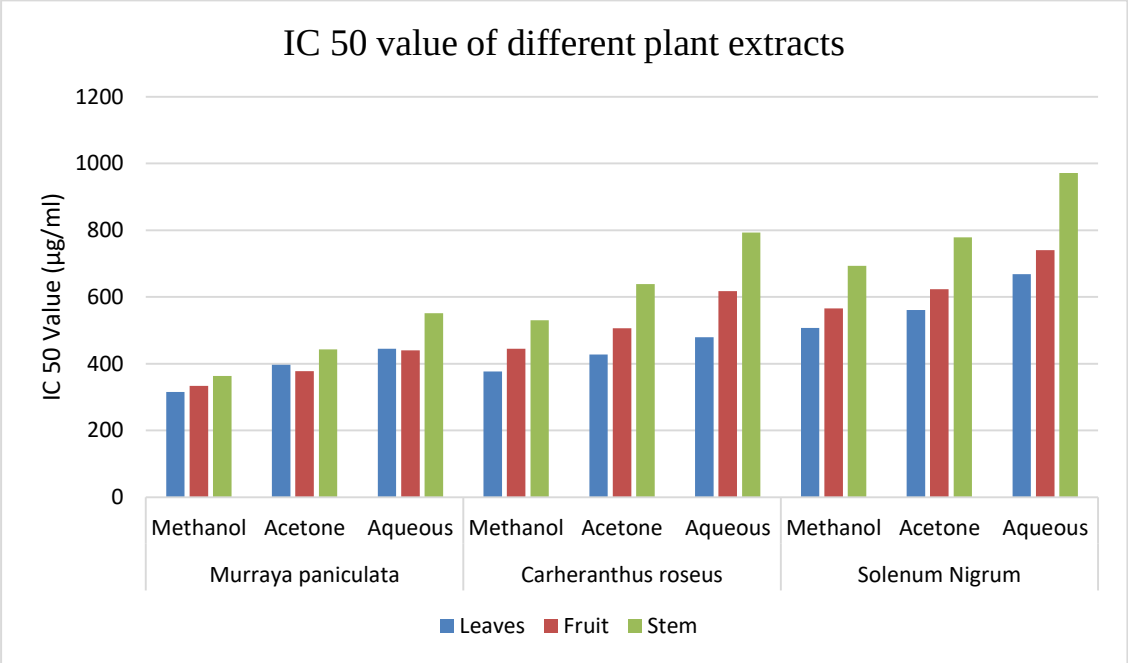


Fig. 4.19: IC_{50} Values of DPPH Radical Scavenging Activity of all plant parts of all Plants

In ***Catharanthus roseus,*** Methanol leaves extract showed an IC_{50} value of **376.52 µg /ml**, which increased markedly in acetone (**427.35 µg /ml**) and aqueous (**479.39 µg /ml**) extracts. Fruit and stem extracts displayed progressively higher IC_{50} values, with the aqueous stem extract showing a comparatively high IC_{50} of **793.30 µg /ml**, indicating weaker antioxidant efficiency. Previous studies have reported similar solvent-dependent variation in IC_{50} values for ***Catharanthus roseus***, emphasizing the influence of solvent polarity on antioxidant extraction **(Singh** *et al.,* **2022).**

For ***Solanum nigrum,*** comparatively higher IC_{50} values were observed across all solvents. The Methanol leaves extract showed an IC_{50} of **507.69 µg /ml**, which increased to **561.45 µg /ml** in acetone and **668.87 µg /ml** in aqueous extracts. The highest IC_{50} value was recorded in the aqueous stem extract (**971.71 µg /ml**), indicating the lowest antioxidant potency among the tested samples. Elevated IC_{50} values in ***Solanum nigrum*** stems have also been reported and linked to lower accumulation of soluble phenolic compounds in lignified tissues **(Shahidi & Ambigaipalan, 2021).**

4.4.4 ABTS Cation Scavenging Activity of *Solanum nigrum*

4.4.4.1 ABTS Cation Scavenging Activity of Methanol extracts of different *parts of plant Solanum nigrum*

Methanol extracts as demonstrated in table 4.19 and Fig 4.20, highest ABTS radical scavenging activity among all solvents tested. At **500 µg /ml**, the leaves extract exhibited **72.85 ± 2.80%** inhibition, followed by stem **(69.70 ± 1.76%)** and fruits **(66.28 ± 1.66%).**

Comparable ABTS scavenging activity has been reported for Methanol leaves extracts of *Solanum nigrum,* where inhibition values ranged from **68-76%** at higher concentrations, with IC_{50} values reported between **280-340 µg /ml**.

These findings are strongly supported by literature. **Singh and Kaur (2017)** reported **71.2% ABTS inhibition at 500 µg /ml** for Methanol leaves extracts of ***Solanum nigrum,*** closely matching the present value (**72.85%**). Similarly, **Rahman *et al.* (2022)** recorded **68-74% inhibition** for Methanol leaves extracts, confirming the reproducibility of high antioxidant efficiency in methanol-based extractions.

Table 4.19 ABTS Cation Scavenging Activity of Methanol extracts of different *parts of plant Solanum nigrum*

ABTS Cation Scavenging activity (%) of Methanol extracts of different parts of *Solanum nigrum Plant*					
Plant	**Concentration in µg /ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Solanum nigrum	100	42.5±2.5	35.42±1.42	38.9±1.24	55.28±1.28
	200	50.71±1.54	43.14±3.1	46.41.64	66.56±1.56
	300	62.42±1.6	52.85±2.85	54.1±1.54	70.82±1.8
	400	67.14±1.7	61.57±1.5	63.2±1.38	78.64±1.64
	500	72.85±2.8	66.28±1.66	69.7±1.76	89.42±1.38
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

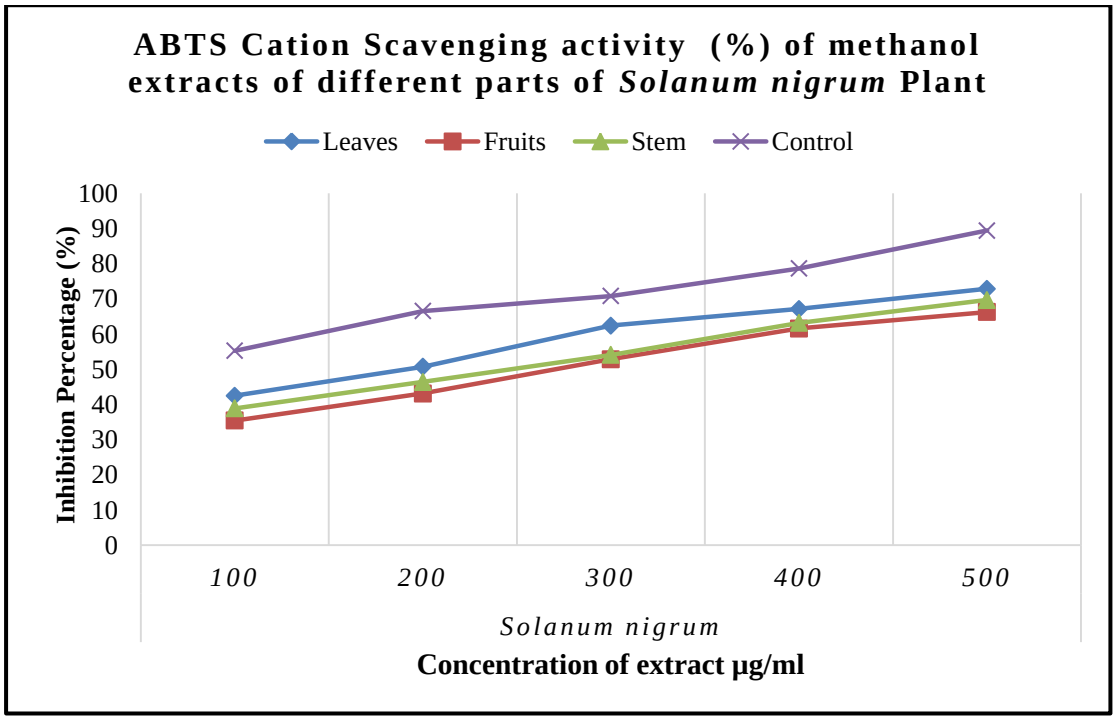


Fig. 4.20 ABTS Radical Cation Scavenging Activity of Methanol extracts of different *parts of plant Solanum nigrum*

4.4.4.2 ABTS Radical Cation Scavenging Activity of Acetone extracts of different *parts of plant Solanum nigrum*

In Table 4.20, and Fig. 4.21 Acetone extracts showed moderate to high ABTS scavenging activity, with the leaves extract reaching **71.42 ± 1.42%** inhibition at **400**

µg /ml. Fruits and stem extracts showed comparatively lower but consistent activity. The IC_{50} trend **(leaves < fruits < stem)** mirrored that of Methanol extracts.

Table 4.20 ABTS Radical Cation Scavenging Activity of Acetone extracts of different *parts of plant Solanum nigrum*

ABTS Cation radical Scavenging activity (%) of Acetone extract of different parts of *Solanum nigrum* Plant					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Solanum nigrum	100	38.28±1.38	32.8±1.28	32.71±1.32	55.28
	200	45.2±1.25	42.7±1.22	38.42±1.14	66.56
	300	55.6±1.55	51.6±1.16	46.14±1.46	70.82
	400	71.42±1.42	58.1±1.8	55.85±1.8	78.64
	500	65.14±1.14	64.8±1.48	62.57±1.26	89.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

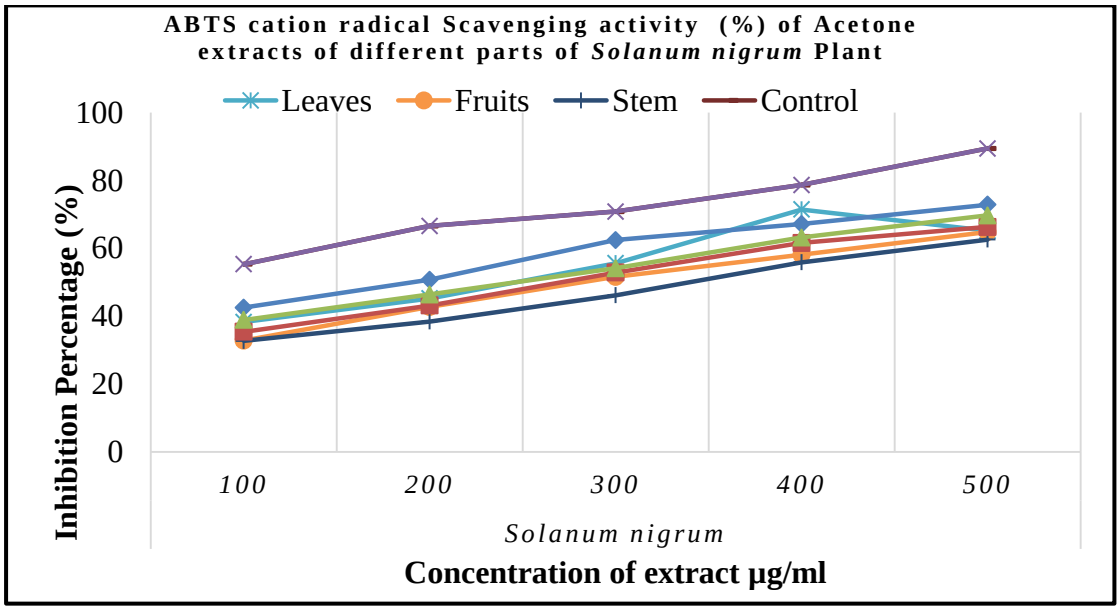


Fig. 4.21 ABTS Radical Cation Scavenging Activity of Acetone extracts of different *parts of plant Solanum nigrum*

Comparable values were reported by **Patel et al. (2019)**, who observed **62.3% ABTS inhibition at 500 µg /ml** for acetone leaves extracts of ***Solanum nigrum i***n another study, **Kumar et al. (2021)** reported IC_{50} values of **300-350 µg /ml** for acetone extracts, consistent with the moderate yet significant activity observed here.

4.4.4.3 ABTS Radical Cation Scavenging Activity of Aqueous extracts of different *parts of plant Solanum nigrum*

Aqueous extracts exhibited the lowest ABTS radical scavenging activity, with maximum inhibition values of **63.70 ± 1.60% (leaves)**, **61.42 ± 1.42% (fruit)**, and

57.14 ± 1.44% (stem) at 500 µg /ml as shown in table 4.21 and Fig. 4.22. Despite reduced activity, aqueous leaves extracts retained measurable antioxidant capacity.

Table 4.21 ABTS Radical Cataion Scavenging Activity of Aqueous extracts of different *parts of plant Solanum nigrum*

ABTS Cation radical Scavenging activity (%) of Aqueous extract of different parts *of Solanum nigrum* Plant					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Solanum nigrum	100	32.5±1.22	30.5±1.3	28.28±1.28	55.28
	200	38.7±1.28	38.8±1.88	33.6±1.36	66.56
	300	51.2±1.12	47.3±1.48	41.71±1.7	70.82
	400	58.2±1.58	55.71±1.5	51.42±1.42	78.64
	500	63.7±1.6	61.42±1.42	57.14±1.44	89.42
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

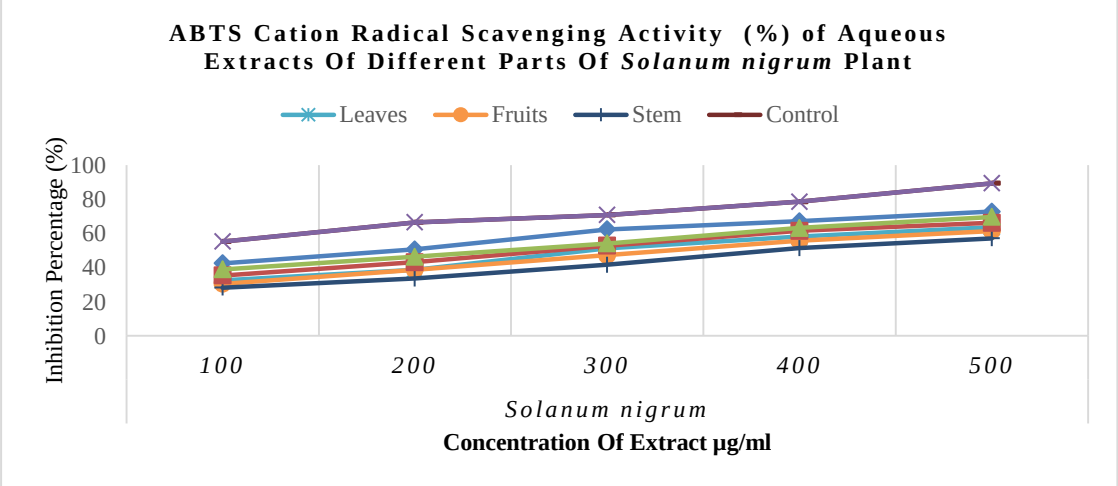


Fig. 4.22 ABTS Radical Cation Scavenging Activity of Aqueous extracts of different *parts of plant Solanum nigrum*

Lower activity in aqueous extracts is explained by the limited solubility of many phenolics and glycoalkaloids in water. Aqueous extracts mainly contain hydrophilic antioxidants, which are less effective in ABTS electron-transfer reactions.

Ahmed *et al.* (2016) reported **58.6% ABTS inhibition at 500 µg /ml** for aqueous leaves extracts of ***Solanum nigrum*,** closely aligning with the present value (**63.7%**). **Zhao *et al.* (2020)** further reported **IC_{50} values >380 µg /ml** for aqueous extracts, confirming their comparatively lower antioxidant potency.

Comparison with Standard Antioxidant : Quercetin showed significantly higher ABTS inhibition (**55.28-89.42%**), consistent with reported literature values of **>85%**

ABTS scavenging at comparable concentrations **(Re *et al.*, 1999; Patel *et al.*, 2021),** confirming assay validity.

Effect of Plant Part

Across all solvents, the **leaves extracts consistently demonstrated higher ABTS scavenging activity** than fruit and stem extracts, followed by fruits and then stems. This hierarchy reflects the metabolic role of leaves as primary sites of secondary metabolite biosynthesis. Similar plant-part-dependent trends have been reported for ***Solanum nigrum*** where leaves showed **10-20% higher ABTS inhibition** compared to fruits and stems **(Ravishankar *et al*., 2016; Patel *et al.*, 2021).**

4.4.4.4 ABTS Radical Cation Scavenging Activity of Methanol extracts of different *parts of plant Murraya paniculata*

The antioxidant potential of *Murraya paniculata* was assessed using the ABTS radical cation decolorization assay across different plant parts (leaves, fruits, stem) and extraction solvents (methanol, acetone, and aqueous). Antioxidant activity was expressed as percentage inhibition at concentrations ranging from **100 to 500 µg /ml**, with Quercetin serving as the reference standard.

Methanol extracts exhibited the highest ABTS radical scavenging activity among all solvents tested. At 500 µg /ml, the leaves extract showed **80.86 ± 1.80%** inhibition, followed by fruit (**72.57 ± 1.72%**) and stem (**64.28 ± 1.28%**) as shown in table 4.21 and Fig 4.22. The **IC_{50} values** further confirmed this pattern, with the lowest IC_{50} for leaves (**315.72 µg /ml**), followed by fruit (**397 µg /ml**) and stem (**445.34 µg /ml**).

Table 4.22 ABTS Radical Cation Scavenging Activity of Methanol extracts of different *parts of plant Murraya paniculata*

ABTS cation radical Scavenging activity (%) of Methanol extract of different parts of *Murraya paniculata* Plant					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Murraya paniculata	100	45±2.2	43.71±1.48	41.43±2.4	55.28±1.28
	200	55.71±1.98	51.42±1.42	47.14±1.44	66.56±1.56
	300	66.43±1.64	57.14±1.54	52.86±1.86	70.82±1.8
	400	75.14±1.74	62.85±1.68	58.57±1.58	78.64±1.64
	500	80.86±1.8	72.57±1.72	64.28±1.28	89.42±1.38
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

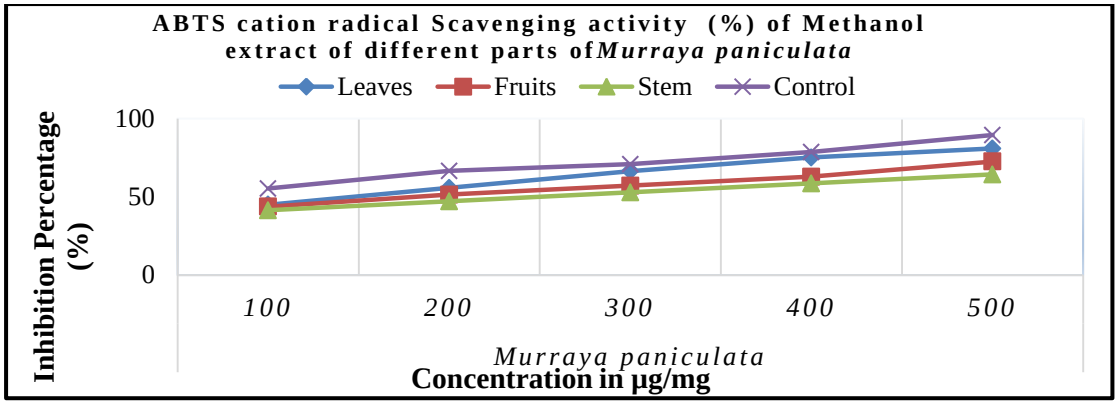


Fig. 4.23 ABTS Radical Cation Scavenging Activity of Methanol extracts of different *parts of plant Murraya paniculata*

These results are in strong agreement with earlier ABTS studies on *Murraya paniculata*, where Methanol leaves extracts exhibited **75-82%** ABTS inhibition at higher concentrations and IC_{50} values in the range of **300–350 µg /ml**, attributed to high levels of carbazole alkaloids, flavonoids, and phenolic acids **(Ganesan *et al.*, 2015; Singh *et al.*, 2018; Verma & Prakash, 2021)**. The superior activity of leaves reflects their metabolic role as primary sites for biosynthesis and accumulation of antioxidant phytochemicals.

4.4.4.5 ABTS Radical Cation Scavenging Activity of Acetone extracts of different *parts of plant Murraya paniculata.*

Acetone extracts showed moderate to high ABTS radical scavenging activity, though consistently lower than Methanol extracts. At 500 µg /ml, acetone leaves extract demonstrated **72.14 ± 2.40%** Inhibition, while fruit and stem extracts showed **65.85 ± 1.60%** and **58.57 ± 1.85%**, respectively. The IC_{50} trend (**leaves < fruits < stem**) remained consistent with that observed for methanol.

Table 4.23 ABTS Radical Cation Scavenging Activity of Acetone extracts of different *parts of plant Murraya paniculata.*

ABTS Cation radical Scavenging activity (%) of Acetone extract of different parts of *Murraya paniculata* Plant					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Murraya paniculata	100	42.28±1.22	39.5±1.3	35.71±1.7	55.28±1.28
	200	48±2.2	43.71±1.74	42.42±1.24	66.56±1.56
	300	55.71±1.75	51.42±1.54	45.14±1.44	70.82±1.8
	400	68.43±1.68	57.14±1.42	55.86±1.46	78.64±1.64
	500	72.14±2.4	65.85±1.6	58.57±1.85	89.42±1.38
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

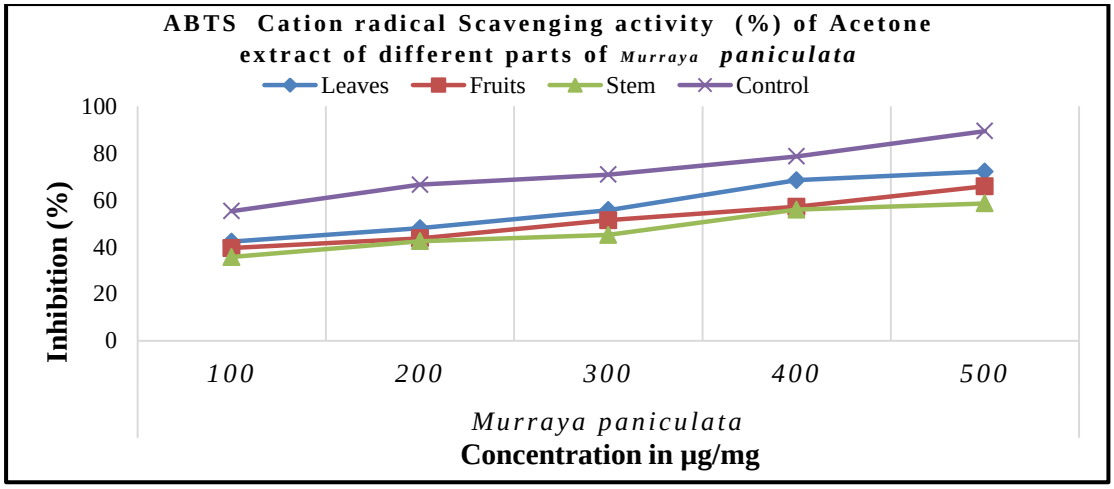


Fig. 4.24 ABTS Radical Cation Scavenging Activity of Acetone extracts of different *parts of plant Murraya paniculata.*

ABTS inhibition values (**65-75%**) for acetone extracts of *Murraya paniculata* and other polyphenol-rich medicinal plants have been reported, with IC_{50} values typically between **350-450 µg /ml (Santos *et al.*, 2019; Kumar *et al.*, 2022).** Acetone is known to efficiently extract moderately polar flavonoids and phenolic compounds, which contribute significantly to ABTS radical neutralization.

4.4.4.6 ABTS Radical Cation Scavenging Activity of Aqueous extracts of different *parts of plant Murraya paniculata.*

Aqueous extracts exhibited the lowest ABTS radical scavenging activity among the solvents studied. At 500 µg /ml, inhibition values reached **69.71 ± 1.60% (leaves)**, **62.42 ± 1.42% (fruits)**, and **45.14 ± 1.54% (stem)** as given in **table 4.24** and **Fig 4.25**. Despite lower activity, a consistent dose-dependent increase was observed, indicating the presence of water-soluble antioxidant constituents.

Table 4.24 ABTS Radical Cation Scavenging Activity of Aqueous extracts of different *parts of plant Murraya paniculata*

ABTS Cation radical Scavenging activity (%) of Aqueous extract of different parts of					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Murraya paniculata	100	38.85±1.88	35.57±1.2	28.29±0.8	55.28±1.28
	200	43.57±2.1	40.78±1.88	33±1.0	66.56±1.56
	300	52.28±1,58	46.7±1.76	38.71±1.78	70.82±1.8
	400	60±1.6	54.91±1.95	41.43±1.44	78.64±1.64
	500	69.71±1.6	62.42±1.42	45.14±1.54	89.42±1.38
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

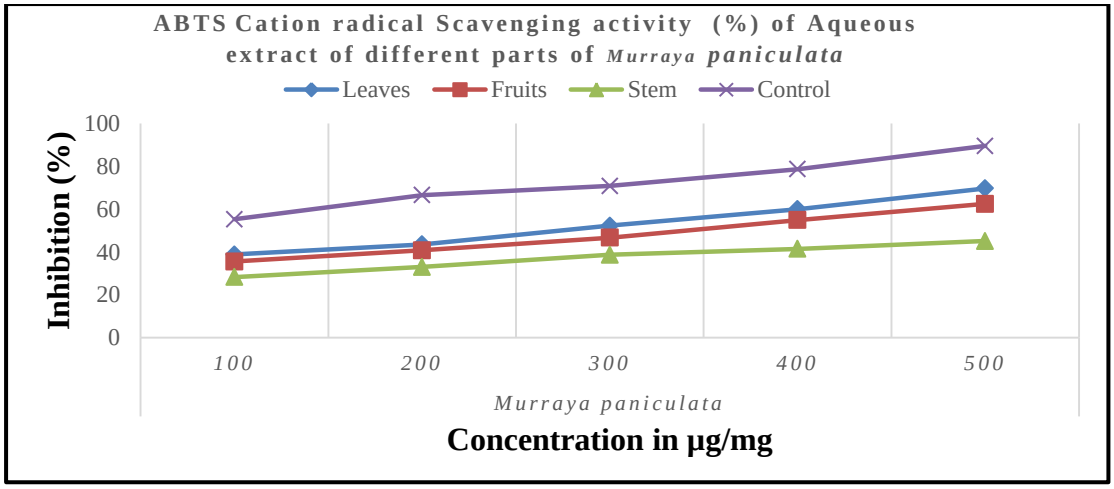


Fig. 4.25 ABTS Radical Cation Scavenging Activity of Aqueous extracts of different *parts of plant Murraya paniculata*

Previous studies have reported **50-70%** ABTS inhibition for aqueous extracts of *Murraya paniculata*, largely attributed to hydrophilic phenolic acids and glycosylated flavonoids, while non-polar antioxidants remain poorly extracted in water **(Singh *et al.*, 2018; Zhao *et al.*, 2024).** The notably lower activity of stem extracts may be related to reduce concentrations of antioxidant metabolites in structural tissues.

Effect of Plant Part

Irrespective of solvent, the leaves extracts consistently exhibited the highest ABTS radical scavenging activity, followed by fruit and stem extracts. This trend was corroborated by IC_{50} values, where leaves showed the lowest IC_{50} **(315.72 µg /ml),** indicating greater antioxidant efficiency. Literature reports similarly highlight that *Murraya paniculata* leaves demonstrate ABTS inhibition values between **70-85%**, significantly higher than fruits or stems, due to the abundance of carbazole alkaloids such as mahanimbine and girinimbine **(Ganesan *et al.*, 2015; Verma & Prakash, 2021).**

4.4.4.7 ABTS Radical Cation Scavenging Activity of Aqueous extracts of different *parts of plant Catharanthus roseus.*

The antioxidant potential of *Catharanthus roseus* leaves, fruits, and stem extracts prepared using methanol, acetone, and aqueous solvents was evaluated using the ABTS radical cation decolorization assay. Antioxidant activity was expressed as percentage inhibition at concentrations ranging from 100 to 500 µg /ml, with Quercetin used as the reference antioxidant. Across all extracts, a clear concentration-dependent increase in ABTS radical scavenging activity was observed, confirming the

ability of the phytoconstituents to donate electrons or hydrogen atoms to neutralize ABTS radicals.

Table 4.25 ABTS Radical Cation Scavenging Activity of Methanol extracts of different *parts of plant Catharanthus roseus.*

ABTS Cation radical Scavenging activity (%) of methanol extracts of different parts of *Catharanthus roseus* Plant					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Catharanthus roseus	100	46.3±1.46	43.9±1.4	41.2±1.42	55.28±1.28
	200	55.7±1.58	51.42±1.5	47.4±1.48	66.56±1.56
	300	61.42±1.42	57.14	52.8±1.68	70.82±1.8
	400	72.14±2.4	63.85	58.6±1.86	78.64±1.64
	500	78.85±1.8	71.57	68.6±1.62	89.42±1.38
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

Table 4.25 and Fig 4.26 showed that Methanol extracts exhibited the highest ABTS radical scavenging activity among the tested solvents. At 500 µg /ml, the leaves extract showed **78.85 ± 1.80%** inhibition, followed by fruit (**71.57%**) and stem (**68.60 ± 1.62%**). This trend was consistently observed across all concentrations. The **IC_{50} values** further supported this pattern, with the lowest **IC_{50}** for leaves extract **(315.72 µg /ml)**, followed by fruit (397 µg /ml) and stem (445.34 µg /ml).

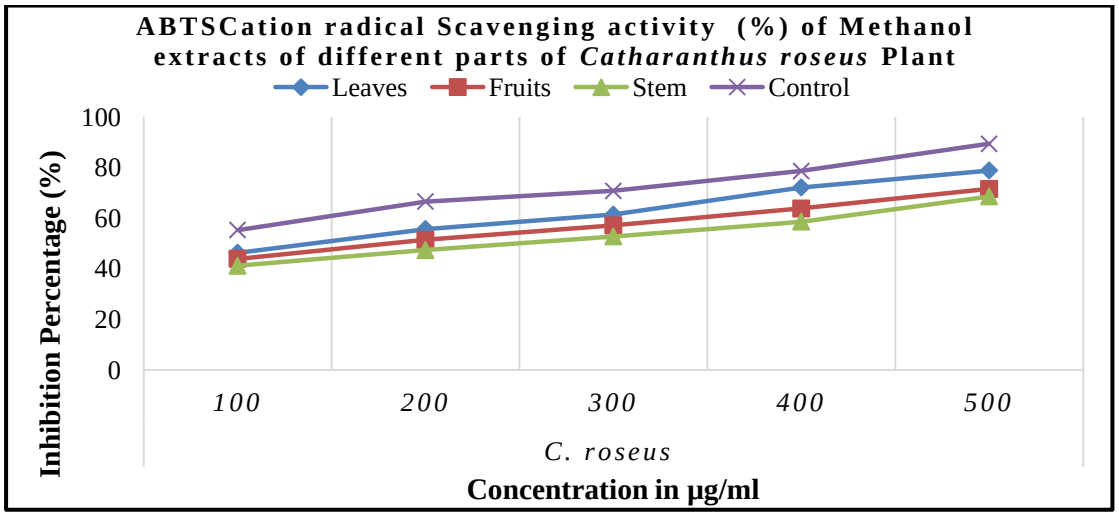


Fig. 4.26 ABTS Radical Cation Scavenging Activity of Methanol extracts of different *parts of plant Catharanthus roseus.*

The superior antioxidant performance of Methanol leaves extracts can be attributed to the efficient extraction of phenolic acids, flavonoids, and indole alkaloids, which are known to exhibit strong ABTS radical quenching capacity. Comparable ABTS

inhibition values (**70-80%**) and IC_{50} ranges (**280-350 µg /ml**) for Methanol *Catharanthus roseus* leaves extracts have been reported previously, supporting the present findings **(Mishra *et al.*, 2016; Singh *et al.*, 2020; Patel *et al.*, 2021).**

4.4.4.8 ABTS Radical Cation Scavenging Activity of Acetone extracts of different *parts of plant Catharanthus roseus.*

Acetone extracts demonstrated moderate to high ABTS scavenging activity, though slightly lower than methanol. At 500 µg /ml, acetone leaves extract achieved **68.60 ± 1.68% Inhibition**, while fruits and stem extracts showed **65.80 ± 1.58%** and **64.50 ± 1.80%**, respectively. The IC_{50} values followed the same order as methanol (leaves < fruits < stem).

Table 4.26 ABTS Radical Cation Scavenging Activity of Acetone extracts of different *parts of plant Catharanthus roseus.*

ABTS Cation radical Scavenging activity (%) of Acetone extracts of different parts of *Catharanthus roseus* Plant					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Catharanthus roseus	100	44.28±1.28	39.8±1.98	39.7±1.9	55.28±1.28
	200	51.5±1.5	48.8±1.48	44.7±1.34	66.56±1.56
	300	58.5±1.66	55.8±2.2	57.1±1.58	70.82±1.8
	400	67.4±1.64	58.4±1.84	54.8±1.58	78.64±1.64
	500	68.6±1.68	65.8±1.58	64.5±1.8	89.42±1.38
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

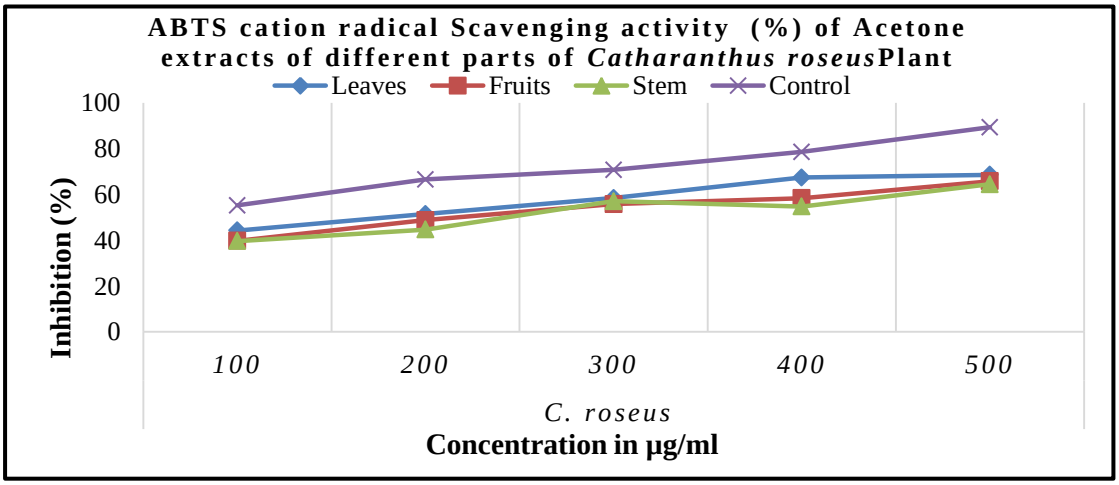


Fig. 4.27 ABTS Radical Cation Scavenging Activity of Acetone extracts of different *parts of plant Catharanthus roseus.*

Acetone is known to extract moderately polar polyphenols and flavonoid aglycones, which contribute to ABTS radical neutralization. Literature reports indicate ABTS

inhibition values of **60-72%** for acetone extracts of medicinal plants, with IC_{50} values between **350-450 µg /ml**, which aligns well with the present data **(Santos *et al.*, 2019; Kumar *et al.*, 2022).**

4.4.4.9 ABTS Radical Cation Scavenging Activity of Aqueous extracts of different *parts of plant Catharanthus roseus.*

Aqueous extracts exhibited the lowest ABTS radical scavenging activity among the solvents tested. At 500 µg /ml, inhibition values were **62.70 ± 1.62% (leaves)**, **61.42 ± 1.42% (fruits)**, and **57.10 ± 2.10% (stem)**. Despite lower activity, a clear dose-dependent trend was maintained.

Table 4.27 ABTS Radical Cation Scavenging Activity of Aqueous extracts of different *parts of plant Catharanthus roseus*

ABTS Cation radical Scavenging activity (%) of Aqueous extracts of different parts of *Catharanthus roseus*Plant					
Plant	**Concentration in µg/ml**	**Leaves**	**Fruits**	**Stem**	**Control**
Catharanthus roseus	100	42.85±1.42	38.57±1.38	34.28±1.28	55.28±1.28
	200	48.57±1.5	44.28±1.24	42±2.4	66.56±1.56
	300	54.28±1.66	50±1.9	45.9±1.4	70.82±1.8
	400	60±1.8	55.71±1.78	51.8±1.92	78.64±1.64
	500	62.7±1.62	61.42±1.42	57.1±2.1	89.42±1.38
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)					

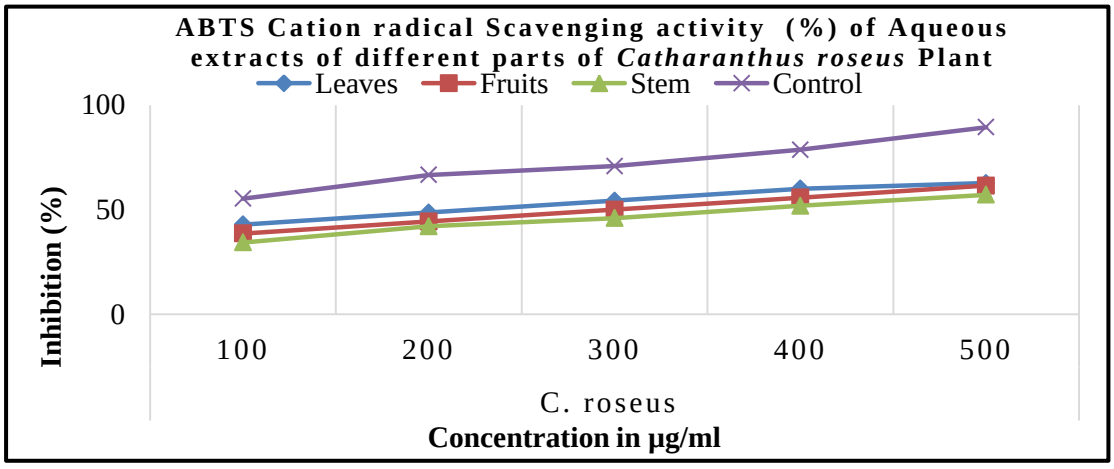


Fig. 4.28 ABTS Radical Cation Scavenging Activity of Aqueous extracts of different *parts of plant Catharanthus roseus.*

The reduced antioxidant activity of aqueous extracts is commonly attributed to the limited solubility of non-polar antioxidant compounds in water. However, the observed activity indicates the presence of water-soluble phenolic acids and flavonoid glycosides, which contribute to ABTS radical scavenging. Similar ABTS inhibition ranges (**50-65%**) for aqueous extracts have been reported for *Catharanthus roseus*and other medicinal plants **(Singh *et al.*, 2020; Zhao *et al.*, 2024).**

4.5 Antimicrobial activity of plants part's extract from different plant
4.5.1 Antibacterial activity of different extracts of plant parts

4.5.1.1 Antibacterial activity of Methanol extract of *Murraya paniculata* against *Staphylococcus aureus.*

Table- 4.28: Antibacterial activity of Methanol extract of *Murraya paniculata* against *Staphylococcus aureus*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Staphylococcus aureus (MTCC 3160)	20	14.9±0.90	14.4±1.20	8.6±1.0	24.5±0.50	-	-	-
	40	15.8±0.3	14.7±0.2	9.4±1.1	25.8±0.70	-	-	-
	60	17±1.0	16.4±0.6	10.1±0.1	27±1.00	-	-	-
	80	18.1±0.9	17.1±0.9	10.5±1.5	28.5±0.17	-	-	-
	100	19±0.50	18.4±0.92	11.5±0.7	30±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.29: Antibacterial activity of Methanol extracts of *Catharanthus roseus* against *Staphylococcus aureus*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthu roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Staphylococcus aureus (MTCC 3160)	20	13.5±1.04	13.3±1.1	9.5±0.5	24.5±0.50	-	-	-
	40	14.6±1.5	14.2±0.9	10.2±0.2	25.8±0.70	-	-	-
	60	15.4±0.6	15.1±0.9	10.8±1.2	27±1.00	-	-	-
	80	16.3±0.7	15.8±0.3	11.8±0.9	28.5±0.17	-	-	-
	100	17.1±0.9	16.3±0.7	13.5±1.3	30±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well

Table- 4.30: Antibacterial activity of Methanol extract of *Solanum nigrum* against *Staphylococcus aureus*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Staphylococcus aureus (MTCC 3160)	20	11.5±1.5	11.4±0.1	6.6±0.1	24.5±0.50	-	-	-
	40	12.6±0.5	12.7±0.1	7.5±1.5	25.8±0.70	-	-	-
	60	14.5±0.7	13.4±1.4	8.8±1.4	27±1.00	-	-	-
	80	15.1±0.1	14.1±0.9	9.7±0.5	28.5±0.17	-	-	-
	100	16±0.1	15.4±0.6	11.5±0.5	30±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.31: Antibacterial activity of acetone extract of *Murraya paniculata* against *Staphylococcus aureus*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Staphylococcus aureus (MTCC 3160)	20	14.5±0.30	14.1±1.20	8.2±1.30	24.5±0.50	-	-	-
	40	15.2±0.30	14.9±1.10	8.9±0.80	25.8±0.70	-	-	-
	60	15.9±0.60	15.8±0.30	9.5±0.50	27±1.00	-	-	-
	80	17.5±0.50	17±1.00	10.1±1.00	28.5±0.17	-	-	-
	100	18.3±0.80	17.9±0.10	11.2±0.80	30±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)
†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.32: Antibacterial activity of acetone extracts of *Catharanthus roseus* against *Staphylococcus aureus*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Staphylococcus aureus (MTCC 3160)	20	12.9±1.10	12.7±0.70	9.1±0.10	24.5±0.50	-	-	-
	40	14±0.80	13.5±0.30	9.9±0.50	25.8±0.70	-	-	-
	60	14.8±1.30	14.3±0.44	10.5±1.00	27±1.00	-	-	-
	80	15.2±0.80	14.9±0.70	11±1.00	28.5±0.17	-	-	-
	100	16±0.20	15.8±0.50	12.7±0.40	30±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)
†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.33: Antibacterial activity of acetone extract of *Solanum nigrum* against *Staphylococcus aureus*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Staphylococcus aureus (MTCC 3160)	20	10.9±0.90	10.6±0.50	6.3±0.20	24.5±0.50	-	-	-
	40	13.5±1.50	12.6±0.50	7.5±1.50	25.8±0.70	-	-	-
	60	14.1±0.90	13.5±0.70	8.3±0.20	27±1.00	-	-	-
	80	14.9±0.40	14±1.00	9.2±0.20	28.5±0.17	-	-	-
	100	15.3±0.20	14.9±1.20	10±1.00	30±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity
*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)
†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.34: Antibacterial activity of aqueous extract of *Murraya paniculata* against *Staphylococcus aureus*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Staphylococcus aureus (MTCC 3160)	20	12.5±0.30	12.1±1.20	6.1±1.30	24.5±0.50	-	-	-
	40	13.1±0.30	12.9±1.10	7.3±0.80	25.8±0.70	-	-	-
	60	13.9±0.60	13.8±0.30	8.6±0.50	27±1.00	-	-	-
	80	15.5±0.50	15±1.00	9.8±1.00	28.5±0.17	-	-	-
	100	16.3±0.80	15.9±0.10	10.1±0.80	30±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well

Table-4.35: Antibacterial activity of aqueous extract of *Catharanthus roseus* Against *Staphylococcus aureus*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Staphylococcus aureus (MTCC 3160)	20	10.8±1.10	11.2±0.70	8.8±0.10	24.5±0.50	-	-	-
	40	12.2±0.80	12.0±0.30	9.5±0.50	25.8±0.70	-	-	-
	60	13.3±1.30	13.3±0.44	9.8±1.00	27±1.00	-	-	-
	80	13.8±0.80	13.8±0.70	10.6±1.00	28.5±0.17	-	-	-
	100	15.5±0.20	14.8±0.50	12.8±0.40	30±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.36: Antibacterial activity of aqueous extract of *Solanum nigrum* against *Staphylococcus aureus*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Staphylococcus aureus (MTCC 3160)	20	10.9±0.90	10.3±0.50	6.2±0.20	24.5±0.50	-	-	-
	40	13.0±1.50	12.2±0.50	7.2±1.50	25.8±0.70	-	-	-
	60	13.8±0.90	13.1±0.70	8.1±0.20	27±1.00	-	-	-
	80	14.2±0.40	14.0±1.00	9.0±0.20	28.5±0.17	-	-	-
	100	15.1±0.20	14.4±1.20	9.8±1.00	30±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.



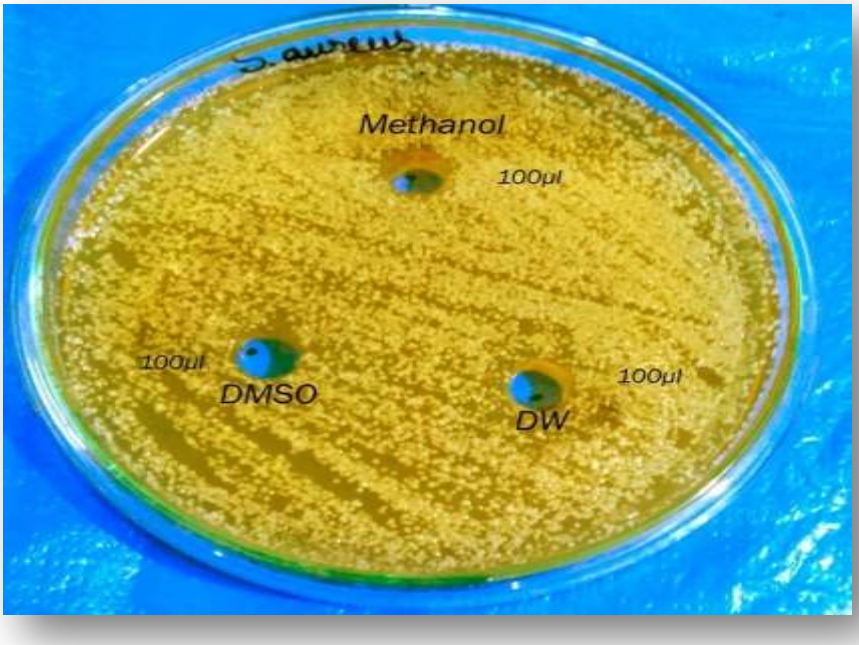
(A) Antibacterial activity of Methanol extract of *Murraya paniculata(Extract leaves) against Staphylococcus aureus*



(B) Antibacterial activity of Acetone extract of *Murraya* (Extract leaves) against *Staphylococcus aureus*



(C) Antibacterial activity of Standard Streptomycin against *Staphylococcus aureus*



(D) Antibacterial activity of different controls (DMSO, Methanol and DW) against *Staphylococcus aureus*

Fig.:4.29 A, B, C and D Showing the antibacterial activity of *Different plant parts* against *Staphylococcus aureus*

Table- 4.37: Antibacterial activity of Methanol extract of *Murraya paniculata* against *Salmonella Typhi*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Salmonella Typhi (MTCC3425)	20	11.0±1.00	9.5±1.50	9.2±0.10	15.0±1.00	-	-	-
	40	11.8±0.60	10.0±0.50	10.9±0.80	16.9±0.90	-	-	-
	60	12.2±0.30	11.4±0.30	11.1±0.80	18.1±0.89	-	-	-
	80	12.9±0.60	11.9±1.10	11.8±1.20	18.9±0.20	-	-	-
	100	14.0±0.80	12.3±0.60	12.1±0.60	20.0±0.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity.

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.38: Antibacterial activities of Methanol extract of *Catharanthus roseus* against *Salmonella Typhi*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Salmonella Typhi (MTCC 425)	20	0.0±0.00	0.0±0.00	0.0±0.00	15.0±1.00	-	-	-
	40	11.5±1.00	0.0±0.00	0.0±0.00	16.9±0.90	-	-	-
	60	12.0±0.50	9.8±1.20	9.9±1.10	18.1±0.89	-	-	-
	80	12.8±0.70	10.2±0.60	10.6±0.80	18.9±0.20	-	-	-
	100	13.8±1.60	12.1±0.40	12.1±0.30	20.0±0.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.39: Antibacterial activity of Methanol extract of *Solanum nigrum* against *Salmonella Typhi*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Salmonella Typhi (MTCC3425)	20	0.0±0.00	6.8±0.30	0.0±0.00	15.0±1.00	-	-	-
	40	7.5±0.70	8.0±1.00	0.0±0.00	16.9±0.90	-	-	-
	60	10.3±0.20	10.8±0.90	6.5±0.70	18.1±0.89	-	-	-
	80	10.9±0.70	11.3±0.70	7.2±0.40	18.9±0.20	-	-	-
	100	11.5±0.60	12±0.40	8.5±1.00	20.0±0.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.40: Antibacterial activity of acetone extract of *Murraya paniculata* against *Salmonella Typhi*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Salmonella Typhi (MTCC 3425)	20	9.9±0.40	9.5±0.40	0.0±0.00	15.0±1.00	-	-	-
	40	10.5±0.70	10.4±0.60	8.4±0.30	16.9±0.90	-	-	-
	60	11.4±0.60	11.5±0.90	9.2±0.50	18.1±0.89	-	-	-
	80	12.1±0.30	11.9±0.60	10.1±0.50	18.9±0.20	-	-	-
	100	12.9±0.10	12.2±0.40	10.9±0.90	20.0±0.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table-4.41: Antibacterial activities of acetone extract of *Catharanthus roseus* against *Salmonella Typhi*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Salmonella Typhi (MTCC 3425)	20	7.0±0.60	10.8±0.60	0.0±0.00	15.0±1.00	-	-	-
	40	9.0±0.80	11.2±0.40	0.0±0.00	16.9±0.90	-	-	-
	60	10.5±0.40	11.9±0.80	9.5±0.50	18.1±0.89	-	-	-
	80	13.0±0.20	12.3±0.20	10.3±0.90	18.9±0.20	-	-	-
	100	13.9±0.40	13.1±0.30	11.9±0.50	20.0±0.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.42: Antibacterial activity of acetone extract of *Solanum nigrum a*gainst *Salmonella Typhi*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Salmonella Typhi (MTCC3425)	20	0.0±0.00	0.0±0.00	0.0±0.00	15.0±1.00	-	-	-
	40	8.0±0.40	10.7±0.50	7.2±0.70	16.9±0.90	-	-	-
	60	10.0±0.80	11.5±0.70	9.8±0.60	18.1±0.89	-	-	-
	80	12.0±0.50	12.1±0.30	10.5±0.40	18.9±0.20	-	-	-
	100	13.0±0.60	12.9±0.40	11.0±0.30	20.0±0.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.43: Antibacterial activity of aqueous extract of *Murraya paniculata* against *Salmonella Typhi*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Salmonella Typhi (MTCC3425)	20	9.9±0.40	9.5±0.40	0.0±0.00	15.0±1.00	-	-	-
	40	10.5±0.70	10.4±0.60	8.4±0.30	16.9±0.90	-	-	-
	60	11.4±0.60	11.5±0.90	9.2±0.50	18.1±0.89	-	-	-
	80	12.1±0.30	11.9±0.60	10.1±0.50	18.9±0.20	-	-	-
	100	12.9±0.10	12.2±0.40	10.9±0.90	20.0±0.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.44: Antibacterial activity of aqueous extract of *Catharanthus roseus* against *Salmonella Typhi*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Salmonella Typhi (MTCC3425)	20	7.0±0.60	10.8±0.60	0.0±0.00	15.0±1.00	-	-	-
	40	9.0±0.80	11.2±0.40	0.0±0.00	16.9±0.90	-	-	-
	60	10.5±0.40	11.9±0.80	9.5±0.50	18.1±0.89	-	-	-
	80	13.0±0.20	12.3±0.20	10.3±0.90	18.9±0.20	-	-	-
	100	13.9±0.40	13.1±0.30	11.9±0.50	20.0±0.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.45: Antibacterial activity of aqueous extract of *Solanum nigrum* against *Salmonella Typhi*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Salmonella Typhi (MTCC 3425)	20	0.0±0.00	0.0±0.00	0.0±0.00	15.0±1.00	-	-	-
	40	7.8±0.40	10.1±0.70	7.3±0.30	16.9±0.90	-	-	-
	60	9.5±0.50	10.5±0.50	9.8±0.80	18.1±0.89	-	-	-
	80	11.7±0.70	11.9±0.90	10.2±0.20	18.9±0.20	-	-	-
	100	12.0±0.80	12.8±0.40	12.0±0.30	20.0±0.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.



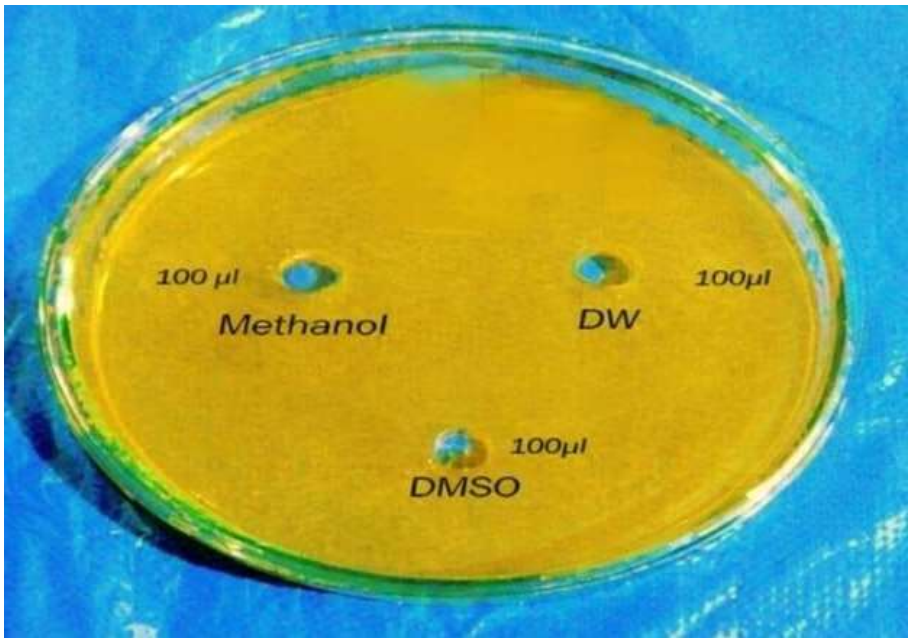
(A) Antibacterial activity of Methanol extract of *Murraya paniculata(Extract leaves) against Salmonella Typhi*



(B) Antibacterial activity of acetone extracts of *Catharanthus roseus* (Extract leaves) against *Salmonella Typhi.*



(C) Antibacterial activity of Standard Streptomycin against *Salmonella Typhi*



(D) Antibacterial activity of different controls (DMSO, Methanol and DW) against *Salmonella Typhi.*

Fig.:4.30 A, B, C and D showing the antibacterial activity of *Different plant parts* against *Salmonella Typhi*

Table- 4.46: Antibacterial activity of Methanol extract of *Murraya paniculata* against *Escherichia coli*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Escherichia coli (MTCC 1610)	20	11.5±0.40	11.1±0.30	10.3±0.20	15.0±0.30	-	-	-
	40	12.9±0.20	11.9±0.60	11.1±0.30	17.0±0.50	-	-	-
	60	14.8±0.50	13.5±0.40	12.9±0.30	20.0±0.50	-	-	-
	80	15.6±0.3	14.2±0.2	13.8±0.3	21.0±0.40	-	-	-
	100	16.2±0.2	15.0±0.2	14.5±0.4	23.0±0.81	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.47: Antibacterial activities of Methanol extract of *Catharanthus roseus* against *Escherichia coli*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Solvents Used*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Escherichia coli (MTCC 1610)	20	10.8±0.30	0.0±0.00	11.1±0.30	15.0±0.30	-	-	-
	40	11.5±0.40	11.0±0.10	11.8±0.30	17.0±0.50	-	-	-
	60	12.9±0.10	12.1±0.25	13.5±0.40	20.0±0.50	-	-	-
	80	13.8±0.1	12.9±0.4	14.1±0.2	21.0±0.40	-	-	-
	100	14.7±0.4	14.7±0.2	15.9±0.3	23.0±0.81	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.48: Antibacterial activities of Methanol extract of *Solanum nigrum* against *Escherichia coli*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Escherichia coli (MTCC 1610)	20	12.5±0.40	11.0±0.30	11.2±0.30	15.0±0.30		-	-
	40	13.4±0.30	12.8±0.50	13.0±0.20	17.0±0.50		-	-
	60	15.1±0.10	15.0±0.20	15.0±0.20	20.0±0.50		-	-
	80	16.0±0.2	15.9±0.2	16.1±0.2	21.0±0.40		-	-
	100	18.0±0.3	17.5±0.3	17.8±0.2	23.0±0.81	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.49: Antibacterial activity of acetone extract of *Murraya paniculata* against *Escherichia coli*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Streptomycin	DMSO	DW	Methanol
Escherichia coli (MTCC 1610)	20	10.9±0.40	10.8±0.20	0.0±0.00	15.0±0.30	-	-	-
	40	12.0±0.30	11.7±0.20	0.0±0.00	17.0±0.50	-	-	-
	60	13.5±0.40	13.2±0.20	10.5±0.40	20.0±0.50	-	-	-
	80	14.6±0.40	14.1±0.30	11.2±0.60	21.0±0.40	-	-	-
	100	15.1±0.40	14.9±0.70	11.9±1.10	23.0±0.81	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.50: Antibacterial activities of acetone extract of *Catharanthus roseus* against *Escherichia coli*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Streptomycin	DMSO	DW	Methanol
Escherichia coli (MTCC 1610)	20	10.5±0.40	0.00±0.00	11.0±0.20	15.0±0.30	-	-	-
	40	11.7±0.30	0.00±0.00	11.9±0.60	17.0±0.50	-	-	-
	60	12.2±0.30	10.9±0.30	12.5±0.30	20.0±0.50	-	-	-
	80	13.1±0.20	11.5±0.70	13.4±0.60	21.0±0.40	-	-	-
	100	13.9±0.70	12.1±0.30	14.2±0.50	23.0±0.81	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.51: Antibacterial activity of acetone extract of *Solanum nigrum* against *Escherichia coli*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Streptomycin	DMSO	DW	Methanol
Escherichia coli (MTCC 1610)	20	8.0±0.70	12.0±0.20	10.0±0.40	15.0±0.30	-	-	-
	40	11.0±0.30	12.9±0.30	11.8±0.60	17.0±0.50	-	-	-
	60	12.0±0.40	14.8±0.40	12.5±0.60	20.0±0.50	-	-	-
	80	14.0±0.60	15.5±0.50	15.0±0.40	21.0±0.40	-	-	-
	100	17.0±0.20	16.0±0.50	16.5±0.50	23.0±0.81	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.52: Antibacterial activity of aqueous extract of *Murraya paniculata* against *Escherichia coli*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Escherichia coli (MTCC 1610)	20	9.9±0.30	9.2±0.40	0.0±0.00	15.0±0.30	-	-	-
	40	11.8±0.20	10.7±0.20	0.0±0.00	17.0±0.50	-	-	-
	60	12.6±0.50	12.1±0.80	10.5±0.40	20.0±0.50	-	-	-
	80	13.6±0.60	13.1±0.30	11.2±0.60	21.0±0.40	-	-	-
	100	15.1±0.40	14.4±0.70	11.9±1.10	23.0±0.81	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.53: Antibacterial activity of aqueous extract of *Catharanthus roseus* against *Escherichia coli*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Escherichia coli (MTCC 1610)	20	10,1±0.40	0.00±0.00	0.0±0.00	15.0±0.30	-	-	-
	40	11.2±0.30	0.00±0.00	0.00±0.60	17.0±0.50	-	-	-
	60	12.1±0.30	10.3±0.30	8.5±0.30	20.0±0.50	-	-	-
	80	12.8±0.20	11.5±0.70	10.4±0.60	21.0±0.40	-	-	-
	100	13.2±0.70	11.9±0.30	12.2±0.50	23.0±0.81	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.54: Antibacterial activity of aqueous extract of *Solanum nigrum a*gainst *Escherichia coli*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Escherichia coli (MTCC 1610)	20	8.2±0.30	7.7±0.20	7.5±0.40	15.0±0.30	-	-	-
	40	10.0±0.20	9.9±0.30	9.8±0.20	17.0±0.50	-	-	-
	60	11.8±0.40	10.8±0.40	10.5±0.80	20.0±0.50	-	-	-
	80	13.8±0.50	13.5±0.50	12.0±0.60	21.0±0.40	-	-	-
	100	15.8±0.20	15.0±0.50	14.5±0.50	23.0±0.81	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.



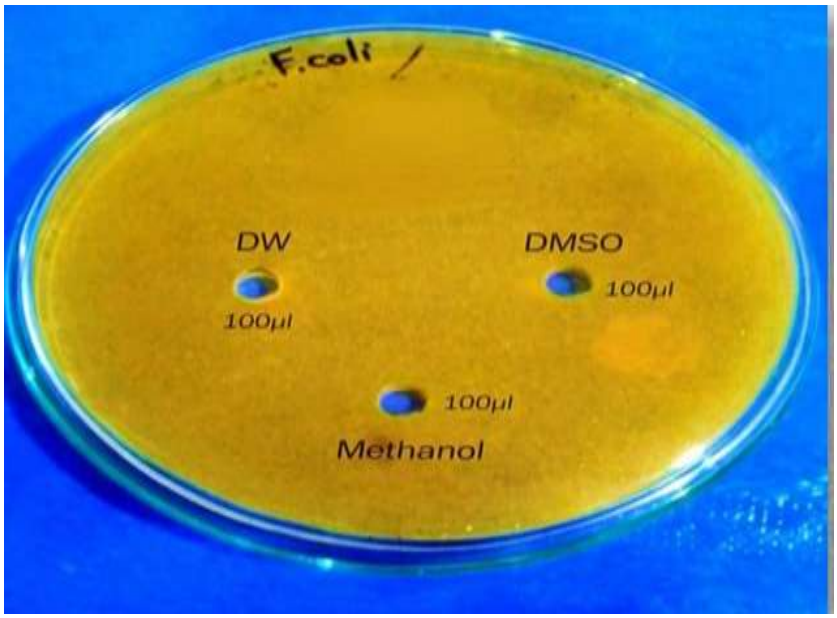
A. **Antibacterial activity of methanol extracts of *Solanum nigrum(Extract leaves)against Escherichia coli***



B. **Antibacterial activity of acetone extract of Solanum nigrum (Extract leaves) against *Escherichia coli***



C. Antibacterial activity of Standard Streptomycin against *Escherichia coli*



D.* Antibacterial activity of different controls (DMSO, Methanol and DW) and DW*)* controls *Escherichia coli.

Fig.:4.31 A, B, C and D showing the antibacterial activity of different plant parts against *Escherichia coli.*

Table- 4.55: Antibacterial activity of Methanol extract of *Murraya paniculata* against *Klebsiella pneumoniae*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Klebsiella pneumoniae (MTCC 3160)	20	15.2±0.90	14.8±1.2	10.2±1.2	23.5±0.40	-	-	-
	40	15.8±0.3	15.2±0.2	10.8±1.2	24.6±0.60	-	-	-
	60	17.5±1.0	16.8±0.4	1151±0.4	26.3±1.00	-	-	-
	80	18.8±0.9	17.6±0.8	12.8±1.4	27.6±0.80	-	-	-
	100	19.0±0.50	18.0±0.2	13.5±0.7	28.2±.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.56: Antibacterial activities of Methanol extract of *Catharanthus roseus* against *Klebsiella pneumoniae*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Klebsiella pneumoniae (MTCC 3160)	20	13.5±1.04	13.3±1.1	9.5±0.5	23.5±0.40	-	-	-
	40	14.6±1.5	14.2±0.9	10.2±0.2	24.6±0.60	-	-	-
	60	15.4±0.6	15.1±0.9	10.8±1.2	26.3±1.00	-	-	-
	80	16.3±0.7	15.8±0.3	11.8±0.9	27.6±0.80	-	-	-
	100	17.1±0.9	16.3±0.7	13.5±1.3	28.2±.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.57: Antibacterial activity of Methanol extract of *Solanum nigrum* against *Klebsiella pneumoniae*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Klebsiella pneumoniae (MTCC 3160)	20	11.5±1.5	11.4±0.1	6.6±0.1	23.5±0.40	-	-	-
	40	12.6±0.5	12.7±0.1	7.5±1.5	24.6±0.60	-	-	-
	60	14.5±0.7	13.4±1.4	8.8±1.4	26.3±1.00	-	-	-
	80	15.1±0.1	14.1±0.9	9.7±0.5	27.6±0.80	-	-	-
	100	16±0.1	15.4±0.6	11.5±0.5	28.2±.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.58: Antibacterial activities of acetone extract of *Murraya paniculata* against *Klebsiella pneumonia*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Klebsiella pneumoniae (MTCC 3160)	20	13.5±0.30	13.1±1.2	7.6±1.30	23.5±0.40	-	-	-
	40	14.4±0.40	13.9±1.1	8.1±0.80	24.6±0.60	-	-	-
	60	15.2±0.60	14.5±0.30	8.8±0.50	26.3±1.00	-	-	-
	80	16.7±0.20	15.8±1.00	09.3±1.00	27.6±0.80	-	-	-
	100	18.1±0.80	16.9±0.20	10.2±0.60	28.2±.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.59: Antibacterial activities of acetone extract of *Catharanthus roseus* against *Klebsiella pneumonia*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Klebsiella pneumoniae (MTCC 3160)	20	12.9±1.10	12.5±0.60	9.2±0.10	23.5±0.40	-	-	-
	40	14±0.80	13.8±0.30	9.7±0.20	24.6±0.60	-	-	-
	60	14.8±1.20	14.2±0.4	10.3±1.00	26.3±1.00	-	-	-
	80	15.2±0.80	14.6±0.70	11.3±1.00	27.6±0.80	-	-	-
	100	16.1±0.50	15.7±0.40	12.5±0.50	28.2±.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.60: Antibacterial activity of acetone extract of *Solanum nigrum* against *Klebsiella pneumonia*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Klebsiella pneumoniae (MTCC 3160)	20	11.9±0.80	10.6±0.50	6.2±0.20	23.5±0.40	-	-	-
	40	13.1±1.50	11.6±0.40	7.4±1.30	24.6±0.60	-	-	-
	60	14.0±0.90	12.1±0.70	8.2±0.50	26.3±1.00	-	-	-
	80	14.5±0.40	12.8±1.00	9.1±0.40	27.6±0.80	-	-	-
	100	15.1±0.60	13.9±1.10	10.2±1.00	28.2±.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.61: Antibacterial activity of aqueous extract of *Murraya paniculata* against *Klebsiella pneumonia*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Klebsiella pneumoniae (MTCC 3160)	20	15.3±0.40	15.0±1.2	11.2±1.2	23.5±0.40	-	-	-
	40	16.2±0.30	15.5±0.2	11.8±1.2	24.6±0.60	-	-	-
	60	17.7±1.10	16.9±0.4	11.51±0.4	26.3±1.00	-	-	-
	80	18.9±0.60	17.8±0.8	12.9±1.4	27.6±0.80	-	-	-
	100	19.1±0.80	18.2±0.2	14.0±0.8	28.2±.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.62: Antibacterial activity of aqueous extract of *Catharanthus roseus* against *Klebsiella pneumonia*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Klebsiella pneumoniae (MTCC 3160)	20	10.8±1.10	11.1±0.70	8.4±0.10	23.5±0.40	-	-	-
	40	11.5±0.80	11.8±0.30	9.2±0.50	24.6±0.60	-	-	-
	60	12.8±1.30	13.2±0.44	9.9±1.00	26.3±1.00	-	-	-
	80	13.9±0.80	13.5±0.70	10.8±1.00	27.6±0.80	-	-	-
	100	15.8±0.20	14.7±0.80	12.8±0.50	28.2±.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

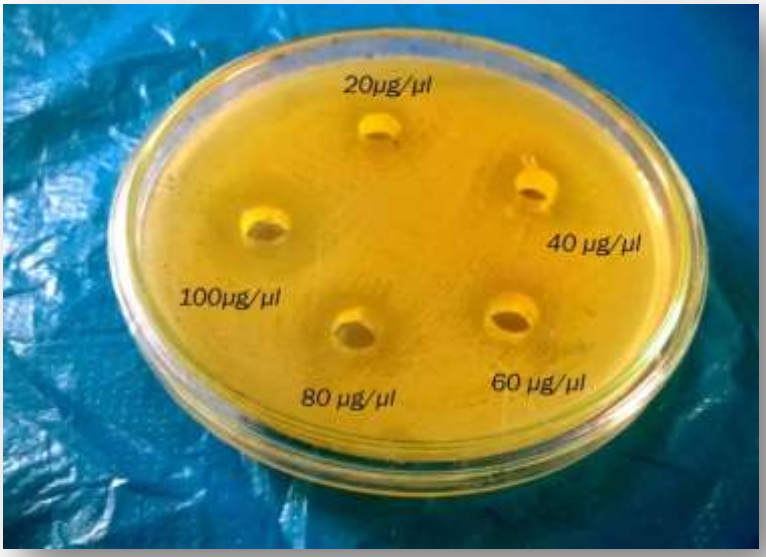
Table- 4.63: Antibacterial activity of aqueous extract of *Solanum nigrum* against *Klebsiella pneumonia*

Bacteria	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Strepto mycin	DMSO	DW	Methanol
Klebsiella pneumoniae (MTCC 3160)	20	10.9±0.90	10.1±0.50	6.5±0.20	23.5±0.40	-	-	-
	40	13.5±1.50	12.2±0.50	7.3±1.50	24.6±0.60	-	-	-
	60	14.1±0.90	12.9±0.70	8.0±0.20	26.3±1.00	-	-	-
	80	14.8±0.40	14.0±1.00	9.1±0.20	27.6±0.80	-	-	-
	100	15.6±0.20	14.2±1.20	9.9±1.00	28.2±.60	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.



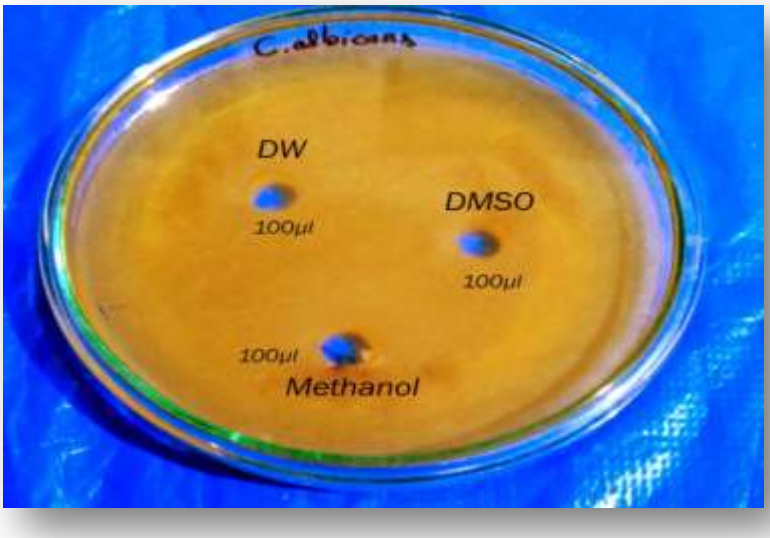
A. Antibacterial activity of Aqueous extract of *Murraya paniculata*(Extract leaves) against *Klebsiella pneumonia*



B. Antibacterial activity of Aqueous extract of *Murraya paniculata* (Extract leaves) against *Klebsiella pneumoniae*



C. Antibacterial activity of Standard Fluconazole against *Klebsiella pneumoniae*



D. Antibacterial l activity of different controls (DMSO, Methanol and DW) against *Klebsiella pneumoniae.*

Fig.:4.32 A, B, C and D showing the antifungal activity of different plant parts against *Klebsiella pneumoniae.*

Table- 4.64: Antifungal activity of Methanol extract of *Murraya paniculata* against *Candida albicans*

Fungi	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Fluconazole	DMSO	DW	Methanol
Candida albicans (MTCC398)	20	9.6±0.40	9.1±0.50	8.5±0.60	9.9±1.2	-	-	-
	40	9.9±0.70	9.7±0.40	9.3±0.60	10.8±0.8	-	-	-
	60	11.2±0.40	10.6±1.10	10.9±0.90	11.6±0.8	-	-	-
	80	12.5±0.50	11.5±0.90	11.3±0.20	12.5±0.9	-	-	-
	100	13.8±0.80	12.9±0.60	11.8±0.10	13.4±0.3	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.65: Antifungal activities of Methanol extract of *Catharanthus roseus* against *Candida albicans*

Fungi	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Fluconazole	DMSO	DW	Methanol
Candida albicans (MTC 3958)	20	9.1±1.00	8.5±0.40	7.5±0.30	15.1±0.70	-	-	-
	40	9.5±0.30	9.2±0.70	9.0±0.80	15.9±0.90	-	-	-
	60	11±0.90	10.5±0.30	9.9±0.10	16.8±0.40	-	-	-
	80	12.3±0.40	11.8±0.20	11.0±0.10	17.2±0.20	-	-	-
	100	13.0±0.70	12.5±0.40	11.9±0.30	18.0±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.66: Antifungal activities of Methanol extract of *Solanum nigrum* against *Candida albicans*

Fungi	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Fluconazole	DMSO	DW	Methanol
Candida albicans (MTCC 3958)	20	7.5±0.10	7.1±0.20	7.5±0.10	15.1±0.70	-	-	-
	40	9.2±0.60	7.9±0.40	9.2±0.60	15.9±0.90	-	-	-
	60	10.1±0.30	8.8±0.60	10.1±0.30	16.8±0.40	-	-	-
	80	11.0±0.90	9.5±0.30	11.0±0.90	17.2±0.20	-	-	-
	100	12.5±0.50	10.6±0.20	12.5±0.50	18.0±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.67: Antifungal activity of acetone extract of *Murraya paniculata* against *Candida albicans*

Fungi	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Fluconazole	DMSO	DW	Methanol
Candida albicans (MTCC 3958)	20	7.9±0.60	7.6±0.60	6.9±0.80	15.1±0.70	-	-	-
	40	8.7±0.60	8.0±0.80	7.8±0.70	15.9±0.90	-	-	-
	60	10.5±0.50	8.9±0.60	8.5±0.40	16.8±0.40	-	-	-
	80	11.9±0.80	10.9±0.90	10.3±0.40	17.2±0.20	-	-	-
	100	12.8±0.40	11.8±0.30	10.5±0.50	18.0±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.68: Antifungal activities of acetone extract of *Catharanthus roseus* against *Candida albicans*

Fungi	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Fluconazole	DMSO	DW	Methanol
Candida albicans (MTCC3958)	20	7.8±0.60	7.0±0.80	7.2±0.70	15.1±0.70	-	-	-
	40	9.4±0.80	7.9±0.80	8.5±0.50	15.9±0.90	-	-	-
	60L	10.9±0.70	8.5±0.30	9.0±0.70	16.8±0.40	-	-	-
	80	11.5±0.70	9.7±0.50	10.2±0.60	17.2±0.20	-	-	-
	100	12.6±0.40	10.4±0.60	10.8±0.40	18.0±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.69: Antifungal activity of acetone extract of *Solanum nigrum* against *Candida albicans*

Fungi	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Fluconazole	DMSO	DW	Methanol
Candida albicans (MTCC 3958)	20	7.1±0.60	6.5±0.70	6.9±0.90	15.1±0.70	-	-	-
	40	8.9±0.50	7.2±0.40	7.8±0.80	15.9±0.90	-	-	-
	60	9.5±0.50	7.8±0.50	8.5±0.40	16.8±0.40	-	-	-
	80	10.4±0.70	8.7±0.70	9.6±0.40	17.2±0.20	-	-	-
	100	11.2±0.20	10.0±0.50	10.5±0.50	18.0±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.70: Antifungal activity of acetone extract of *Murraya paniculata* against *Candida albicans*

Fungi	Extract in µg/µl	Zone of inhibition (mm)						
		Murraya paniculata			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Fluconazole	DMSO	DW	Methanol
Candida albicans MTCC 3958)	20	7.2±0.60	7.0±0.60	6.9±0.80	15.1±0.70	-	-	-
	40	8.0±0.50	7.5±0.80	7.1±0.80	15.9±0.90	-	-	-
	60	10.2±0.50	8.9±0.40	7.8±0.60	16.8±0.40	-	-	-
	80	11.1±0.80	9.9±0.90	8.3±0.40	17.2±0.20	-	-	-
	100	11.7±0.30	10.8±020	9.5±0.80	18.0±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.71: Antifungal activities of acetone extract of *Catharanthus roseus* against *Candida albicans*

Fungi	Extract in µg/µl	Zone of inhibition (mm)						
		Catharanthus roseus			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Fluconazole	DMSO	DW	Methanol
Candida albicans (MTCC 3958)	20	6.8±0.30	6.6±0.40	6.2±0.70	15.1±0.50	-	-	-
	40	7.4±0.70	7.1±0.80	7.5±0.50	15.9±0.20	-	-	-
	60	8.9±0.70	8.2±0.30	8.0±0.70	16.8±0.40	-	-	-
	80	9.5±0.70	9.1±0.50	9.2±0.60	17.2±0.20	-	-	-
	100	10.6±0.50	9.4±0.80	9.8±0.70	18.0±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.

Table- 4.72: Antifungal activities of acetone extract of *Solanum nigrum* against *Candida albicans*

Fungi	Extract in µg/µl	Zone of inhibition (mm)						
		Solanum nigrum			*Standard*	*Control*		
		Extract L	Extract F	Extract S	Fluconazole	DMSO	DW	Methanol
Candida albicans (MTCC 3958)	20	6.1±0.60	6.5±0.70	6.1±0.90	15.1±0.70	-	-	-
	40	6.9±0.50	7.2±0.40	6.8±0.80	15.9±0.90	-	-	-
	60	8.5±0.50	7.5±0.50	7.2±0.40	16.8±0.40	-	-	-
	80	8.9±0.70	8.7±0.70	8.1±0.40	17.2±0.20	-	-	-
	100	10.2±0.20	9.0±0.50	8.5±0.50	18.0±1.00	NA	NA	NA

Extract used: L= leaves; F=Fruits; S=Stem; NA= No activity

*Each presented value in table are expressed as mean ± SD Means of triplicate analyses (n=3)

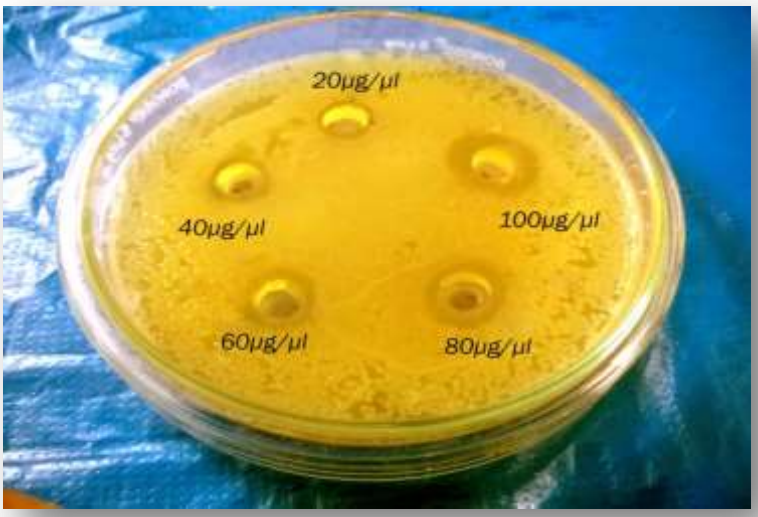
†Solvents DMSO, DW and Methanol were used as control at a volume of 100 µl to each well.



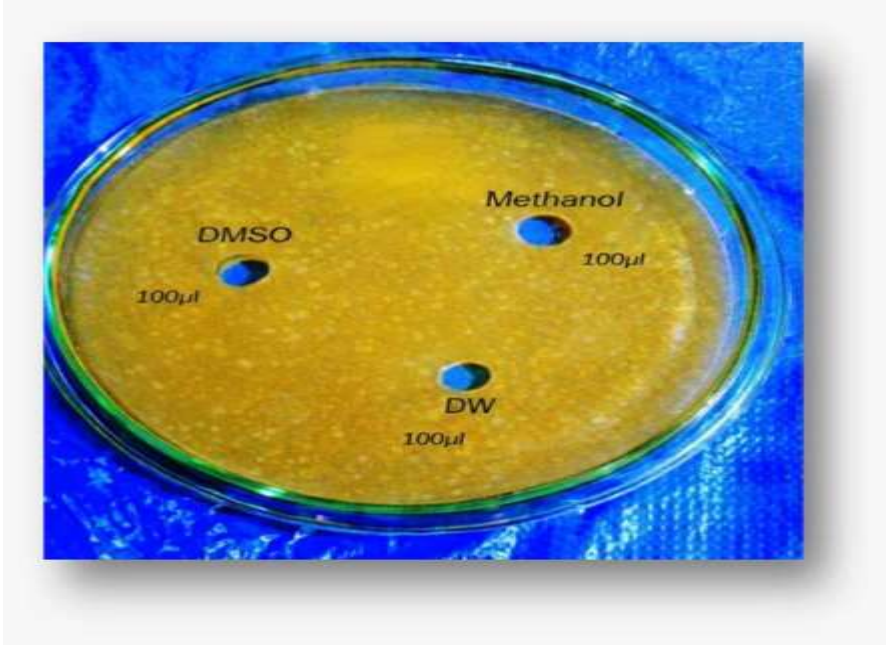
A. Antifungal activity of Methanol extract of *Murraya paniculata* (Extract leaves) against *Candida albicans*



B.* Antifungal activity of Acetone extract of *Murraya paniculata* (Extract Stem) against *Candida albicans.



C. Antifungal activity of Standard Fluconazole against *Candida albicans.*



D. Antifungal activity of different controls (DMSO, Methanol and DW)) against *Candida albicans.*

Fig.:4.33 A, B, C and D showing the antifungal activity of *different plant parts* .against *Candida albicans.*

4.6 Brief Description

The present study provides a broad comparative evaluation of present phytochemicals and antioxidant potentiality of *Murraya paniculata*, *Catharanthus roseus*, and *Solanum nigrum* using their different parts (leaves, fruits, and stem) and extractive solvents (methanol, acetone, and aqueous). The discussion included the extractive yield, qualitative phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and in vitro antioxidant assays (DPPH and ABTS), thereby offering a complete elucidation of solvent dependent bioactivity. The present findings collectively emphasize the principal role of solvent polarity and plant part specificity in determining phytochemical and antioxidant efficiency.

4.6.1 Influence of Solvent Polarity on Extractive Yield

The extractive yield data discussed in this section are taken from **Table 4.1** and the corresponding graphical representations **(Fig. 4.1-4.3).** The observed solvent-dependent variation was found similar and consistent with earlier studies on polar solvent efficiency **(Altemimi *et al.*, 2020; Ahmed *et al.*, 2021).** Extractive value is a primary indicator of solvent efficiency in recovery of bioactive constituents. the present study concluded that methanol and aqueous solvents consistently produced higher extractive yields compared to acetone across all three plant species. This is thought to the higher polarity of methanol and water, which empowers effective solubilization of a wide range of polar and moderately polar phytochemicals, including phenolic, flavonoids, tannins, glycosides, and alkaloids. **Altemimi *et al.* (2020)** also observed the similar solvent-dependent extraction efficiencies and suggested that the methanol is the most effective solvents for comprehensive phytochemical extraction.

Leaves showed noticeably higher extractive values than fruits and stems, regardless of solvent used. These findings indicated the leaves are physiologically active metabolic sites for biosynthesis and accumulation of secondary metabolites further involved in defense, stress tolerance, and antioxidative protection **(Harborne, 1998).** The comparatively lower extractive values observed that higher lignin and cellulose content in stems may be attributed to their structural function which reduced the metabolic activity.

Acetone’s intermediate polarity and limited capacity to dissolve highly polar

compounds showed lower extractive yields. While acetone is found effective for extracting certain flavonoid aglycones and low-molecular-weight phenolic, its overall extraction range is narrower comparatively to methanol **(Bhuyan *et al.*, 2022).** These findings highlight the importance of solvent selection based on the required category of phytochemicals.

4.6.2 Qualitative phytochemical screening

The qualitative screenings of phytochemical groups were summarized in **Table 4.2**, and the plant part specific metabolite distribution concerned with classical phytochemical localization theories **(Harborne, 1998; Kokate, 2019).** Qualitative phytochemical screening showed the discrete differences in bio metabolite distribution depending on plant species, plant part, and solvent system Methanol extracts exhibited the broadest range of localized phytoconstituents, followed by aqueous extracts, whereas acetone extracts showed limited phytochemical range. The higher performance of methanol substantiates earlier reports emphasizing its effectiveness in disrupting plant cell matrices and extracting both polar and semi-polar compounds **(Ahmed *et al.*, 2021).**

Across all species, leaves showed strong to moderate presence of alkaloids, flavonoids, phenolic, tannins, and glycosides. This manner has been widely attributed to the leaves which synthesize protective secondary metabolites in response to environmental stress such as ultraviolet radiation, pathogens, and oxidative stress **(Kokate, 2019).**

In *Catharanthus roseus*, the noticeable presence of alkaloids in Methanol leaves extracts is predominantly remarkable as the plant's well-established status as a source of pharmaceutically important in dole alkaloids. The little presence of phytochemicals in acetone extracts sues further highlights the selective nature of solvent extraction and highlights the risk of underestimating phytochemical potential when inappropriate solvents are employed.

4.6.3 Total Phenolic Content

The quantitative phenolic contents discussed here are based on Methanol (**Table 4.3**), acetone (**Table 4.4**), and aqueous (**Table 4.5**) extracts, with graphical comparison provided in **Fig. 4.4-4.6**. Similar solvent-dependent phenolic findings have been

reported by **Kumar *et al*. (2020)** and **Bhuyan *et al*. (2022).** Phenolic compounds are the most important part of antioxidant activity due to their redox properties and ability to scavenge free radicals. In the present study, TPC values varied significantly across plant types and organ specific distribution, solvents with a consistent movement of **leaves > fruits > stem**. Methanol extracts demonstrated the highest TPC values, followed by acetone and aqueous extracts.

The higher phenolic content estimated in Methanol leaves extracts of *Murraya paniculata* suggests a high presence of phenolic acids, flavonoids, and related compounds. Similar findings have been reported by **Kumar *et al*. (2020),** who demonstrated primary storage of phenolic in photosynthetically active tissues. The reduced TPC values in aqueous extracts indicate that it is less efficient for moderately polar phenolic compounds.

The lower phenolic content observed in stems across all species is consistent with their primary structural role. Phenolic biosynthesis is closely related to metabolic activity, which is significantly higher in leaves compared to stems. These findings suggested that leaves are the most appropriate material for antioxidant phytochemical studies.

4.6.4 Total Flavonoid Content and Solvent Efficiency

Estimated Total flavonoid content values in this section are found correspond to Methanol (**Table 4.6**), acetone (**Table 4.7**), and aqueous (**Table 4.8**) extracts, as illustrated in **Fig. 4.7-4.9.** The solvent efficiency hierarchy similar to the previous flavonoid extraction studies **(Alam *et al*., 2023; Patel & Shah, 2020).** Flavonoids play a crucial role in antioxidant defense mechanisms. The quantification of TFC using standard quercetin revealed is found with patterns similar to TPC observes. Consequently Methanol extracts gave high yield of the flavonoid content, comparatively to acetone and aqueous extracts.

The higher TFC values in Methanol extracts can be due to solubility of both flavonoid glycosides and aglycones. The intermediate polarity specially flavonoid aglycones, results in moderate flavonoid recovery in acetone solvent. In contrast, aqueous extracts exhibited comparatively lower TFC values because of limited solubility of many flavonoid aglycones in water **(Alam *et al*., 2023).**

Among the taken plant species, *Murraya paniculata* leaves exhibited the highest

flavonoid content, followed by *Catharanthus roseus* and *Solanum nigrum* the cause of this variation may be the species-specific metabolic pathways and genetic regulation of flavonoid biosynthesis. The strong correlation between TFC and antioxidant activity observed in subsequent assays highlights the functional significance of flavonoids in free radical scavenging.

4.6.5 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity were supported by **Table 4.9-Table 4.17** and the associated figures **(Fig. 4.10-4.18).** The DPPH assay is a widely accepted method for evaluating the hydrogen-donating ability of antioxidants. The present study shows that all extracts demonstrated the concentration-dependent DPPH radical scavenging activity by confirming their antioxidant potential. Methanol extracts exhibited the strongest activity, followed by acetone and aqueous extracts.

Leaves extracts consistently showed higher scavenging activity than fruits and stems, which show a relationship with their higher phenolic and flavonoid content. The strong DPPH activity of Methanol extracts can be of the synergistic action of multiple antioxidant compounds efficiently extracted by methanol **(Singh *et al.*, 2022).**
Quercetin, a positive control, showed significantly higher scavenging activity than all plant extracts, validating the sensitivity of the assay. However, comparatively high DPPH scavenging activity was observed for Methanol leaves extracts indicated the significant antioxidant potentiality and support their possible use as natural antioxidant sources.

4.6.6 IC_{50} Values and Comparative Antioxidant Efficiency

The IC_{50} values were also summarized in **Table 4.18** and comparatively explained in **Fig. 4.19.** IC_{50} values provide a quantitative measure of antioxidant strength, with lower values indicating higher efficacy. In the present study, Methanol leaves extracts of all three plants showed the lowest IC_{50} values, followed by fruit and stem extracts. Aqueous stem extracts consistently exhibited the highest IC_{50} values, indicating weaker antioxidant efficiency.

The lower IC_{50} values observed in Methanol extracts are because of their higher phenolic and flavonoid content. Similar IC_{50} values have been reported by **Shahidi and Ambigaipalan (2021),** who emphasized the role of solvent polarity in

antioxidant extraction.

4.6.7 ABTS Radical Cation Scavenging Activity

The estimated ABTS radical cation scavenging activity in the present study is given in **Table 4.19-Table 4.27** and showed in **Fig. 4.20-4.27.** The ABTS assay complements the DPPH method by assessing both hydrophilic and lipophilic antioxidant capacity. In present study, Methanol extracts revealed higher ABTS radical scavenging activity, followed by acetone and aqueous extracts. Leaves extracts found effective comparatively fruits and stems across all solvents.

The strong ABTS scavenging activity showed by Methanol leaves extracts of *Murraya paniculata* and *Catharanthus roseus* is indicative of a diverse antioxidant profile, including phenolic, flavonoids, and alkaloids. Comparable inhibition values have been reported in earlier studies, strengthening the reproducibility and reliability of the present findings **(Ganesan *et al.*, 2015; Verma & Prakash, 2021).**

4.6.8 Comparative Evaluation among Plant Species

Among the three studied species, *Murraya paniculata* consistently exhibited superior antioxidant potential, followed by *Catharanthus roseus* and *Solanum nigrum.* This hierarchy may be attributed to differences in phytochemical composition, metabolic specialization, and accumulation of antioxidant constituents. The results validate the traditional medicinal use of these plants and provide scientific evidence supporting their antioxidant properties.

The findings of the present study clearly exhibited that solvent polarity and plant part selection critically influence phytochemical extraction and antioxidant activity. Methanol leaves extract emerged as the most potent source of antioxidants across all evaluated parameters. The strong correlation between phenolic/flavonoid content and antioxidant activity underscores the importance of these compounds in free radical scavenging mechanisms. The study provides a robust scientific foundation for the further investigation of these plants as natural sources of antioxidant compounds and supports their potential application in pharmaceutical, nutraceutical, and functional food formulations.

4.6.9 Antimicrobial Activity of Plant Part Extracts

The antimicrobial evaluation of ***Murraya paniculata*, *Catharanthus roseus*,** and ***Solanum nigrum*** showed clear **plant-part-specific, solvent-dependent, and microorganism-specific activity,** as given in **Tables 4.28–4.69** and corresponding **figures** (**Fig. 4.29–4.32**). The results collectively revealed that leaves extracts, particularly those prepared using **methanol,** exhibit superior antibacterial and antifungal efficacy compared to fruits and stem extracts.

4.6.9.1 Antibacterial Activity against *Staphylococcus aureus*

4.6.9.1.1 Methanol Extracts

As shown in **Tables 4.28–4.30**, Methanol leaves extracts of all three plants exhibited antibacterial activity against ***Staphylococcus aureus*** (MTCC 3160), with Inhibition zones increasing in a **range of Concentratipns (20-100 µg/µl)**. Among the tested species, ***Murraya paniculata*** leaves extract produced the highest inhibition (19.0 ± 0.50 mm at 100 µg/µl; **Table 4.28**), followed by ***Catharanthus roseus*** (17.1 ± 0.9 mm; **Table 4.29**) and ***Solanum nigrum*** (16.0 ± 0.1 mm; **Table 4.30**).

The superior activity of Methanol leaves extracts can be attributed to the efficient extraction of **phenolic, flavonoids, alkaloids, and tannins,** which are known to disrupt bacterial cell walls, inhibit protein synthesis, and interfere with nucleic acid metabolism **(Cushnie & Lamb, 2011; Altemimi *et al.*, 2020).** The consistently lower activity of stem extracts reflects their reduced metabolic role and lower accumulation of bioactive secondary metabolites.

4.6.9.1.2 Acetone and Aqueous Extracts

Acetone extracts showed moderate antibacterial activity (**Tables 4.31-4.33**), while aqueous extracts exhibited comparatively lower inhibition (**Tables 4.34-4.36**). This solvent-dependent trend supports earlier findings that organic solvents are more efficient than water in extracting antibacterial phytochemicals **(Ahmed *et al.*, 2021).** The negligible activity observed in solvent controls confirms that the inhibition is solely attributable to plant metabolites.

4.6.9.2 Antibacterial Activity against *Salmonella Typhi* spp.

The antibacterial activity against ***Salmonella Typhi*** (MTCC 425) was also found similar to that observed for ***Staphylococcus aureus*.** Methanol leaves extracts again exhibited the highest Inhibition zones (**Tables 4.37-4.39**), with ***Murraya paniculata*** demonstrating superior efficacy (14.0 ± 0.80 mm at 100 µg/µl; **Table 4.37**). Acetone and aqueous extracts showed reduced but measurable activity (**Tables 4.40-4.45**).

The relatively lower susceptibility of ***Salmonella Typhi*** compared to ***Staphylococcus aureus*** can be explained by its **Gram-negative cell wall structure,** which possesses an outer lipopolysaccharide layer that restricts the penetration of antimicrobial compounds **(Nikaido, 2003).** Nevertheless, the ability of Methanol extracts to inhibit ***Salmonella Typhi*** highlights their potential as sources of broad-spectrum antibacterial agents.

4.6.9.3 Antibacterial Activity against *Escherichia coli*

Tables 4.46-4.54 presented the all three plant species exhibited antibacterial activity against ***Escherichia coli*** (MTCC 1610), with Methanol leaves extracts showing the highest zones of inhibition. Notably, ***Solanum nigrum*** leaves extract displayed strong activity (18.0 ± 0.3 mm at 100 µg/µl; **Table 4.48**), suggesting a higher concentration of Gram-negative-active phytochemicals.

The enhanced antimicrobial activity of Methanol extracts against ***Escherichia coli*** supports previous reports indicating that **flavonoids and phenolic acids** can inhibit essential enzymatic pathways in Gram-negative bacteria **(Daglia, 2012; Kumar *et al.*, 2020).**

4.6.9.4 Antibacterial Activity against *Klebsiella pneumoniae*

Extracts showed moderate ***against Klebsiella pneumoniae*** (MTCC 3160) as given in **Tables 4.55-4.63**. Methanol leaves extracts of ***Murraya paniculata*** showed the highest Inhibition (19.1 ± 0.80 mm at 100 µg/µl; **Table 4.61**), whereas aqueous stem extracts showed minimal activity.

The comparatively reduced susceptibility of ***Klebsiella pneumoniae*** may be attributed to its **capsular polysaccharide**, which confers resistance to antimicrobial agents **(Podschun & Ullmann, 1998).** Despite this, the consistent inhibition by leaves extracts underscores their therapeutic relevance.

4.6.9.5 Antifungal Activity against *Candida albicans*

The antifungal activity against ***Candida albicans*** (MTCC 3958) is summarized in **Tables 4.64-4.69**. Methanol leaves extracts again exhibited superior activity, with ***Murraya paniculata*** showing inhibition comparable to fluconazole at higher concentrations (**Table 4.64**). Acetone extracts showed moderate activity, while aqueous extracts were least effective.

The antifungal efficacy of leaves extracts may be linked to the presence of **phenolic compounds and alkaloids,** which disrupt fungal cell membranes and inhibit ergo sterol biosynthesis **(Pinto *et al.*, 2009; Sharma *et al.*, 2023).**

4.6.9.6 Comparative Evaluation of Plant Parts and Solvents

Across all tested microorganisms, a consistent trend was observed:

Leaves > Fruits > Stem

Methanol > Acetone > Aqueous

This hierarchy confirms that **leaves**, being the primary sites of secondary metabolite biosynthesis, are the most potent sources of antimicrobial compounds. Methanol proved to be the most effective extraction solvent due to its ability to solubilize a wide spectrum of bioactive constituents.

4.6.9.7 Comparison with Standard Drugs

Although streptomycin and fluconazole exhibited higher inhibition zones than plant extracts, the comparatively strong activity of Methanol leaves extracts-particularly of

Murraya paniculata-suggests their potential as **natural antimicrobial agents.** Importantly, plant-based antimicrobials may reduce the risk of resistance development and adverse side effects associated with synthetic drugs **(WHO, 2019).**

4.6.9.8 Significance

The antimicrobial results presented in **Tables 4.28-4.69** and **Fig. 4.29-4.32** provide strong scientific validation for the traditional medicinal use of the studied plants. The findings highlight the importance of **solvent selection and plant-part choice** in antimicrobial research and support further purification and characterization of active compounds from Methanol leaves extracts.

4.6 Brief Description

The present study provides a broad comparative evaluation of present phytochemicals and antioxidant potentiality of *Murraya paniculata*, *Catharanthus roseus*, and *Solanum nigrum* using their different parts (leaves, fruits, and stem) and extractive solvents (methanol, acetone, and aqueous). The discussion included the extractive yield, qualitative phytochemical screening, total phenolic content (TPC), total flavonoid content (TFC), and in vitro antioxidant assays (DPPH and ABTS), thereby offering a complete elucidation of solvent dependent bioactivity. The present findings collectively emphasize the principal role of solvent polarity and plant part specificity in determining phytochemical and antioxidant efficiency.

4.6.1 Influence of Solvent Polarity on Extractive Yield

The extractive yield data discussed in this section are taken from **Table 4.1** and the corresponding graphical representations **(Fig. 4.1-4.3).** The observed solvent-dependent variation was found similar and consistent with earlier studies on polar solvent efficiency **(Altemimi *et al.*, 2020; Ahmed *et al.*, 2021).** Extractive value is a primary indicator of solvent efficiency in recovery of bioactive constituents. the present study concluded that methanol and aqueous solvents consistently produced higher extractive yields compared to acetone across all three plant species. This is thought to the higher polarity of methanol and water, which empowers effective solubilization of a wide range of polar and moderately polar phytochemicals, including phenolic, flavonoids, tannins, glycosides, and alkaloids. **Altemimi *et***

***al.* (2020)** also observed the similar solvent-dependent extraction efficiencies and suggested that the methanol is the most effective solvents for comprehensive phytochemical extraction.

Leaves showed noticeably higher extractive values than fruits and stems, regardless of solvent used. These findings indicated the leaves are physiologically active metabolic sites for biosynthesis and accumulation of secondary metabolites further involved in defense, stress tolerance, and antioxidative protection **(Harborne, 1998).** The comparatively lower extractive values observed that higher lignin and cellulose content in stems may be attributed to their structural function which reduced the metabolic activity.

Acetone's intermediate polarity and limited capacity to dissolve highly polar compounds showed lower extractive yields. While acetone is found effective for extracting certain flavonoid aglycones and low-molecular-weight phenolic, its overall extraction range is narrower comparatively to methanol **(Bhuyan *et al.*, 2022).** These findings highlight the importance of solvent selection based on the required category of phytochemicals.

4.6.2 Qualitative phytochemical screening

The qualitative screenings of phytochemical groups were summarized in **Table 4.2**, and the plant part specific metabolite distribution concerned with classical phytochemical localization theories **(Harborne, 1998; Kokate, 2019).** Qualitative phytochemical screening showed the discrete differences in bio metabolite distribution depending on plant species, plant part, and solvent system Methanol extracts exhibited the broadest range of localized phytoconstituents, followed by aqueous extracts, whereas acetone extracts showed limited phytochemical range. The higher performance of methanol substantiates earlier reports emphasizing its effectiveness in disrupting plant cell matrices and extracting both polar and semi-polar compounds **(Ahmed *et al.*, 2021).**

Across all species, leaves showed strong to moderate presence of alkaloids, flavonoids, phenolic, tannins, and glycosides. This manner has been widely attributed to the leaves which synthesize protective secondary metabolites in response to environmental stress such as ultraviolet radiation, pathogens, and oxidative stress **(Kokate, 2019).**

In *Catharanthus roseus*, the noticeable presence of alkaloids in Methanol leaves extracts is predominantly remarkable as the plant's well-established status as a source of pharmaceutically important in dole alkaloids. The little presence of phytochemicals in acetone extracts sues further highlights the selective nature of solvent extraction and highlights the risk of underestimating phytochemical potential when inappropriate solvents are employed.

4.6.3 Total Phenolic Content

The quantitative phenolic contents discussed here are based on Methanol (**Table 4.3**), acetone (**Table 4.4**), and aqueous (**Table 4.5**) extracts, with graphical comparison provided in **Fig. 4.4-4.6**. Similar solvent-dependent phenolic findings have been reported by **Kumar *et al*. (2020)** and **Bhuyan *et al*. (2022).** Phenolic compounds are the most important part of antioxidant activity due to their redox properties and ability to scavenge free radicals. In the present study, TPC values varied significantly across plant types and organ specific distribution, solvents with a consistent movement of **leaves > fruits > stem**. Methanol extracts demonstrated the highest TPC values, followed by acetone and aqueous extracts.

The higher phenolic content estimated in Methanol leaves extracts of *Murraya paniculata* suggests a high presence of phenolic acids, flavonoids, and related compounds. Similar findings have been reported by **Kumar *et al*. (2020),** who demonstrated primary storage of phenolic in photosynthetically active tissues. The reduced TPC values in aqueous extracts indicate that it is less efficient for moderately polar phenolic compounds.

The lower phenolic content observed in stems across all species is consistent with their primary structural role. Phenolic biosynthesis is closely related to metabolic activity, which is significantly higher in leaves compared to stems. These findings suggested that leaves are the most appropriate material for antioxidant phytochemical studies.

4.6.4 Total Flavonoid Content and Solvent Efficiency

Estimated Total flavonoid content values in this section are found correspond to Methanol (**Table 4.6**), acetone (**Table 4.7**), and aqueous (**Table 4.8**) extracts, as illustrated in **Fig. 4.7-4.9.** The solvent efficiency hierarchy similar to the previous

flavonoid extraction studies **(Alam *et al.*, 2023; Patel & Shah, 2020).** Flavonoids play a crucial role in antioxidant defense mechanisms. The quantification of TFC using standard quercetin revealed is found with patterns similar to TPC observes. Consequently Methanol extracts gave high yield of the flavonoid content, comparatively to acetone and aqueous extracts.

The higher TFC values in Methanol extracts can be due to solubility of both flavonoid glycosides and aglycones. The intermediate polarity specially flavonoid aglycones, results in moderate flavonoid recovery in acetone solvent. In contrast, aqueous extracts exhibited comparatively lower TFC values because of limited solubility of many flavonoid aglycones in water **(Alam *et al.*, 2023).**

Among the taken plant species, *Murraya paniculata* leaves exhibited the highest flavonoid content, followed by *Catharanthus roseus* and *Solanum nigrum* the cause of this variation may be the species-specific metabolic pathways and genetic regulation of flavonoid biosynthesis. The strong correlation between TFC and antioxidant activity observed in subsequent assays highlights the functional significance of flavonoids in free radical scavenging.

4.6.5 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity were supported by **Table 4.9-Table 4.17** and the associated figures **(Fig. 4.10-4.18).** The DPPH assay is a widely accepted method for evaluating the hydrogen-donating ability of antioxidants. The present study shows that all extracts demonstrated the concentration-dependent DPPH radical scavenging activity by confirming their antioxidant potential. Methanol extracts exhibited the strongest activity, followed by acetone and aqueous extracts.

Leaves extracts consistently showed higher scavenging activity than fruits and stems, which show a relationship with their higher phenolic and flavonoid content. The strong DPPH activity of Methanol extracts can be of the synergistic action of multiple antioxidant compounds efficiently extracted by methanol **(Singh *et al.*, 2022).**

Quercetin, a positive control, showed significantly higher scavenging activity than all plant extracts, validating the sensitivity of the assay. However, comparatively high DPPH scavenging activity was observed for Methanol leaves extracts indicated the significant antioxidant potentiality and support their possible use as natural antioxidant sources.

4.6.6 IC_{50} Values and Comparative Antioxidant Efficiency

The IC_{50} values were also summarized in **Table 4.18** and comparatively explained in **Fig. 4.19.** IC_{50} values provide a quantitative measure of antioxidant strength, with lower values indicating higher efficacy. In the present study, Methanol leaves extracts of all three plants showed the lowest IC_{50} values, followed by fruit and stem extracts. Aqueous stem extracts consistently exhibited the highest IC_{50} values, indicating weaker antioxidant efficiency.

The lower IC_{50} values observed in Methanol extracts are because of their higher phenolic and flavonoid content. Similar IC_{50} values have been reported by **Shahidi and Ambigaipalan (2021),** who emphasized the role of solvent polarity in antioxidant extraction.

4.6.7 ABTS Radical Cation Scavenging Activity

The estimated ABTS radical cation scavenging activity in the present study is given in **Table 4.19-Table 4.27** and showed in **Fig. 4.20-4.27.** The ABTS assay complements the DPPH method by assessing both hydrophilic and lipophilic antioxidant capacity. In present study, Methanol extracts revealed higher ABTS radical scavenging activity, followed by acetone and aqueous extracts. Leaves extracts found effective comparatively fruits and stems across all solvents.

The strong ABTS scavenging activity showed by Methanol leaves extracts of *Murraya paniculata* and *Catharanthus roseus* is indicative of a diverse antioxidant profile, including phenolic, flavonoids, and alkaloids. Comparable inhibition values have been reported in earlier studies, strengthening the reproducibility and reliability of the present findings **(Ganesan *et al.*, 2015; Verma & Prakash, 2021).**

4.6.8 Comparative Evaluation among Plant Species

Among the three studied species, *Murraya paniculata* consistently exhibited superior antioxidant potential, followed by *Catharanthus roseus* and *Solanum nigrum.* This hierarchy may be attributed to differences in phytochemical composition, metabolic specialization, and accumulation of antioxidant constituents. The results validate the traditional medicinal use of these plants and provide scientific evidence supporting their antioxidant properties.

The findings of the present study clearly exhibited that solvent polarity and plant part selection critically influence phytochemical extraction and antioxidant activity. Methanol leaves extract emerged as the most potent source of antioxidants across all evaluated parameters. The strong correlation between phenolic/flavonoid content and antioxidant activity underscores the importance of these compounds in free radical scavenging mechanisms. The study provides a robust scientific foundation for the further investigation of these plants as natural sources of antioxidant compounds and supports their potential application in pharmaceutical, nutraceutical, and functional food formulations.

4.6.9 Antimicrobial Activity of Plant Part Extracts

The antimicrobial evaluation of ***Murraya paniculata*, *Catharanthus roseus*,** and ***Solanum nigrum*** showed clear **plant-part-specific, solvent-dependent, and microorganism-specific activity,** as given in **Tables 4.28–4.69** and corresponding **figures** (**Fig. 4.29–4.32**). The results collectively revealed that leaves extracts, particularly those prepared using **methanol,** exhibit superior antibacterial and antifungal efficacy compared to fruits and stem extracts.

4.6.9.1 Antibacterial Activity against *Staphylococcus aureus*

4.6.9.1.1 Methanol Extracts

As shown in **Tables 4.28–4.30**, Methanol leaves extracts of all three plants exhibited antibacterial activity against ***Staphylococcus aureus*** (MTCC 3160), with Inhibition zones increasing in a **range of Concentratipns (20-100 µg/µl)**. Among the tested species, ***Murraya paniculata*** leaves extract produced the highest inhibition (19.0 ± 0.50 mm at 100 µg/µl; **Table 4.28**), followed by ***Catharanthus roseus*** (17.1 ± 0.9 mm; **Table 4.29**) and ***Solanum nigrum*** (16.0 ± 0.1 mm; **Table 4.30**).

The superior activity of Methanol leaves extracts can be attributed to the efficient extraction of **phenolic, flavonoids, alkaloids, and tannins,** which are known to disrupt bacterial cell walls, inhibit protein synthesis, and interfere with nucleic acid metabolism **(Cushnie & Lamb, 2011; Altemimi *et al.*, 2020).** The consistently lower activity of stem extracts reflects their reduced metabolic role and lower accumulation of bioactive secondary metabolites.

4.6.9.1.2 Acetone and Aqueous Extracts

Acetone extracts showed moderate antibacterial activity (**Tables 4.31-4.33**), while aqueous extracts exhibited comparatively lower inhibition (**Tables 4.34-4.36**). This solvent-dependent trend supports earlier findings that organic solvents are more efficient than water in extracting antibacterial phytochemicals **(Ahmed *et al.,* 2021).** The negligible activity observed in solvent controls confirms that the inhibition is solely attributable to plant metabolites.

4.6.9.2 Antibacterial Activity against *Salmonella Typhi* spp.

The antibacterial activity against ***Salmonella Typhi*** (MTCC 425) was also found similar to that observed for ***Staphylococcus aureus*.** Methanol leaves extracts again exhibited the highest Inhibition zones (**Tables 4.37-4.39**), with ***Murraya paniculata*** demonstrating superior efficacy (14.0 ± 0.80 mm at 100 µg/µl; **Table 4.37**). Acetone and aqueous extracts showed reduced but measurable activity (**Tables 4.40-4.45**).

The relatively lower susceptibility of ***Salmonella Typhi*** compared to ***Staphylococcus aureus*** can be explained by its **Gram-negative cell wall structure,** which possesses an outer lipopolysaccharide layer that restricts the penetration of antimicrobial compounds **(Nikaido, 2003).** Nevertheless, the ability of Methanol extracts to inhibit ***Salmonella Typhi*** highlights their potential as sources of broad-spectrum antibacterial agents.

4.6.9.3 Antibacterial Activity against *Escherichia coli*

Tables 4.46-4.54 presented the all three plant species exhibited antibacterial activity against ***Escherichia coli*** (MTCC 1610), with Methanol leaves extracts showing the highest zones of inhibition. Notably, ***Solanum nigrum*** leaves extract displayed strong activity (18.0 ± 0.3 mm at 100 µg/µl; **Table 4.48**), suggesting a higher concentration of Gram-negative-active phytochemicals.

The enhanced antimicrobial activity of Methanol extracts against ***Escherichia coli*** supports previous reports indicating that **flavonoids and phenolic acids** can inhibit essential enzymatic pathways in Gram-negative bacteria **(Daglia, 2012; Kumar *et al.,* 2020).**

4.6.9.4 Antibacterial Activity against *Klebsiella pneumoniae*

Extracts showed moderate ***against Klebsiella pneumoniae*** (MTCC 3160) as given in **Tables 4.55-4.63**. Methanol leaves extracts of ***Murraya paniculata*** showed the highest Inhibition (19.1 ± 0.80 mm at 100 µg/µl; **Table 4.61**), whereas aqueous stem extracts showed minimal activity.

The comparatively reduced susceptibility of ***Klebsiella pneumoniae*** may be attributed to its **capsular polysaccharide**, which confers resistance to antimicrobial agents **(Podschun & Ullmann, 1998).** Despite this, the consistent inhibition by leaves extracts underscores their therapeutic relevance.

4.6.9.5 Antifungal Activity against *Candida albicans*

The antifungal activity against ***Candida albicans*** (MTCC 3958) is summarized in **Tables 4.64-4.69**. Methanol leaves extracts again exhibited superior activity, with ***Murraya paniculata*** showing inhibition comparable to fluconazole at higher concentrations (**Table 4.64**). Acetone extracts showed moderate activity, while aqueous extracts were least effective.

The antifungal efficacy of leaves extracts may be linked to the presence of **phenolic compounds and alkaloids,** which disrupt fungal cell membranes and inhibit ergo sterol biosynthesis **(Pinto *et al.*, 2009; Sharma *et al.*, 2023).**

4.6.9.6 Comparative Evaluation of Plant Parts and Solvents

Across all tested microorganisms, a consistent trend was observed:

Leaves > Fruits > Stem

Methanol > Acetone > Aqueous

This hierarchy confirms that **leaves**, being the primary sites of secondary metabolite biosynthesis, are the most potent sources of antimicrobial compounds. Methanol

proved to be the most effective extraction solvent due to its ability to solubilize a wide spectrum of bioactive constituents.

4.6.9.7 Comparison with Standard Drugs

Although streptomycin and fluconazole exhibited higher inhibition zones than plant extracts, the comparatively strong activity of Methanol leaves extracts-particularly of ***Murraya paniculata***-suggests their potential as **natural antimicrobial agents.** Importantly, plant-based antimicrobials may reduce the risk of resistance development and adverse side effects associated with synthetic drugs **(WHO, 2019).**

4.6.9.8 Significance

The antimicrobial results presented in **Tables 4.28-4.69** and **Fig. 4.29-4.32** provide strong scientific validation for the traditional medicinal use of the studied plants. The findings highlight the importance of **solvent selection and plant-part choice** in antimicrobial research and support further purification and characterization of active compounds from Methanol leaves extracts.

CHAPTER – 5

SUMMARY & CONCLUSION

5.1 Summary

The rapid advancement of modern medicine has significantly improved disease management; however, the **emergence of antibiotic-resistant microorganisms**, increasing prevalence of **cancer**, and the **adverse side effects of synthetic drugs** remain major global health challenges. These limitations have intensified scientific interest in **natural products**, particularly **medicinal plants**, as alternative and complementary sources of safer and cost-effective therapeutic agents.

Medicinal plants have been used since ancient times as primary healthcare resources, especially in developing countries where access to modern medicine is limited. According to the World Health Organization, a large proportion of the global population relies on traditional medicine for disease prevention and treatment. The therapeutic efficacy of medicinal plants is attributed to their rich content of **phytochemicals**, including alkaloids, phenolic, flavonoids, tannins, saponins, and terpenoids, which exhibit diverse pharmacological activities such as **antioxidant, antimicrobial, anti-inflammatory, and anticancer effects**

Oxidative stress caused by excessive generation of reactive oxygen and nitrogen species plays a key role in the development of chronic diseases such as cardiovascular disorders, diabetes, cancer, and neurodegenerative conditions. Plant-derived antioxidants, particularly phenolic compounds and flavonoids, are increasingly recognized for their ability to neutralize free radicals and reduce oxidative damage, offering a safer alternative to synthetic antioxidants known for their toxic side effects.

The introduction highlights the medicinal significance of three important plants-***Murraya paniculata***, ***Catharanthus roseus***, and ***Solanum nigrum***-which are widely used in traditional medicine systems. *Catharanthus roseus* is well known for its anticancer alkaloids such as vincristine and vinblastine, along with its antioxidant and anti-inflammatory properties. *Solanum nigrum* possesses a broad spectrum of bioactive compounds with reported antimicrobial, antioxidant, and anticancer activities, despite concerns related to toxicity at high doses. *Murraya paniculata* (curry leaf plant) is valued both as a culinary and medicinal plant, rich in carbazole

alkaloids, flavonoids, and phenolic, and is traditionally used for treating infections, inflammation, and metabolic disorders.

The increasing problem of **antimicrobial resistance** further underscores the urgent need to explore plant-based bioactive compounds as potential alternatives or adjuncts to existing antibiotics. Despite extensive traditional use and preliminary scientific evidence, systematic and comparative studies on the phytochemical composition and biological activities of different plant parts and solvent extracts remain limited.

Therefore, the present study was undertaken to scientifically evaluate the **phytochemical profile, antioxidant potential, and antibacterial activity** of different extracts of selected medicinal plants, thereby providing experimental evidence to support their traditional uses and to explore their potential application in the development of natural therapeutic agents.

The present study generated comprehensive quantitative data on extractive yield, phytochemical content, antioxidant activity, and antibacterial efficacy of different plant parts of *Murraya paniculata*, *Catharanthus roseus*, and *Solanum nigrum*. All values are expressed as **mean ± standard deviation**, reflecting good experimental precision and reproducibility.

5.1.1 Extractive Yield

The extractive value varied markedly with plant species, plant part and solvent used. In all three plants, **leaves consistently yielded significantly higher extractive values** than fruits and stems. Among solvents, methanol and water proved more efficient than acetone.

The highest extractive yield was observed in the **aqueous leaf extract of *Solanum nigrum*** (670.55 ± 1.8 mg/g), followed by its methanolic leaf extract (630.38 ± 2.5 mg/g). Similarly, *Murraya paniculata* leaves showed high methanolic extractive value (600.29 ± 7.78 mg/g), while *Catharanthus roseus* leaves recorded 610.7 ± 5.8 mg/g in aqueous extract and 590.85 ± 3.2 mg/g in methanolic extract. In contrast, acetone extracts of all plant parts showed comparatively lower extractive values, confirming the limited efficiency of acetone for extracting highly polar constituents.

5.1.2 Total Phenolic Content (TPC)

Total phenolic content exhibited clear dependence on both plant part and solvent. Across all species, **leaf extracts contained the highest phenolic concentration**, followed by fruits and stems.

Methanol extracts showed the highest TPC values overall. *Murraya paniculata* leaves recorded the maximum TPC (32.1 ± 0.03 mg GAE/g), followed by fruits (30.1 ± 0.3 mg GAE/g) and stems (27.7 ± 0.2 mg GAE/g). In *Catharanthus roseus*, leaves extracts showed 28.4 ± 0.4 mg GAE/g, while fruits and stem contained 23.8 ± 0.3 and 20.0 ± 0.5 mg GAE/g, respectively. *Solanum nigrum* leaves exhibited 27.7 ± 0.1 mg GAE/g, whereas fruits and stem showed lower values of 18.8 ± 0.5 and 16.0 ± 0.8 mg GAE/g.

Acetone extracts showed moderate phenolic content, with *Murraya paniculata* leaves extract recording 26.4 ± 0.03 mg GAE/g, while aqueous extracts showed comparatively lower TPC, the highest being 25.4 ± 0.03 mg GAE/g in *Murraya paniculata* leaves. The consistently low SD values indicate reliable phenolic estimation.

5.1.3 Total Flavonoid Content (TFC)

Flavonoid content followed trends similar to phenolic content. Methanol extracts exhibited the highest flavonoid levels, particularly in leaf tissues.

Murraya paniculata leaves showed the highest TFC (22.1 ± 0.2 mg QE/g), followed by *Catharanthus roseus* leaves (19.4 ± 0.6 mg QE/g) and *Solanum nigrum* leaves (17.7 ± 1.1 mg QE/g). Fruits and stem showed progressively lower values, with the lowest flavonoid content observed in the stem extract of *Catharanthus roseus* (10.0 ± 0.5 mg QE/g).

Acetone and aqueous extracts showed reduced flavonoid content compared to methanol. However, leaf extracts still retained relatively higher values, such as 16.8 ± 0.12 mg QE/g in acetone leaves extracts and 15.9 ± 1.3 mg QE/g in aqueous leaves extract of *Murraya paniculata*.

5.1.4 Antioxidant Activity (DPPH Radical Scavenging Assay)

All extracts demonstrated **concentration-dependent DPPH radical scavenging activity**, indicating increasing antioxidant capacity with increasing extract concentration.

Methanol extracts exhibited the strongest antioxidant activity. At 500 µg/ml, methanol leaves extract of *Murraya paniculata* showed 69.75 ± 1.36% Inhibition, followed by fruit (63.25 ± 2.50%) and stem (51.25 ± 1.98%). Acetone extracts showed comparable but slightly lower activity, with leaf extract showing 68.75 ± 1.86% Inhibition at the same concentration. Aqueous extracts showed lower activity, with maximum inhibition of 63.75 ± 1.75% observed in leaf extract.

Quercetin, used as the standard, exhibited higher scavenging activity (81.42 ± 1.42% at 500 µg/ml), validating the assay. The relatively low standard deviation values across all concentrations indicate consistent antioxidant measurements.

5.1.5 Antibacterial Activity

Antibacterial activity varied with extract concentration, solvent, and plant part. **Leaves extracts consistently showed larger zones of inhibition** compared to fruits and stem.

Against *Staphylococcus aureus*, the methanol leaves extract of *Murraya paniculata* showed a zone of Inhibition of 19.0 ± 0.50 mm at 100 µg/µl, while acetone and aqueous leaves extracts showed 18.3 ± 0.80 mm and 16.3 ± 0.80 mm, respectively. Streptomycin produced a significantly higher zone (30.0 ± 1.00 mm).

Against *Salmonella*, methanol leaves extract of *Murraya paniculata* showed 14.0 ± 0.80 mm inhibition at the highest concentration, whereas acetone and aqueous extracts showed approximately 12.9 ± 0.10 mm.

Against *Escherichia coli*, *Solanum nigrum* methanol leaves extract exhibited the highest activity (18.0 ± 0.3 mm at 100 µg/µl).

In all cases, solvent controls showed no inhibition, confirming the antimicrobial role of plant-derived bioactive compounds.

A major outcome of the study is the clear demonstration that **solvent polarity and plant part selection play decisive roles** in determining extractive yield, phytochemical richness, and biological activity. Methanol and aqueous solvents consistently produced higher extractive yields than acetone, which is attributed to their greater polarity and ability to solubilize a wide spectrum of bioactive phytochemicals such as phenolic, flavonoids, tannins, glycosides, and alkaloids. Leaves showed significantly higher extractive yields than fruits and stems, reflecting their role as metabolically active tissues involved in secondary metabolite biosynthesis

Qualitative phytochemical screening revealed marked variation among species, solvents, and plant parts. **Methanol leaves extracts** exhibited the broadest range of phytochemicals across all species, while acetone extracts showed limited phytochemical recovery. The high abundance of alkaloids in methanol leaves extracts of *Catharanthus roseus* supports its well-established pharmacological importance, particularly as a source of anticancer indole alkaloids. These observations reinforce earlier theories on tissue-specific localization of secondary metabolites and the importance of appropriate solvent selection.

Quantitative estimation of **total phenolic content (TPC)** and **total flavonoid content (TFC)** further confirmed solvent- and tissue-dependent variation. Methanol extracts, especially from leaves, exhibited the highest TPC and TFC values, followed by acetone and aqueous extracts. The consistent trend of **Leaves > Fruits > Stem** observed across all solvents reflects higher metabolic and biosynthetic activity in foliar tissues. Lower phenolic and flavonoid levels in stems were attributed to their primarily structural role and reduced metabolic activity.

Antioxidant activity assessed through **DPPH and ABTS radical scavenging assays** showed a strong concentration-dependent response in all extracts. Methanol leaves extracts demonstrated the highest antioxidant activity, which closely correlated with their elevated phenolic and flavonoid content. Lower IC_{50} values recorded for methanol leaves extracts further confirmed their superior antioxidant efficiency. The

strong agreement between DPPH and ABTS results highlights the presence of both hydrophilic and lipophilic antioxidant compounds in these extracts and supports the reliability of the findings.

The antimicrobial evaluation revealed distinct **plant-part-specific, solvent-dependent, and microorganism-specific** activity patterns. Methanol leaves extracts consistently exhibited the strongest antibacterial and antifungal activity against *Staphylococcus aureus*, *Salmonella spp*., *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans*. Among the studied plants, *Murraya paniculata* showed superior antimicrobial efficacy, followed by *Catharanthus roseus* and *Solanum nigrum*. Reduced susceptibility of Gram-negative bacteria was attributed to their complex cell wall structure, while the strong activity of methanolic extracts was linked to the synergistic action of phenolics, flavonoids, alkaloids, and tannins.

Overall, the discussion establishes that **methanol leaves extracts are the most potent source of antioxidant and antimicrobial compounds** among the tested samples. The strong correlation between phytochemical content and biological activity provides scientific validation for the traditional medicinal use of these plants. The findings emphasize the importance of solvent selection and plant-part choice and provide a robust foundation for future studies focused on **isolation, characterization, and formulation of bioactive compounds** for pharmaceutical, nutraceutical, and functional food applications.

5.2 Conclusion:

The present study was carried out to evaluate the phytochemical composition, antioxidant potential, and antimicrobial activity of *Murraya paniculata*, *Catharanthus roseus*, and *Solanum nigrum* using different plant parts and extraction solvents. The study was conducted to evaluate comparative findings on extractive efficiency, distribution of secondary metabolites, and biological activity, thereby fulfilling the stated objectives.

The findings clearly showed that both plant-part selection and solvent polarity significantly influence phytochemical screening and biological activity. Leaves consistently showed higher extractive values and a broader spectrum of

phytochemicals compared to fruits and stem. Methanol found to be the most effective solvent for extracting bioactive compounds, resulting in higher phenolic and flavonoid content as well as enhanced antioxidant and antimicrobial activity, followed by *acetone* and *aqueous* extracts.

Antioxidant evaluation was done using DPPH and ABTS assays showed free radical scavenging activity across all extracts, with methanol leaves extracts showed lower IC_{50} values, indicating stronger antioxidant efficiency. Likewise, antimicrobial screening also revealed that leaves extracts, particularly those prepared in methanol, produced larger zones of inhibition against tested bacterial and fungal strains, suggesting the presence of potent antimicrobial compounds.

Comparative analysis among the three plant species indicated that *Murraya paniculata* exhibited the highest overall biological activity, followed by *Catharanthus roseus* and *Solanum nigrum*. The observed correlation between phenolic and flavonoid content and both antioxidant and antimicrobial activities highlights the central role of these phytochemicals in biological efficacy.

In conclusion, the study findings supported the antioxidant and antimicrobial potential of the selected medicinal plants. The results elucidated the importance of solvent and plant-part specificity in phytochemical and bioactivity studies. This study contributes significance to the field of medicinal plant research and supports the rational use of these plants in traditional and modern therapeutic applications.

CHAPTER – 6

RECOMMENDATIONS & FUTURE SCOPE OF RESEARCH

6.1 Recommendations

Based on the comprehensive evaluation of antioxidant and antimicrobial activities of *Murraya paniculata*, *Catharanthus roseus*, and *Solanum nigrum*, the following recommendations are proposed:

1. The consistently superior antioxidant and antimicrobial performance of methanol leaves extracts suggests that future research should prioritize these extracts for bioactivity-guided fractionation. Leaves should be considered the primary plant material due to their higher accumulation of phenolic, flavonoids, and antimicrobial metabolites.
2. Further a biochemical characterization is required for isolation and characterization of active compounds from extract. Advanced chromatographic and spectroscopic techniques such as HPLC, LC-MS/MS, GC-MS, and NMR are recommended to isolate and structurally characterize the specific bioactive compounds responsible for the observed antioxidant and antimicrobial activities.
3. Further studies are required to elucidate the molecular mechanisms underlying antimicrobial action, including membrane disruption, enzyme inhibition, oxidative stress induction, and interaction with microbial DNA or proteins. Such studies will strengthen the pharmacological relevance of the findings.
4. Since plants extracts contain complex mixtures of phytochemicals, studies on synergistic interactions between isolated compounds or between plants extracts and conventional antibiotics are recommended. This approach may contribute to overcoming antimicrobial resistance.
5. Prior to pharmaceutical or nutraceutical application, comprehensive toxicity studies including cytotoxicity, acute toxicity, and chronic toxicity assessments are recommended to ensure safety and dosage optimization.
6. The promising bioactivity of methanol leaves extracts supports their further development into herbal formulations such as ointments, gels, capsules, or functional food additives. Stability and shelf-life studies should accompany formulation research.

7. For validation of antioxidant and antimicrobial efficacy under physiological conditions
   requires in vivo studies using suitable animal models are recommended to.
8. Future studies may explore additional biological activities such as anti-inflammatory, wound-healing, anticancer, and immunomodulatory effects of these plants extracts using the present observed strong antioxidant–antimicrobial correlation.
9. To ensure reproducibility, consistency, and regulatory acceptance of plant-based products derived from these species needs Development of standardized extraction protocols and quality control parameters.

6.1.1 Research Recommendations

Findings of the present study proposed the following research recommendations are proposed:

1. **Use of Leaves Material**

   Subsequently leaves showed higher phytochemical content and antioxidant activity, future research should highlight leaves extracts for both analytical and biological studies.

2. **Selection of Solvent Based on Research Objective**

   In present findings Methanol was found to be the most effective solvent for extracting phenolics and flavonoids; however, aqueous extracts demonstrated significance for traditional and safety-oriented applications. In Further studies solvents should be selected strategically based on intended use.

3. **Integration of Multidisciplinary Approaches**

   Further work can combine phytochemistry, molecular biology, pharmacology, and computational modeling (in silico docking and QSAR studies) is recommended to explore comprehensive understanding into bioactivity mechanisms.

4. **Comparative Studies**

   Future research should investigate the influence of geographical location, climatic conditions, and seasonal variation on phytochemical composition and antioxidant potential.

5. **Correlation between Phytochemical Content and Biological Activity** Further studies may include Quantitative structure–activity relationship (QSAR) analysis and recommended to establish strong correlations between specific phytoconstituents and observed antioxidant or pharmacological effects.

6.2 Future Scope of Research work

Further study involving isolation, purification, and structural characterization of individual bioactive compounds are recommended. In vivo studies and evaluations may help to establish therapeutic efficacy, safety, and mode of action, facilitating the development of plant-based antimicrobial and antioxidant formulations for pharmaceutical and nutraceutical use.

The present work has established the phytochemical presence, antioxidant and anti-microbial efficacy of selected medicinal plants using different extracting solvents and plant parts. Thus the findings point out a strong biochemical base and several advanced research progress that can significantly spread the scientific and pragmatic importance of this work.

1. **Isolation and Characterization of Bioactive Compounds:** Forthcoming studies should be focussed on the isolation, purification, and structural elucidation of specific bioactive compounds responsible for the observed antioxidant activity. Advanced analytical techniques such as HPLC, LC-MS/MS, GC-MS, and NMR spectroscopy can be used to identify and characterize specific phenolic compounds, flavonoids and alkaloids derivatives. This establishing a correlation of individual phytochemicals and their biological efficacy.

2. **Antioxidant Studies:** Even though the present findings estimated free radical scavenging capacity using DPPH and ABTS assays, upcoming research may explore cellular and molecular reactions of antioxidant action. This study involves the reactive oxygen species (ROS) modulation, lipid peroxidation inhibition, mitochondrial protection, and redox-signalling pathways using cell culture models would provide appropriate validation of antioxidant efficacy.
3. **Pharmacological Evaluation: P**hytochemical containing extracts, particularly methanol leaves extracts, and could be further evaluated for specific pharmacological properties incliding anti-inflammatory, antimicrobial, anticancer, antidiabetic, hepatoprotective, and neuroprotective activities. Pharmacological studies would expand the therapeutic applicapabilty of present medicinal plants including antioxidant activity.
4. **Toxicological Assessment:** To support the therapeutic applications, future studies should be carried out to evaluate acute, sub-chronic, and chronic toxicity following OECD guidelines. Cytotoxicity study must be essential to establish safe dosage limits and therapeutically usage of plant extracts and isolated compounds.
5. **In vivo Validation and Bioavailability Studies: F**or In vivo studies shall be could be done on appropriate animal models to confirm antioxidant efficacy under physiological conditions. Furthermore, bioavailability, metabolism, and pharmacokinetic studies of major phytoconstituents could help in understanding the systemic effectiveness.
6. **Green Extraction Technologies:** Future study may investigate the environmentally sustainable extraction techniques such as ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, or enzyme-assisted extraction. These approaches may enhance extraction efficiency while reducing solvent usage and environmental impact.
7. **Standardization of Herbal Extracts:** Use of chemical markers in Development of standardized extract profiles can enhance reproducibility and industrial applicability. Appropriate analytical validation procedures can established a batch-to-batch consistency and also essential for pharmaceutical and nutraceutical development.
8. **Formulation and Product Development:** The antioxidant rich extracts can be supported for formulation into nutraceuticals, functional foods, herbal

supplements. Stability and shelf life studies would further support commercialization potential.

REFERENCES

- **Abd El Moneim, D., Alqahtani, M.M., Abdein, M.A. and Germoush, M.O. (2020).** Drought and Salinity Stress Response in Wheat: Physiological and TaNAC Genes Expression Analysis of Contrasting Egyptian Wheat Genotypes. Journal of Plant Biotechnology, 47, 1-14.
- **Abdein, M.A. (2018).** Genetic Diversity between Pumpkin Accessions Growing in the Northern Border Region in Saudi Arabia Based on Biochemical and Molecular Parameters. Egyptian Journal Botany, 58, 463-476.
- **Abdein, M.A. and Osman, A.K.E. (2020).** Plant Diversity Assessment of Wadi Al-Hilali, Northern Border Region, Saudi Arabia. International Journal of Botany Studies, 5, 87-95.
- **Abdein, M.A., Wrda, H.N. and Osman, A.K. (2020).** Genetic Characterization of Genus Tephrosia Pers. Based on Molecular Markers in KSA. International Journal of Botany Studies, 5, 203-209.
- **Abdel-Mageed, A.M., Osman, A.K.E., Awad, N.S. and Abdein, M.A. (2019).** Evaluation of Antidiabetic Potentiality of Truffles and Balanites Aegyptiaca among Strep- tozotocin Induced Diabetic Rats. International Journal of Pharmaceutical Research & Allied Sciences, 8, 53-58.
- **Abera B (2014)** Medicinal plants used in traditional medicine by Oromo people, Ghimbi District**, Southwest Ethiopia. J Ethnobiol Ethnomed 10(1):**1–15.
- **Abualreish, M.J.A. and Abdein, M.A. (2014).** The Analytical Applications and Biological Activity of Hydroxamic Acids.Journal of Advances in Chemistry, 10, 2118- 2125.
- **Adebayo, O.C., Ram, V., and Vasudeva., S. (2008).** Peroxidase activity, chlorophylls and antioxidant profile of two leaf vegetables (*Solanum nigrum* L. and *Amaranthus cruentus* L.) under six pretreatment methods before cooking. Int. J. Food Sci. Technol.; 43(1): 173-178.
- **Adebiyi, O.E., Olayemi, F.O, Ning-Hua, T., Guang-Zhi, Z.B. (2017).** Sciences A. In vitro antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of Grewia carpinifolia. Beni-Suef Univ J Basic Appl. Sci. 2; 6(1):10–4.

- **Ahluwalia, V., Sisodia, R., Walia, S., Sati, O. P., Kumar, J., & Kundu, A. (2013).**Chemical analysis of essential oils of *Eupatorium adenophorum* and their antimicrobial, antioxidant and phytotoxic properties. Journal of Pest Science, 87(2), 341–349.
- **Ahmed, D., Khan, M. M., & Saeed, R. (2021).** Comparative evaluation of phenolic content and antioxidant activity of medicinal plants using different solvents. ***Journal of Herbal Medicine***, 28, 100451.
- **Ahmed, M.F., Kazim, S.M., Ghori, S.S., Mehjabeen, S.S., Ahmed, S.R., Ali, S.M., Ibrahim, M., (2010**). Antidiabetic Activity of Vinca rosea Extracts in Alloxan-Induced Diabetic Rats. Int J Endocrinol; 2010:841090.
- **Alam, M.K., Rana, Z.H., Islam, S.N., Akhtaruzzaman, M.J.P. (2019).** Total phenolic content and antioxidant activity of methanolic extract of selected wild leafy vegetables grown in Bangladesh: A cheapest source of antioxidants. Potravinarstvo; 13:1.
- **Allah, K., Rehab, M., Yousef, M., et al. (2019).** Utilization of Black Berry Juice to Reduce the Oxidative Stress in Rats Treated with Acrylamide. Asian Journal of Biological Sciences,12, 9-16.
- **Allamsetty, J., Pedada, S.P., Pedada, N., Dhanunjayarao, K. (2020).** A basic review on *Vinca rosea*. *Int J Pharmacogn Chem.*; 1(1):31-6.
- **Almagro, L., Fernandez Perez, F., Pedreno, M.A. (2015).** Indole alkaloids from *Catharanthus roseus*: bioproduction and their effect on human health. *Molecules;* 20(2):2973-3000.
- **Al-Omar, M.S., Mohammed, H.A., Mohammed, S.A.A., et al. (2020).** Anti-Microbial, Anti-Oxidant, and α-Amylase Inhibitory Activity of Traditionally-Used Medicinal Herbs: A Comparative Analyses of Pharmacology, and Phyto constituents of Regional Halophytic Plants' Diaspora. Molecules, 25,5457.
- **Al-Qahtani, H., Alfarhan, A.H. and Al-Othman, Z.M. (2020).** Changes in Chemical Composition of Zilla spinosa Forssk. Medicinal Plants Grown in Saudi Arabia in Response to Spatial and Seasonal Variations. Saudi Journal of Biological Sciences,27, 2756-2769.
- **Alqethami, A., Hawkins, J.A. and Teixidor-Toneu, I. (2017).** Medicinal Plants Used by Women in Mecca: Urban, Muslim and Gendered Knowledge. Journal of Ethnobiology and Ethnomedicine, 13, 62.

- **Al-Qirim, T., Zaidi, S.M., Shahwan, M., Shattat, G., Banu, N.(2008).** Effect of *Solanum nigrum* on immobilization stress induced antioxidant defense changes in rat. Res. Biol. Sci., 3, 1426–1429.
- **AlSalhi, M.S.; Elangovan, K.; Ranjitsingh, A.J.A.; Murali, P.; Devanesan, S.** Synthesis of silver nanoparticles using plant derived 4-N-methyl benzoic acid and evaluation of antimicrobial, antioxidant and antitumor activity. Saudi J. Biol. Sci. **2019**, 26, 970–978.
- **Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2020).** Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. ***Plants***, 9(12), 1621.
- **Anywar, G. (2020).** Historical Use of Toxic Plants. In: Mtewa, A.G., et al., Eds., Poisonous Plants and Phytochemicals in Drug Discovery, Wiley, Hoboken, 1-17.
- **Aouadi, A., Saoud, D.H., Rebiai, A., ABD, F.M., Hadjadj, S., Mustafa, A.A. (2024).** Impact of different extraction solvents and concentrations on the total phenolics content and bioactivity of the Algerian lemongrass (*Cymbopogon citratus)* extracts. Ovidius Univ. Ann Chem.; 35:16–26.
- **Arulvendhan, V., Saravana, B. P. and Rajaganesh, R. (2024).** Molecular identification and phytochemical analysis and bioactivity assessment of *Catharanthus roseus* leaf extract: Exploring antioxidant potential and antimicrobial activities, *Appl Biochem Biotechnol*,, 1-28.
- **Asema, U.K. Syeda, Q. B. (2014).** "Seasonal Variation in Glycine Content of Two Medicinal Plants," Int J Of Sci & Tech, vol. 2(12), pp. 219 – 221.
- **Ashrafudoulla,M., Bellah,S.F., Alam,F., Faisal,S.S., Abdullah, M., Fuad,H.K.F., (2016).** Phytochemical screening of *Solanum nigrum* L, S. myriacanthus Dunal, Solanum melongena and Averrhoa bilimbi in Bangladesh, Journal of Medicinal Plants Studies Journal of Medicinal Plants Studies; 4(1): 35-38.
- **Augusti, K.T.,(1996).** Therapeutic values of onion and garlic, Indian J Exp Biol.; 34(3):634-640.
- **Augusti, K.T.,(1996).** Therapeutic values of onion and garlic, Indian J Exp Biol.; 34(3):634-640

- **Ayyanar, M. (2013).** Traditional Herbal Medicines for Primary Healthcare among Indigenous People in Tamil Nadu, India. Journal of Homeopathy & Ayurvedic Medicine, 2(5).
- **Baba, S.A., Malik, S.A. (2015).** Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J Taibah Univ Sci.; 9(4):449–54.
- **Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008).** Biological effects of essential oils—A Review. Food Chem. Toxicol., 46,446–475
- **Barah, F. (2013).** Non-Antibiotic Biocides: An Updated Review. Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education; Méndez-Vilas, A.,Ed.; Formatex Research Centre: Badajoz, Spain, 2013; Volume 1, pp. 598–607.
- **Barnett, C.J., Cullinan, G.J., Gerzon, K., Hoying, R.C., Jones, W.E., Newlon, W.M., Poore, G.A., Robinson, R.L., Sweeney, M.J., Todd, G.C., Dyke, R.W., Nelson, R.L. (1978).** Structure-activity relation-ships of dimeric *Catharanthus* alkaloids.1.Deacetylvinblastine amide (vindesine) sulfate. JMed Chem 21: 88.
- **Barron, D., Di Pietro, A., Dumontet, C., McIntosh, D.B., (2002).** Isoprenoid flavonoids are new leads in the modulation of chemoresistance. Phytochem Rev.1:325–32.
- **Betoni, J.E., Mantovani, R.P., Barbosa, L.N., Di Stasi, L.C., Fernandes Junior, A. (2006).** Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Mem. Inst. Oswaldo Cruz., 101, 387–390.
- **Bhambhani, S., Kirtikumar, R., Kond Hare, Ashok, P. (2021).** Diversity in chemical structures and biological properties of plant alkaloids. *Molecules.* 2021; 26(11):3374.
- **Bhandari, P.R. (2012)** "Curry leaf (Murraya Koenigii) or Cure leaf: Review of its curative properties," J Med Nutraceut, vol. 1(9), pp. 2 – 7.
- **Bhattacharyya, A., Chattopadhyay, R., Mitra, S., Crowe, S.E. (2014).** Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev.; 94(2):329–54.
- **Bhuyan, D. J., Vuong, Q. V., Bond, D. R., & Chalmers, A. C. (2022).** Phenolic compounds and antioxidant activity of medicinal plant extracts: Influence of plant parts and solvents. ***Antioxidants***, **11(5), 987.**

- **Bodart P and Noirfalise A: Glycoalkaloids in potatoes. Rev Med Liege 2003**; 58: 25-32.
- **Campisi, A., Acquaviva, R., Raciti, G., Duro, A., Rizzo, M., Santagati, N.A.(2019).** Antioxidant Activities of *Solanum nigrum* L. Leaf Extracts Determined in In Vitro Cellular Models. Foods, 8, 63.
- **Chagas, C.M.; Alisaraie, L.** Metabolites of Vinca alkaloid vinblastine: Tubulin binding and activation of nausea-associated receptors. ACS Omega **2019**, 4, 9784–9799.
- **Chandrashekhar, R., Pagadala, J.C., Bhavani, N.L. (2021).** Vinca alkaloids from Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don): an overview. *Everymans Sci.*; LV (5):187-92.
- **Chaturvedi, V., S. Goyal, M. Mukim, M. Meghani, F. Patwekar, M. Patwekar, S.K. Khan and G.N. Sharma. 2022.** A comprehensive review on *Catharanthus roseus* L. (G.) Don: clinical pharmacology, ethnopharmacology and phytochemistry. J. Pharmacol. Res. Dev. 4:17-36.
- **Chaudhary, K., Parihar, S., Sharma, D. (2021).** A Critical Review on Nanoscience Advancement: In Treatment of Viral Infection. Journal of Drug Delivery and Therapeutics. 2021 Nov; 11(6):225-237.
- **China Pharmacopoeia Committee (2020).** Pharmacopoeia of the people's Republic of China. Vol I. 11. Beijing: China Chemical Industry Press.
- **Cowan, M.M. (1999).** Plant products as antimicrobial agents. Clin. Microbial. Rev.; 12: 564–582
- **D'Costa, V.M.; King, C.E.; Kalan, L.; Morar, M.; Sung, W.W.L.; Schwarz, C.; Froese, D.; Zazula, G.; Calmels, F.; Debruyne, R.; *et al.*** Antibiotic resistance is ancient. Nature **2011**, 477, 457–461.
- **Dai, G., Shi, H., McBride, M.B., Fu, H., Li, Z., Wang, and X., *et al.*, (2024).** Biogeochemical processes in heterogeneous soil-*Solanum nigrum* L. system control lead partitioning: Roles of strengite and oxalated zero-valent iron nanoparticle. J Clean Prod.; 450:141993.
- **Das, S, Sharangi, A.B. (2017).** Madagascar periwinkle (*Catharanthus roseus* L.): diverse medicinal and therapeutic benefits to humankind. *J Pharmacogn Phytochem.*; 6(5):1695-701.
- **Deepika, M.S.; Thangam,R.;Vijayakumar, T.S.;Sasirekha,R.;Vimala, R.T.V.; Sivasubramanian,S.;Arun,S.Babu, M.D.; Thirumurugan, R.**

Antibacterial synergy between rutin and florfenicol enhances therapeutic spectrum against drug resistant Aeromonas hydrophila. Microb. Pathogen. **2019**, 135.

- **Dhellot, J.R.; Matouba, E.; Maloumbi, M.G.; Nzikou, J.M.; Dzondo, M.G.; Nzikou, J.M.; Dzondo, M.G.; Linder, M.; Parmentier, M.; Desobry, S.(2006).** Extraction and nutritional properties of *Solanum nigrum* L. seed oil. Afr. J. Biotechnol.; 5, 987–991.
- **Djeussi, D.E., Noumedem, J.A., Seukep, J.A., Fan,kam, A.G., Voukeng, I.K., Tankeo, S.B., Nkuete, A.H., Kuete, V. (2013).** Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complement. Altern. Med., 13, 164.
- **Dubey, A., Ghosh, N.S., Singh, R. (2022).** Zebrafish as An Emerging Model: An Important Testing Platform for Biomedical Science. J Pharm Negative Results; 13(3): 1-7.
- **Dubey, A., Kushwaha, R. (2020).** A discussion on vinca plant. *Journal of Pharmacognosy and Phytochemistry;* 9 (5):27-31.
- **Dukes, J.A. (1985).** CRC Handbook of Medicinal Herbs, 1st ed.; CRC Press, Inc.: Boca Raton, FL, USA.
- **Duraipandiyan, V., Ayyanar, M., Ignacimuthu, S., (2006).** Antimicrobial Activity of Some Ethnomedical Plants used by Paliyar Tribe from Tamil Nadu, India. BMC Complementary and alternative medicine, 365.
- **Edeoga H.O., Okwu, D.E., Mbaebie, B.O., (2005).** Phytochemical constituents of some Nigerian medicinal plants, African Journal of Biotechnology; 4(7):685-688.
- **Edmonds, J.M., Chweya, J.A. (1997).** Black Nightshades—*Solanum nigrum* L. and Related Species; International Plant Genetics Research Institute: Rome, Italy; ISBN 10-9290433213.
- **Egbuna, C., Kumar, S., Ifemeje, J.C., et al. (2019).** Phytochemicals as Lead Compounds for New Drug Discovery. Elsevier,Amsterdam.
- **Ekor, M., (2014).** The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol.; 4:177.
- **El-Refai, A.A., Sanad, M.I., Ramdan, A.-H.M. and Hikal, D.M. (2010).** Antimicrobial Activity of Natural Anthocyanins and Carotenoids Extracted from Some Plants and Wastes. Journal of Food and Dairy Science, 1, 413-427.

- **Elujoba AA, Ajulo OO, Iwabo GO (1989).** Chemical and Biological analysis of Nigeria Cassia species for laxative activity .J. Pharm. Biomed. Anal. 12: 1453-1457.
- **Emmanuela, N., Muhammad, D.R., Iriawati, Wijaya, C.H., Ratnadewi, Y.M.D., Takemori, H., *et al.*, (2024).** Isolation of plant-derived exosome-like nanoparticles (PDENs) from *Solanum nigrum* L. berries and Their Effect on interleukin-6 expression as a potential anti- inflammatory agent. PLoS One.; 19(1):e0296259.
- **Evans, WC. and Trease, GE.(1989).** Pharmacognosy. 11th ed. BraillIar Tiridel Can. Macmilliann Publishers. .
- **Ferreres, F., Pereira, D.M., Valentao, P., Andrade, P.B., Seabra, R.M., Sottomayor, M. (2008).** New phenolic compounds and antioxidant potential of *Catharanthus roseus. J Agric Food Chem.*; 56(21):9967-74.
- **Friedman M:** Potato glycoalkaloids and metabolites: Roles in the plant and in the diet. J Agric Food Chem 2006; 54:8655-8681.
- **Gabrani, R., Jain, R., Sharma, A., Sarethy, I.P., Dang, S., Gupta, S.(2012).** Antiproliferative Effect of *Solanum nigrum* on Human LeukemicCell Lines. Indian J. Pharm. Sci., 74, 451–453.
- **Gahlawat, D. K., Jakhar, S., & Dahiya, P. (2014).** *Murraya koenigii* (L.) Spreng: An ethnobotanical, phytochemical and pharmacological review. Journal of Pharmacognosy and Phytochemistry, 3(3), 109-119.
- **Ganeshpurkar, A.;** Saluja, A.K. The pharmacological potential of rutin. Saudi Pharm. J. **2017**, 25, 149–164.
- **Gaonkar, V.P., Hullatti, K. (2020).** Indian Traditional medicinal plants as a source of potent Anti-diabetic agents: A Review. *J. Diabetes Metab. Disord.19*, 1895–1908
- **Gautam, M. K., Gangwar, M., Nath, G., Rao, C. V., and Goel, R. K. (2012a).** *In vitro* antibacterial activity on human pathogens and total phenolic, flavonoid contents of *Murraya paniculata* Linn. Leaves. Asian pac. J. Trop. bio. 2, S1660–S1663.
- **Gębarowska, E.; Łyczko, J.; Rdzanek, M.; Wiatrak, B.; Pląskowska, E.; Gołębiowska, H.; Kuźniarski, A.; Gębarowski, T.** Evaluation of Antimicrobial and Chemopreventive Properties and Phytochemical

Analysis of Solanum nigrum L. Aerial Parts and Root Extracts. Appl. Sci. **2022**, 12, 6845. https://doi.org/10.3390/ app12146845

- **Ghasemzadeh, A., Jaafar, H., Rahmat, A., (2011).** Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (Zingiber officinale roscoe) extracts. J Med Plants Res. 5(7):1147–54.
- **Ghasemzadeh, A., Jaafar, H.Z., Rahmat, A. and Devarajan, T. (2014).** Evaluation of bioactive compounds, pharmaceutical quality, and anticancer activity of curry leaf (*Murraya koenigii* L.). Evidence Based Complement Alternative Medicine.;
- **Ghosh, D., Sarkar, S., & Chatterjee, S. (2023).** Phenolic profiling and antioxidant properties of ***Murraya koenigii*** and related medicinal plants. ***South African Journal of Botany***, 155, 310–318.
- **Glossman-Mitnik D**: CHIH-DFT determination of the molecular structure
- **Godos, J.; Castellano, S.; Ray, S.; Grosso, G.; Galvano, F. (2018).** Dietary polyphenol intake and depression: Results from the Mediterranean healthy eating, lifestyle and aging (MEAL) study. Molecules, 23, 999.
- **Górniak, I., Bartoszewski, R., Króliczewski, J.(2019).** Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem.Rev., 18, 241–272.
- **Goswami, S., A. Ali, M.E. Prasad and P. Singh. 2024.** Pharmacological significance of *Catharanthus roseus* in cancer management: a review. Pharmacol. Res. Mod. Chin. Med. 11, 100444.
- **Grosso, G.; Micek, A.; Godos, J.; Pajak, A.; Sciacca, S.; Galvano, F.; Giovannucci, E.L.(2017).** Dietary flavonoid and lignan intake and mortality in prospective cohort studies: Systematic review and dose-response meta-analysis. Am. J. Epidemiol., 185, 1304–1316.
- **Guang, C.G., Yun Z.H., Lu, L. , Li Z.Y., Wei SC, Ji, G.U., Bang, S.W., Ping, L.Y., Dong, L.X.,(2016).** Non- alkaloid constituents of Vinca major, CHENG Gui-Guang ; 14(1):56-60.
- **Guo, T., He, D., Liu, Y., Li, J., Wang, F. (2024).** Lanthanum promotes *Solanum nigrum* L. growth and phytoremediation of cadmium and lead through endocytosis: Physiological and biochemical response, heavy metal uptake and visualization. Sci Total Environ. 912:168915.

- **Gurib-Fakim, A. (2006).** Medicinal Plants: Traditions of Yesterday and Drugs of Tomorrow. Molecular Aspects of Medicine, 27, 1-93.
- **Hall, R.D., Yeoman, M.M. (1986).** Factors determining anthocyanin yield in cell cultures of *Catharanthus roseus* (L.) G. Don. *New Phytol. 103*:33–43.
- **Hamilton, A. C. (2004).** Medicinal plants, conservation and livelihoods. Biodiversity and Conservation, 13(8), 1477–1517.
- **Hamilton, V. (2018).** The Encyclopaedia of Herbs and Spices. Reference Reviews, 32(7/8), 31.
- **Hashim, M., H. Arif, B. Tabassum, S. Rehman, P. Bajaj, R. Sirohi and M.F.A. Khan. 2024**. An overview of the ameliorative efficacy of *Catharanthus roseus* extract against Cd^{2+} toxicity: implications for human health and remediation strategies. Front. Public Health 12, 1327611.
- **Heo KS, Lee S and Lim K T :** Cytotoxic effect of glycoprotein isolated from *Solanum nigrum* L. through the inhibition of hydroxyl radicalinduced DNA-binding activities of NF-kappa B in HT-29 cells. Environ. Toxicol. Pharmacol. **2004**; 17(1): 45-54.
- **Heo KS, Lee SJ, Ko JH, Lim K and Lim KT:** Glycoprotein isolated from *Solanum nigrum* L. Inhibits the DNA- binding activities of NFB and AP-1, and increases the production of nitric oxide in TPAstimulated MCF-7 cells. Toxicol. *In Vitro* 2004; 18(6): 755-763.
- **Hossain, M.M., Mondal, M., Hossain, M.P., Uddin, M.S., Uddin, N., Hasan, M.M., Hossain, M.S., Islam, A.F.M.M., Rana, M.S.,(2015).** In vitro antioxidant, brine shrimp lethality and antimicrobial activities of methanol and petroleum ether extract of Cassia renigera fruit. Journal of Food Measurement and Characterization. 9(1):68–75.
- **Ibrahim, M., Mehjabeen, S.M. & Lakshmi, M. (2011).** *International of Pharmaceutical Applications. 2,* 165-173.
- **Imran, M.; Rauf, A.; Shah, Z.A.; Saeed, F.; Imran, A.; Arshad, M.U.; Ahmad, B.; Bawazeer, S.; Atif, M.; Peters, D.G.; et al.(2019).** Chemo preventive and therapeutic effect of the dietary flavonoid kaempferol: A comprehensive review. Phytother. Res., 33, 263–275.

- **Iwalokun, B.A., Usen, U.A., Otunba, A.A. and Olukoya, D.K. (2007).** Comparative phytochemical evaluation, antimicrobial and antioxidant properties of Pleurotus ostreatus. Afr. J. Biotechnol, 6(15): 1732-1739.
- **Iyenger, M.H, (1995),** "Study of ocrude drug", 8:2,
- **Jamal, Q. M. S. and Ahmad, V. (2024).** Identification of metabolites from *Catharanthus roseus* leaves and stem extract, and *In vitro* and *In silico* antibacterial activity against food pathogens, *J Pharm*, **17**(4), 450,
- **Jamshidi-Kia, F., Lorigooini, Z. and Amini-Khoei, H. (2018).** Medicinal Plants: Past History and Future Perspective. Journal of Herb med Pharmacology, 7, 1-7.
- **Jayaprakash, G.K., Singh, R.P.and Sakariah, K.K. (2001)** J.Agric Food Chem., 55: 1018-1022.
- **Jeeva, S., Anlin Sheebha, Y. (2014).** A review of antidiabetic potential of ethnomedicinal plants. *Med. Aromat. Plants*. *3*, 1–8.
- **Jhanji, R.; Bhati, V.; Singh, A.; Kumar, A**. Phytomolecules against bacterial biofilm and efflux pump: An in silico and in vitro study. J. Biomol. Struct. Dyn. **2019**.
- **Ji YB: Antitumor effects in traditional Chinese medicine.** In:Pharmacological Action, Application of Available Composition of Traditional Chinese Medicine. 1st edition. Heilongjiang Science and Technology Press, Harbin, Heilongjiang 1995; 433.
- **Jian L, Qing-Wang L, Da-Wei G, Zeng-Sheng H and Wen- Zong L**: Antitumor and immunomodulating effects of polysaccharides isolated from *Solanum nigrum* Linne. Phytother. Res 2009; 23(11): 1524-1530.
- **Jithesh, M.N., Prashanth, S.R., Sivaprakash, K.R. and Parida, A.K. (2006).** Antioxidative Response Mechanisms in Halophytes: Their Role in Stress Defence. Journal of Genetics, 85, 237.
- **Jyoti, R., Naveen,G., (2016)**"Phytochemical Analysis and Antibacterial Activity of Different Leaf Extracts of Murraya koenigii," Int. J. of Biochem and Biomol, vol. 2(2), pp. 1-5.
- **Kameswrarao, B., Giri, R., Kesavalu, M. M. and Apparao, Ch., (1997).** "Herbal medicine," in The Management by Indigenous Resources**,** ***J. S.***

***Bajaj, Ed.*, 1997;375–377,** Diabetes Mellitus in Developing Countries. Interprint., New Delhi, India. 25.

- **Karthikeyan A, Shanthi V, Nagasathaya A.** Preliminary phytochemical and antibacterial screening of crude extract of the leaf of Adhatoda Vasica L. Int J Green Pharm.2009; 3:78-80.
- **Karthikeyan, A., Shanthi, V., Nagasathaya, A. (2009).** Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda Vasica* L. Int J Green Pharm.; 3:78-80.
- **Karthikumar,S., Vigneswari, K Jegatheesan, K., (2007).** Screening of antibacterial and antioxidant activities of leaves Eclipta prostrate (L) Sci Res Essay; 2(4):101-104.
- **Karunamoorthi, K., Jegajeevanram, K., Vijayalakshmi, J. and Mengistie, E. (2013).** Traditional Medicinal Plants: A Source of Phytotherapeutic Modality in Resource- Constrained Health Care Settings. Evidence-Based Complementary and Alternative Medicine, 18, 67-74.
- **Kaur, C., Kapoor, H.C.(2002).** Antioxidant activity and total phenolic content of some asian vegetables. Int J Food Sci Tech.37:153–62.
- **Kaur, D., Kaur, A., and Arora, S. (2016).** Delineation of attenuation of oxidative stress and mutagenic stress by *Murraya exotica* L. leaves. Springerplus 5, 1037.
- **Khan, A., K. Maparu and K.R. Aran. 2024.** *Catharanthus roseus*: a comprehensive review of its phytochemicals, therapeutic potential, and mechanisms of action. Nat. Cell Sci. 2: 224-237
- **Khan, A.A., Manzoor, K.N., Sultan, A., Saeed, M., Rafique, M., Noushad, S.,Talib, A., Rentschler, S., Deigner, H.P.(2021).** Pulling the Brakes on Fast and Furious Multiple Drug-Resistant (MDR) Bacteria. Int. J. Mol. Sci., 22, 859.
- **Khatun, A., Rahman, M., and Jahan, S. (2014).** Preliminary phytochemical, cytotoxic, and thrombolytic and antioxidant activities of the methanol extract of *Murraya exotica* Linn. leaves. Orient. Pharm. Exp. Med. 14, 223–229.
- **Kikuchi, H.; Yuan, B.; Hu, X.; Okazaki, M.(2019).** Chemo preventive and anticancer activity of flavonoids and its possibility for clinical use by combining with conventional chemotherapeutic agents. Am. J. Cancer Res., 9, 1517–1535.

- **Kinoshita, T., and Firman, K. (1996a**). Prenylcoumarin derivatives from the leaves of an Indonesian medicinal plant *murraya paniculata*(rutaceae). Chem. Pharm. Bull. 44, 1261–1262.
- **Kodamatani H, Saito K, Niina N, Yamazaki S and Tanaka Y:** Simple and sensitive method for determination of glycoalkaloids in potato tubers by high-performance liquid chromatography with chemiluminescence detection. J Chromatogr A 2005; 1100: 26-31.
- **Korpan YI, Nazarenko EA, Skryshevskaya IV, Martelet C,Jaffrezic-Renault N and El'skaya AV:** Potato glycol- alkaloids: True safety or false sense of security? Trends Biotechnol 2004; 22: 147-151.
- **Krishnaiah D, Sarbatly R, Bono A (2007)** Phytochemical antioxidants for health and medicine a move towards nature. Biotechnol Mol Biol Rev 2(4):97–104.
- **Krishnaiah, D., Sarbatly, R., Nithyanandam, R.J.F.(2011).** Processing b. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process.; 89(3):217–33.
- **Kumar, G., Kumar, R., Gautam, G.K., Rana, H. (2021).** The Phytochemical and Pharmacological Properties of *Catharanthus roseus (Vinca*), 4, Page No.: 429-434.
- **Kumar, A., Gupta, R., Mishra, R.K., Shukla, A.C., Dikshit, A., (2012).** Pharmaco-phylogenetic investigation of *Micromeria biflora* Benth and *Citrus reticulata* Blanco. Nat Acad Sci Lett. 35(4):253–7.
- **Kumar, S., Mittal, A., Babu, D. and Mittal, A. (2021).** Herbal Medicines for Diabetes Management and its Secondary Complications. Curr Diabetes Rev; 17(4): 437-456.
- **Kumar, S., Pandey, A. K., & Singh, R. (2020).** Antioxidant, antibacterial and phenolic content of medicinal plants. ***Biocatalysis and Agricultural Biotechnology***, 27, 101685.
- **Kumar, N.S., Simon, N.,(2016)** "Invitro Antimicrobial Activity and Phytochemical Analysis of *Murraya Koenigii* (L) Leaf Extracts," Global J of Sci Frontier R Biol Sci, vol. 16(1), pp. 28 - 32.
- **Lee, S.J. Lim, K.T. (2006).** 150 kDa glycoprotein isolated from *Solanum nigrum* Linne stimulates caspase-3 activation and reduces inducible nitric oxide production in HCT-116 cells. Toxicol. *in vitro*; 20(7): 1088-1097.

- **Levêque, D. & Jehl, F. (2007).** "Molecular pharmacokinetics of *Catharanthus* (Vinca) alkaloids," The *Journal of Clinical Pharmacology*. 47(*5*): 579–588.
- **Liu, B. Y., Zhang, C., Zeng, K. W., Li, J., Guo, X. Y., Zhao, M. B., *et al*. (2015b).** Exotines A and B, two heterodimers of isopentenyl-substituted indole and coumarin derivatives from *Murraya exotica*. Org. Lett. 17, 4380–4383.
- **Liu, B. Y., Zhang, C., Zeng, K. W., Li, J., Guo, X. Y., Zhao, M. B., *et al*. (2018).** Anti- inflammatory prenylated phenylpropenols and coumarin derivatives from *Murraya exotica*. J. Nat. Prod. 81, 22–33.
- **Liu, B., and Jiang, Y. (2015a).** Simultaneous determination of three main analytes of *Murraya exotica* by HPLC. J. Chin. Pharm. Sci. 24, 88–94. **Lu, M., Du, Z., Yuan, S., Ma, Q., Han, Z., Tu, P., et al. (2021b).** Comparison of the preventive effects of *Murraya exotica* and *Murraya paniculata* on alcohol-induced gastric lesions by pharmacodynamics and metabolomics. J. Ethnopharmacol. 281, 114567.
- **Lu, M., Liang, H., Tu, P., and Jiang, Y. (2021a).** Pharmacodynamic comparison of two different source plants of Murrayae Folium et Cacumen. J. Chin. Pharm. Sci. 30, 49–57.
- **Maillard, J. (2018).** Resistance of Bacteria to Biocides. Microbiol. Spectrum. 6, 19.
- **Malar, Retna, A., Ethalsha, P. (2013).** A review of the taxonomy ethnobotany chemistry and pharmacology of *Catharanthus roseus* (Apocyanaceae). *Int J Eng Res Technol.*; 2(10):3899-912.
- **Malhotra, M., H. Rana and S. Tandon. 2024**. Exploring the therapeutic potential of *Catharanthus roseus*: unveiling its diverse phytochemicals and mechanisms of action for chronic and infectious diseases. Int. J. Curr. Pharm. Res.16: 1-8.
- **Maruca, A., Catalano, R.., Bagetta, D., Mesiti, F., Ambrosio, F.A., Romeo, I., *et al*., (2019).** The mediterranean diet as source of bioactive compounds with multi-targeting anti-cancer profile. Eur. J Med Chem.; 181:111579.
- **Mazandarani, M., Zarghami, P., Zolfaghari, M., Ghaemi, E., Bayat, H., (2012**). Effects of solvent type on phenolics and flavonoids content and antioxidant activities in *Onosma dichroanthum* Boiss. J Med Plants Res.6(28):4481–8.

- **Meira, C.S.; Guimarães, E.T.; dos Santos, J.A.F.; Moreira, D.R.M.; Nogueira, R.C.; Tomassini, T.C.B.; Ribeiro, I.M.; de Souza, C.V.C.; dos Santos, R.R.; Soares, M.B.P.** In vitro and in vivo antiparasitic activity of Physalis angulata L. concentrated ethanolic extract against *Trypanosoma cruzi*. Phytomedicine **2015**, 22, 969–974.
- **Mendonce, K.C., N. Palani, S. Rajadesingu, K. Radhakrishnan, M. Ayyar and L.S. Priya. 2025.** Pharmacological potential of bioactive compounds in *Catharanthus roseus* extract: a comprehensive review. Toxicol. Rep. 14, 101998.
- **Mendoza, N. and Escamilla, E. (2018).** Introduction to Phytochemicals: Secondary Metabolites from Plants with Active Principles for Pharmacological Importance. In: Asao, T. and Asaduzzaman, M., Eds., Phytochemicals: Source of Antioxidants and Role in Disease Prevention, IntechOpen, London, 25-47.
- **Menezes, C. D. A., de Oliveira Garcia, F. A., de Barros Viana, S., Pinheiro, P. G., Felipe, C. F. B., de Albuquerque, T. R., et al. (2017).** *Murraya paniculata* (L.) (Orange jasmine): potential nutraceuticals with ameliorative effect in alloxan-induced diabetic rats. Phytother. Res. 31, 1747–1756.
- **Merwid-Lad, A.; Ksia¸dzyna, D.; Hałon´ , A.; Szkudlarek, D.; Trocha, M.; Szandruk-Bender, M.; Matuszewska, A.; Nowak, B.; Sozan´ ski, T.; Kuz´niar, A.; et al.** Morin-5'-Sulfonic Acid Sodium Salt (NaMSA) Attenuates Cyclophosphamide-Induced Histological Changes in Genitourinary Tract in Rats-Short Report. Pharmaceuticals **2021**, 14, 192.
- **Mirzaei, H.; Naseri, G.; Rezaee, R.; Mohammadi, M.; Banikazemi, Z.; Mirzaei, H.R.; Salehi, H.; Peyvandi, M.; Pawelek, J.M.; Sahebkar, *A. Curcumin*:** A new candidate for melanoma therapy? Int. J. Can. **2016**, 139, 1683-1695.
- **Misganaw Tegegne, Enkuahone Abiyu, Solomon Libesu, Belete Bedemo & Mekuanint Lewoyehu (2021)** Phytochemical investigation, antioxidant and antibacterial activities of the fruit extracts of *Solanum anguivi* , Biotechnology & Biotechnological Equipment, 35:1, 1480-1491,
- **Mishra, S.K. and Shankar, R. (2019).** Diversity and Conservation Strategies of Pharmaceutically important Medicinal plants of Bundelkhand region, India. American Journal of Pharmatech Research.; 9(04): 35-48.

- **Moga, M.A., Dimienescu, O.G., Arvatescu, C.A., Mironescu, A., Dracea, L., Ples, L.(2016).** The Role of Natural Polyphenols in thePrevention and Treatment of Cervical Cancer—an Overview. Molecules, 21, 1055.
- **Mohy-ud-dint, A., Khan, Z., Ahmad, M., Kashmiri, M.A.(2010).** Chemotaxonomic value of alkaloids in Solanum *nigrum* complex.Pak. J. Bot., 42, 653–660.
- **Mojab, F., Kamalinijad, M., Ghaderi, N., Vahidipour, H., (2003)** Phytochemical Screening of Some Iranian Plants, Iranian Journal of Pharmaceutical Research. 77-82.Mol Biomol Spectrosc 2007; 66: 208-211.
- **Molly, J., Edison, S., Vijajaraghavan, R..,(2017)** "Effect of Murraya koennigii (Curry Leaves) powder on the liver and renal functions in women with hyperlipidemia," Int J Health Sci R, vol. 7(1), pp.188-192.
- **Mulatu G (2020)** Antibacterial activities of *Calpurnia aurea* against selected animal pathogenic bacterial strains. Adv Pharmacol Pharm Sci.
- **Muto M, Mulabagal V, Huang HC, Takahashi H, Tsay HS and Huang JW:** Toxicity of black nightshade (*Solanum nigrum*) extracts on (*Alternaria brassicicola*, causal agent of black leaf spot of Chinese cabbage (*Brassica pekinensis*). J. Phytopathol. 2006; 154 (1): 45-50.
- **Naseem U, Muhammad Z, Farhat Ali K, Shazeb K (2014)** A review on general introduction to medicinal plants, its phytochemicals and role of heavy metal and inorganic constituents. Life Sci 11(7s):520–527
- **Nawaz, A., Jamal, A., Arif, A., Parveen, Z. (2021).** In vitro cytotoxic potential of *Solanum nigrum* against human cancer cell lines. Saudi. J.Biol. Sci., 28, 4786–4792.
- **Negi, N., Abou-Dough, A. M., Kurosawa, M., Kitaji, Y., Saito, K., Ochi, A., et al. (2015).** Coumarins from Murraya exotica collected in Egypt. Nat. Prod. Commun. 10,1934578X1501000–620.
- **Ng, M., Abdulhadi Noaman, Y., Cheah, Y., Yeap, S., and Alitheen, N. (2012).** Bioactivity studies and chemical constituents of *Murraya paniculata* (Linn) Jack. Int. Food Res. J. 19, 1307–1312.
- **Osman, A.K. and Abdein, M.A. (2019).** Karyological and Molecular Studies between Six Species of Plantago in the Northern Border Region at Saudi Arabia. Journal of Taibah University for Science, 13, 297-308.

- **Osman, A.K.E. and Abdein, M.A. (2019).** Floristic Diversity of Wadi Ar'ar, Saudi Arabia. Journal of Taibah University for Science, 13, 772-789.
- **Panda, S. K., Das, R., Lavigne, R., and Luyten, W. (2020).** Indian medicinal plant extracts to control multidrug-resistant *S. aureus*, including in biofilms. S. Afr. J. Bot. 128,283–291.
- **Pandey, B.P., Thapa, R., Upreti, A., (2017).** Chemical composition, antioxidant and antibacterial activities of essential oil and methanol extract of Artemisia vulgaris and Gaultheria fragrantissima collected from Nepal. Asian Pac J Trop Med.;10(10):952–9.
- **Parekh, J., Chanda, V., (2007).** In vitro antimicrobial activity and phytochemical analysis of some indian medicinal plants. Turkish J Biol. 31:53–8.
- **Parihar, S., Sharma, D. (2021).** Cynodondactylon: A Review of Pharmacological Activities. Sch Acad J Pharm.; 11:183-189.
- **Parihar, S., Sharma, D. (2021).** Navagraha (nine planets) plants: the traditional uses and the therapeutic potential of nine sacred plants of india that symbolises nine planets. IJRAR; 8(4):96-108.
- **Park, Y.K., Koo, M.H., Ikegaki, M. and Contado, J.L.; (1997).** Arquivos de Biologiae Techno. 40 (1): 97-106.
- **Partridge, S.R., Kwong, S.M., Firth, N., Jensen, S.O. (2018).** Mobile genetic elements associated with antimicrobial resistance. Clin.Microbiol. Rev., 31, e00088-17.
- **Pârvu, M.; Vlase, L.; Fodorpataki, L.; Pârvu, O.; Bartha, C.; Roş ca-Casian, O.; Barbu-Tudoran, L.; Pârvu, A.E.(2013).** Chemical composition of celandine (*Chelidonium majus* L.) extract and its effects on Botrytis tulipae (Lib.) Lind fungus and the tulip. Not. Bot. Hort. Agrobot. 41, 414–426.
- **Pate, S., Dube,y A., Gupta, A. k., Ghosh, N.S., (2023).** Evaluation of Antimicrobial Activity of *Calotropis gigantea* extracts on Two Main Skin Infection Causing Bacteria - *Escherichia Coli* and *Staphylococcus aureus*. 12(1):145-157.
- **Patterson,J., Verghese, M.,(2015).** "Anticancer and Toxic Effects of Curry Leaf (Murraya koenigii) Extracts," J. Pharmacol. Toxicol, vol. 10 (2): pp. 49-59.

- **Pearce, H.L. (1990). Medicinal chemistry of bisindole alkaloids from *Catharanthus.* In:** Brossi A, SunessM (eds) The alkaloids ± antitumor bisindole alkaloids from *Catharanthus roseus* (L.). Academic Press, San Diego, p 145.
- **Pendhari, R.K., Nitave,S.A., Magdum,. J.J., Trust's, (2019).** Pharmacological activity of Catharanthus roseus. World J Pharm Pharm Sci ; 8(2): 299-308.
- **Pham, H.N., Vuong, Q.V., Bowyer, M.C., Scarlett, C.J.(2020).** Phytochemicals derived from *Catharanthus roseus and* their health benefits. *Technologies;* 8(4):80.
- **Prabha Lahare, R., Yadav, H.S., Dashahre, A.K., Kumar Bisen, Y. (2020).** An updated review on phytochemical and pharmacological properties of *Catharanthus roseus*. *SJMPS*; 6(12):759-66.
- **Pradhan, S., Huidrom, P. (2024).** Antimicrobial properties and phytochemical analysis of some medicinal plants: A review. Int J Sci. Res.; 13:508–11.
- **Prajapati, N.D., Purohit, S.S., Sharma, A.K., Kumar, T. (2002).** A hand book of medicinal plants". 1st ed. India: *Agrobios, 2003 IDMA. Indian Herbal Pharmacopoeia*. Mumbai: 2002. 14.
- **Prestinaci, F., Pezzotti, P., Pantosti, A. (2015).** Antimicrobial resistance: A global multifaceted phenomenon. Pathog. Glob. Health.,109, 309–318
- **Rahman, M. A., Hasanuzzaman, M., Uddin, N., and Shahid, I. (2010).** Antidiarrhoeal and anti-inflammatory activities of Murraya *paniculata* (L). Jack. Pharmacol. 3,768–776.
- **Rai, P.P., Obayemi, O.M. (1973).** Anthraquinones from the leaves of Cassia podocarpa. Cur. Sci. p. 47.
- **Rai, S.P., Rai, S.K. (2021).** Periwinkle M. Horticultural and therapeutic potential of *Catharanthus roseus*: a model plant for terpenoid indole alkaloid synthesis. *Agric Food;* 3(8):375-8.
- **Raimi CO, Oyelade AR, Adesola OR (2020)** phytochemical screening and in- vitro antioxidant activity on *Vernonia amygdalina* (ewuro- bitter leaf). Eur J Agric for Res 8(2):12–17
- **Rajalakshmi A and Jayachitra A:** Antioxidant, antibacterial effects of solanine isolated from *Solanum nigrum* and its cytotoxic activity on the HEP-2 and AGS cell lines. Int J Pharm Sci Res 2017; 8(7): 2932-39.

- **Raju, R., Rao, C.V. (2012).** Diosgenin, a Steroid Saponin Constituent of Yams and Fenugreek: Emerging Evidence for Applications in Medicine. Bioact. Compd. Phytomedicine, 125, 143.
- **Ramakrishnan, K., Prasannan, G. and Rajan, R. (1994).**Text book of medical biochemistry".
- **Rana, P., Kumar, A., Choudhary, A., Kaur, H. and Singh, R. (2021).** The wisdom of prevention: Holistic, preventive herb approach for healing of the globe, The Pharma Innovation Journal; 10(1): 29-46.
- **Ranjan R, Kumar M. Biochemical profile of *Vinca rosea* Linn (*Catharanthus roseus* G. Don).** IOSR J Biotechnol Biochem 2020; 6(5):13-26.
- **Rao, L.J.M., Ramalakshmi, K., Borse, B.B., Raghavan, B.(2007).** Antioxidant and radical-scavenging carbazole alkaloids from the oleoresin of curry leaf (*Murraya koenigii* Spreng.). Food Chem.; 100(2):742–7.
- **Rayan, A.M., Abdein, M.A. and Ibrahim, A.A. (2020).** Associated Weeds of Some Agroecosystems in the Northern Border Region, KSA. International Journal of Botany Studies, 5, 345-351.
- **Redda YT, Kebede E, Cruz C, Gugsa G, Awol N, Mengeste B (2014)** Potential antibacterial activity of crude extracts from Aloe vera, Zingiber officinale and Vinca major medicinal plants. Intl J 5(3):202–207
- **Regassa R (2013)** Assessment of indigenous knowledge of medicinal plant practice and mode of service delivery in Hawassa city, southern Ethiopia. J Med Plants Res 7(9):517–535
- **Rehman, F., Khan, M., and Khan, I., (2013).** Molecular interactions of an alkaloid euchrestifoline as a new acetylcholinesterase inhibitor. Bangl. J. Pharmacol. 8, 361–364.
- **Retna AM, EthalshaP.** A review of the taxonomy, Ethnobotany, chemistry and pharmacology of *Catharanthus roseus* (Apocyanaceae). IJERT **2013;** 2(10):3899-3912.
- **Rodanant, P., Khetkam, P., Suksamrarn, A., and Kuvatanasuchati, J. (2015).** Coumarins and flavonoid from *Murraya paniculata* (L.) Jack: antibacterial and anti- inflammation activity. Pak J. Pharm. Sci. 28, 1947–1951.
- **Rodríguez, E., Ramis-Ramos, G., Vander Heyden, Y., Simo-Alfonso, E., Lerma- Garcia, M., Saucedo-Hernandez, Y., *et al.* (2012).** Chemical

composition, antioxidant properties and antimicrobial activity of the essential oil of *Murraya paniculata* leaves from the mountains of central Cuba. Nat. Prod. Commun. 7, 1934578X1200701–1530.

- **Russell-Smith J, Karunaratne NS, Mahindapala R (2006)** Rapid inventory of wild medicinal plant populations in Sri Lanka. Biol Cons 132(1):22–32
- **Saikia, S., Tamuli, K. J., Narzary, B., Bordoloi, M., and Banik, D. (2021).** Chemical composition, antimicrobial activity and cytotoxicity of Murraya paniculata (L.) Jack leaf essential oil from Assam, India: the effect of oil on cellular morphology of micro-organisms. Arch. Microbio. 204, 99.
- **Sak, K. (2017).** Intake of individual flavonoids and risk of carcinogenesis: Overview of epidemiological evidence. Nutr. Cancer, 69.
- **Sak, K.** Intake of individual flavonoids and risk of carcinogenesis: Overview of epidemiological evidence. Nutr. Cancer **2017,** 69, 1119–1150.
- **Sangkaew, A., Samritsakulchai, N., Sanachai, K., Rungrotmongkol, T., Chavasiri, W., and Yompakdee, C. (2020).** Two flavonoid-based compounds from *Murraya paniculata* as novel human carbonic anhydrase isozyme II inhibitors detected by a resazurin yeast-based assay. J. Microbio. Biotechnol. 30, 552–560.
- **Saqib, F., Ahmed, M. G., Janbaz, K. H., Dewanjee, S., Jaafar, H. Z. E., and Zia-Ul-Haq, M. (2015).** Validation of ethnopharmacological uses of *Murraya paniculata* in disorders of diarrhea, asthma and hypertension. BMC Complement. Altern. Med. 15, 319.
- **Satish, C. S., Gopu, B"A(2016).** Review on Curry Leaves (Murraya koenigii): Versatile Multi-Potential Medicinal Plant," American J of Phytomedicine and Clin Therapeutics, vol. 3(4), pp. 363-368.
- **Selestino Neta, M. C., Vittorazzi, C., Guimarães, A. C., Martins, J. D., Fronza, M., Endringer, D. C., et al. (2017).** Effects of β-caryophyllene and Murraya paniculata essential oil in the murine hepatoma cells and in the bacteria and fungi 24-h time-kill curve studies. Pharm. Biol. 55, 190–197.
- **Selvamani, S., Balamurugan, S.(2015).** Phytochemical screening and GCMS analysis of acetone leaf extract of Acalypha indica (Linn.). Int. J. Res. Stud. Biosci. ; 3(5):229–32.
- **Shah, K., S. Seeley, C. Schulz, J. Fisher and S. Gururaja Rao. 2022**. Calcium channels in the heart: disease states and drugs. Cells 11, 943.

- **Shankar, R. and Rawat, M.S.(2013).** Conservation and cultivation of threatened and high value medicinal plants in North East India. International Journal Biodiversity Conservation; 5(9): 572-579.
- **Shankar, R., Tripathi, A.K., Neyaz, S. and Anku, G. (2016**). Distribution, and conservation of medicinal plants in Kohima, Mokokchung, Tuensang and Zunheboto districts of Nagaland. World Journal of Pharmceutical Research; 3(5): 1225-1237
- **Sharifi-Rad, M., Anil Kumar, N.V., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., *et al.*,** Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. Front Physiol.; 11:694.
- **Sharma, P., Rana, J. C., & Sharma, R. (2024).** Plant part-dependent variation in phenolic content and antioxidant activity of traditional medicinal plants. ***Journal of Applied Research on Medicinal and Aromatic Plants***, 36, 100480.
- **Sharma, S., and Arora, S. (2015).** A clinical evaluation of atrial fibrillation in rheumatic heart disease. Int. J. Pharm. Res. Rev. 4, 22–25.
- **Shindo T, Ushiyama H, Kan K, Yasuda K and Saito K:** Contents and its change during storage of alpha-solanine and alpha-chaconine in potatoes. Shokuhin Eiseigaku Zasshi 2004; 45: 277-282.
- **Shukla, S.; Mehta, A.** Anticancer potential of medicinal plants and their phytochemicals: A review. Braz. J. Bot. **2015**, 38, 199–210.
- **Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer Statistics, 2019**. CA Cancer J. Clin. **2019**, 69, 7–34.
- **Silva, F. F. A. d., Fernandes, C. C., Santiago, M. B., Martins, C. H. G., Vieira, T. M., Crotti, A. E. M., et al. (2020).** Chemical composition and in vitro antibacterial activity of essential oils from Murraya paniculata (L.) Jack (Rutaceae) ripe and unripe fruits against bacterial genera Mycobacterium and Streptococcus. Braz. J. Pharm. Sci. 56.
- **Silva, N., Fernandes Júnior, A. (2010).** Biological properties of medicinal plants: A Review of their antimicrobial activity. J. Venom. Anim. Toxins Incl. Trop. Dis., 16, 402–413.
- **Sindhu, R.K., Arora, S. (2012).** Evaluation of phenolic contents and antioxidant potential of *Murraya koenigii* (L.) Spreng roots. J Appl Pharm Sci. 2(11):120–2.

- **Singh, K.B.M., Sarkar, P.K. and Chaturvedi, O.P. (2019).** Improved livelihood, and Ecosystem Singh R, Dev I, Tewari RK, Rizvi RH, Garg KK, Singh AK, Dwivedi RP, Sridhar Services through Agroforestry based Watershed intervention in Bundelkhand region of Central India. In Agroforestry for Climatic Resilience and Rural Livelihood, Scientific Publishers; 242-250.
- **Singh, P., Mahadi, F., Roy, A., Sharma, P.B.(2009)** Reactive oxygen species, reactive nitrogen species and antioxidants in etiopathogenesis of diabetes mellitus type-2. Indian J Clin. Biochem.; 24:324–42.
- **Singh, S.N., Vats, P., Suri, S., Shyam, R., Kumari, M.M., Ranganathan, S. (2001).** Effects of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in streptozotocin induced diabetic rats. J Ethnopharmacol, 001; 76:269-77.
- **Sofowara, A. (1993)** Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Ltd., Ibadan, Nigeria. pp. 289- 300.
- **Sofowora, A. (1986).** The state of Medicinal Plants Research in Nigeria. University Press, Ibadan, Nigerian 86.Taiwo O, H.V. H., Lee, S. (1999). Antibacterial activities of extracts from Nigerian chewing sticks. phytotherapy. Res. 13: 675-679.
- **Son YO, Kim J, Lim JC, Chung Y, Chung GH and Lee JC**: Ripe fruit of *Solanum nigrum* L. inhibits cell growth and induces apoptosis in MCF-7 cells. Food Chem Toxicol **2003**; 41: 1421-1428.
- **Sporn, M.B., Liby, K.T. (2020).** Chemoprevention of Cancer: Past, Present, and Future. In Natural Products for Cancer Chemoprevention; Pezzuto, J., Vang, O., Eds.; Springer: Cham, Switzerland.
- **Stessy. Ann. Punnen, Maleeka Begum, S. F. and Vinitha Lakshmi, P. (2016).** Review article "Ethnopharmacological update on *Catharanthus Roseus.L*" in *World Journal of Pharmaceutical Research*, 5(10): 244-257.
- **Stevanovic, Z.D., Stankovic, M.S., Stankovic, J., et al. (2019).** Use of Halophytes as Medicinal Plants: Phytochemical Diversity and Biological Activity. In: Halophytes and Climate Change: Adaptive Mechanisms and Potential Uses, CABI, Wallingford, 343.
- **Stobiecki M, Matysiak-Kata I, Frański R, Skała J and Szopa J**: Monitoring changes in anthocyanin and steroid alkaloid glycoside content in lines

of transgenic potato plants using liquid chromatography/mass spectrometry. Phytochemistry 2003; 62: 959-969.

- **Subramani, R., Narayanasamy, M., Feussner, K.D. (2017).** Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. Biotech. 7, 172.
- **Sultana S, Perwaiz S, Iqbal M and Athar M:** Crude extracts of hepatoprotective plants, *Solanum nigrum* and *Cichorium intybus* inhibit free radical-mediated DNA damage. J. Ethnopharmacol. **1995**; 45(3): 189-192.
- **Sultana, S., Ashwini, B. S., Ansari, M. A., Alomary. M. N., Amous, Y. F., *et al*., (2024).** *Catharanthus roseus*-assisted bio-fabricated zinc oxide nanoparticles for promising antibacterial potential against *Klebsiella pneumoniae*, *Bioproc Biosyst Eng*, 1-11, doi: 10.1007/s00449-024-03001-8.
- **Sumalatha, D., Nithya, T.G. (2014).** *Invitro* anti-oxidant and anticancer activity of *Murraya koenigii* against human colon cancer HT-29 cell lines. Asian J. Pharm. Clin. Res.; 7(SUPPL. 1):83–6.
- **Sumedh, V. W., and Tanuja, M. N., (2016).** "A Review on Role of Murraya Koenigii (Curry Leaf) In (Diabetes Mellitus – Type Ii) Prameha," Int. J of Development R, vol. 6(4), pp. 7468-7469.
- **Sutrisna, E. (2015).** In a review article "A Controversial Medicinal Plant in Indonesia" *Int. J. Res. Ayurveda Pharm.6* (5).
- **Szandruk-Bender, M., Rutkowska, M., Merwid-La˛d, A., Wiatrak, B., Szela˛g, A., Dzimira, S., Sobieszczan´ ska, B., Krzystek- Korpacka, M., Kucharska, A.Z., Matuszewska, A., et al. (2010).** Cornelian Cherry Iri-doid-Polyphenolic Extract Improves Mucosal Epithelial Barrier Integrity in Rat Experimental Colitis and Exerts Antimicrobial and Antiadhesive Activities in Vitro. Oxid. Med. Cell. Longev.2020, 7697851.
- **Tachibana, Y., Kikuzaki, H., Lajis, N., Nakatani, N. (2001).** Antioxidative activity of carbazoles from *Murraya koenigii* leaves. J Agric Food Chem. 1; 49:5589–94.
- **Tai, C.J., Wang, C.K., Chang, Y.J., Lin, C.S., Tai, C.J. (2012).** Aqueous Extract of *Solanum nigrum* Leaf Activates Autophagic Cell Death and Enhances Docetaxel-Induced Cytotoxicity in Human Endometrial Carcinoma Cells. Evid.-Based Complem. Altern. Med., 10, 859185.

- **Tamene S (2020)** Ethnobotanical study of indigenous knowledge on medicinal plant uses and threatening factors around the Malga District, Southern Ethiopia. Int J Biodivers Conserv 12(3):215–226.
- **Tanabe, S., O'Brien, J., Tollefsen, K.E., Kim, Y., Chauhan, V., Yauk, C., *et al., (2022).*** Reactive oxygen species in the adverse outcome pathway framework toward creation of harmonized consensus key events. Front Toxicol.; 4:887135.
- **Tapiero, .H, Ba, G.N., Couvreur, P., Tew, K.D., (2002).** Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. Biomed Pharmacother.56:215–22.
- **Tolambiya P, Mathur S**. A Study on Potential Phyto pharmaceuticals Assets in *Catharanthus roseus* L. (Alba). Int J Life Sci Biotech Pharma Res 2016;5(1):1-6.
- **Trease, G.E., Evans, W.C. (1983).** Textbook of Pharmacognosy. 12th ed. Bailliere Tindal, London 343-383.
- **Uniyal, G.C., Bala, S., Mathur, A.K., & Kulkarni, R.N. (2001).**Symmetry C18 column: A better choice for the analysis of indole alkaloids of *Cathranthu*s *roseus*. *Phytoche Anal. 12*:206-10.
- **Uruguiaga, I., Leighton, F.,(2000)** Plant poly phenol antioxidant and oxidative stress, Biological Research; 33:159-165.
- **Van der Heijden, R., Jacobs, D.I., Snoeijer, W., Hallard, D., Verpoorte, R.(2004).** The *Catharanthus* alkaloids: *pharmacognosy and biotechnology. Curr. Med. Chem.*; 11(5):607-28.
- **Vani, T. Rajani, M. Sarkar, S. Shishoo, C. J.; (1997).** Inter. J. Pharmacognosy. 35: 313-317.
- **Varuna, K.M., Khan, M.U., & Sharma, P.K. (2010).** Review on vinca *rosea. J. Pharmacy Res.*, 3(*4*):714-717.
- **Wallis, T.E. (1967).** Text Book of phrmacolognosy. 5th Edition, London J.A. Chiorchhill Ltd. pp. 81-82.
- **Wang S, Panter KE, Gaffield W, Evans RC and Bunch TD**: Effects of steroidal glycoalkaloids from potatoes (*Solanum tuberosum*) on *in vitro* bovine embryo development. Anim Reprod Sci 2005; 85: 243-250.
- **Wang, X., Liang, H., Zeng, K., Zhao, M., Tu, P., Li, J., et al. (2019a).** Panitins A G: coumarin derivatives from *Murraya paniculata* from Guangxi Province, China show variable NO inhibitory activity. Phytochemistry 162, 224–231.

- **Wang, X., Sun, Z., Wang, X., Li, M., Zhou, B., Zhang, X. (2024).** *Solanum nigrum* L. berries extract ameliorated the alcoholic liver injury by regulating gut microbiota, lipid metabolism, inflammation, and oxidative stress. Food Res Int.; 188:114489.
- **Wang, Y., Lv, H., Cui, Q., Tu, P., Jiang, Y., and Zeng, K. (2019b).** Isosibiricin inhibits microglial activation by targeting the dopamine D1/D2 receptor-dependent NLRP3/ caspase-1 inflammasome pathway. Acta Pharmacol. Sin. 41, 173–180.
- **World Health Organization**. Lack of new antibiotics threatens global efforts to contain drug-resistant infections. Available from: https://www.who.int/news-room/detail/17-01-2020-lack-of-new-antibiotics-threatens-global-efforts-to-Contain-drug-resistant-infections.
- **Yao, M., Yuan, B., Wang, X., Sato, A., Sakuma, K., Kaneko, K,; Komuro, H., Okazaki, A., Hayashi, H., Toyoda, H., *et al.*, (2017).** Synergistic cytotoxic effects of arsenite and tetrandrine in human breast cancer cell line MCF-7. Int. J. Oncol., 51, 587–598.
- **Yeap, S.K., Abu, N., Mohamad, N.E., Beh, B.K., Ho, W.Y., Ebrahim, S., Yusof, H.M., Ky, H., Tan, S.W. and Alitheen, N.B.(2015).** Chemo preventive and immunomodulatory effects of *Murraya koenigii* aqueous extract on 4T1 breast cancer cell-challenged mice. BMC Complement Alternative Med.: 15(1): 306.
- **Youssef, S.M., Sayed S.N., Mahmoud A.M.M., et al., (2020)** Response of Stressed China Aster (*Callistephus chinensis*) cv. Kamini Plants to Foliar Application of Benzyl adenine (BA) and Cycocel (CCC). The Seybold Report, 15, 30-42.
- **Yu, M., Gouvinhas, I., Rocha, J., Barros, A. (2021).** Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. Sci. Rep. 2021; 11(1):10041.
- **Zanoaga, O., Braicu, C., Jurj, A., Rusu, A., Buiga, R., Berindan-Neagoe, I. (2019).** Progress in Research on the Role of Flavonoids in LungCancer. Int. J. Mol. Sci., 20, 4291.
- **Zhang, J. Y., Li, N., Che, Y. Y., Zhang, Y., Liang, S. X., Zhao, M. B., *et al.* (2011).** Characterization of seventy polymethoxylated flavonoids (PMFs) in the leaves of *Murraya paniculata* by on-line high-performance liquid

chromatography coupled to photodiode array detection and electrospray tandem mass spectrometry. J. Pharm. Biomed. Anal. 56, 950–961.

- **Zhou, M.L., Shao, J.R., Tang, Y.X. (2009).** Production and metabolic enginnering of terpenoid indole alkaloids in cell cultures of the medicinal plant *Catharanthus* (L.) G. Don (Madagascar periwinkle). *Biotechnol Appl Biochem. 52*:313-23.
- **Zhu, J., Wang, M., Wen, W., Yu, R. (2015).** Biosynthesis and regulation of terpenoid indole alkaloids in *Catharanthus roseus*. *Pharmacogn Rev.*; 9(17):24-8.
- **Zou, J., Yu, X., Qu, S., Li, X., Jin, Y., and Sui, D. (2014).** Protective effect of total flavonoids extracted from the leaves of *Murraya paniculata* (L.) Jack on diabetic nephropathy in rats. Food Chem. Toxicol. 64, 231–237.
- **Zou, J., Sui, D., Fu, W., Li, Y., Yu, P., Yu, X., et al. (2021).** Total flavonoids extracted from the leaves of *Murraya paniculata* (L.) Jack alleviate oxidative stress, inflammation and apoptosis in a rat model of diabetic cardiomyopathy. J. Funct. Foods 76, 104319.
- **Zywicki B, Catchpole G, Draper J and Fiehn O**: Comparison of rapid liquid chromatography-electrospray ionization-tandem mass spectrometry methods for determination of glycoalkaloids in transgenic field-grown potatoes. Anal Biochem 2005; 336: 178-186.

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Invitro Phytochemical Screening and Antibacterial Potentiality of *Catharanthus roseus* Leaves Extract Against Some Pathogenic Bacteria

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A B S T R A C T

Catharanthus roseus is one of the most important plant as described in ayurveda. This plant is known as sadabahar is grown in every home throughout India as ornamental plant. This plant comprises a no of significant bioactive compounds to cure various critical diseases as described in ayurveda. These incurable diseases have necessitated its continued exploration and consumption. The presence of bioactive compounds also has antimicrobial activity. Therefore, this study is carried out to evaluate the antimicrobial property and phytochemical components of *Catharanthus roseus* plant's leaf extract. Moreover about the study phytochemical screening of different extracts namely methanol extracts of *C.roseus* plant's leaves was done. Antimicrobial activity of Methanols extract against Salmonella, *Staphyloccus aureus* and *E.coli* was carried out. The 100 µg/µl methanolic extract showed good activity against *Salmonella* and *Staphylococcus aureus* comparing to *E.coli* organisms.

Introduction

Ayurveda is playing a significant role as natural remedies from ancient times in Indian subcontinent. Currently the demand for Traditional medicine system in India is growing. The commercial drug producers are now focusing on the medicinal potential of plants and natural products.

In India a vast diversity persists among plant grown in temperate as well as tropical area under the different climatic condition. A wide variety of plants having therapeutic importance can be found all over the world. Many weeds in our environment are unknown to us, comprises highly effective medicinal charaterstics that can cure a variety of significant health issues (Parihar *et al.,* 2021; Chaudhary *et al.,* 2021). From ancient times, India has long been known as a potential source of natural remedies among ancient cultures (Parihar *et al.,* 2021).

In present scenario, exhaustive usages of chemically synthesized antibiotics have badly affected the human immune system by generating drug resistance pathogenecity. This problem has led the extensive search of natural remedies to withstand such conditions. The growing demand for novel drugs that can overcome the

problem of drug resistance contribute to a revival of interest in plant based compounds for drug discovery. Nature endows medicinal plants for our health and survival (Prajapati *et al.,* 2002). In present time, the use of herbal has become even more important, while pharmaceuticals are making synthetic drugs based on natural constituents from medicinal plant. A great part of herbal compounds constitutes the main part in drug discovery to produce synthetic molecular analog. This specifies the importance of study of phytochemicals in diversity oriented synthesis (DOS) of natural product like pharma compounds.

Medicinal plants grow worldwide, which are intended for medicinal purpose. Among those *Catharantus roseus* is a globally significant plant which found in tropical areas. Ayurveda states the use of plant as traditional medicine system in India that comprises the therapeutic importance of plants and natural products. Some plants of family Apocynaceae *are medicinally important including Catharanthus roseus* L.

Catharanthus roseus is a perennial tropical medicinal plant (Malar Retna *et al.,* 2013). Family Apocynaceae comprises eight species of *Catharanthus*, out of which, seven species are endemic to Madagascar and *C. coriaceus, C. lanceus, C. ln\ifolius, C. ovalis, and C. roseus* widely spread in India (Chandrashekhar *et al.,* 2021). Furthermore, about *C. roseus* plant, this produces more than 130 terpenoids indole alkaloids (TIAs). Some terpenoids indole alkaloids are very useful pharmaceutical and medicinal plants due to their strong and important pharmacological activities (Van der Heijden *et al.,* 2004; Almagro *et al.,* 2015).

Catharanthus roseus is a significant curative plant (Das *et al.,* 2017). This dicotyledonous angiospermic plant integrates two therapeutically important compounds terpene indole alkaloids: vinblastine and vincristine, which are the major constituents of the plant and fight against a variety of diseases. *Catharanthus* is a greek word that means "unadulterated blossom." It has many synonyms as Madagascar periwinkle, bright eyes, Cape periwinkle, graveyard plant, old maid, pink periwinkle, and rose periwinkle myrtle. In India this is frequently referred to as "Nayantara" or "Sadabahar" (Rai *et al.,* 2021; Dubey *et al.,* 2020).

Catharanthus roseus is a well-recognized herbal medicinal plant (Jamal *et al.,* 2024). *Catharanthus roseus* is widely distributed in South Africa and Australia and grows well in the United States and Southern Europe. This is an ornamental plant, widely distributed throughout India. Different parts of *C. roseus* are used in folklore herbal medicines to treat many types of infectious diseases. Vinblastine and Vincristine, these two vinca alkaloids, anticancer chemicals, are abundant in *C. roseus* leaves (Arulvendhan *et al.,* 2024). Previous researches reveal that *Catharanthus roseus* contains more than 130 alkaloids. the presence of active components vincristine and vinblastine make this plant specific and are widely used in the treatment of various cancers, such as breast cancer, lung cancer, and melanoma (Leveque *et al.,* 2007; Ferreres *et al.,* 2008). *Catharanthus roseus* leaves contain 70 different bioactive compounds including ajmalicine, reserpine, and serpentine. Alkaloids are the main therepeutic compound of *C. roseus* and are significantly used in the remedy of severe complications like diabetes, high blood pressure, dysmenorrhea, asthma, constipation, and cancer (Pham *et al.,* 2020). Two major derivatives terpene indole alkaloids, namely vincristine and vinblastine are produced in the plants (Bhambhani *et al.,* 2021) along with large amounts of volatile and phenolic compounds such as cafe oil, quinic acid, and flavone glycosides. These compounds confer antioxidant activity against reactive oxygen species and play an important role in the plant's defence system (Prabha *et al.,* 2020). Plants compounds which impart antioxidant activity are mainly used for hypertension, diabetes, blood cancer, malaria, non-small-lung cancer, Hodgkin's lymphoma, and improving memory. These plant synthesized compounds also has antimicrobial, antioxidant, anti-diarrheal, and hypolipidimic activity, as well as wound-healing activity (Allamsetty *et al.,* 2020). The current work is focused on the phytochemicals and antimicrobial activity of methanolic extract of *Vinca rosea*.

Materials and Methods

This work was carried out at Advanced Environmental Testing & Research Lab (P) Ltd, Gwalior M.P. The plant was a perennial so, there was no difficulty in getting the material for this study.

Microbes used

A total of three microbes *Salmonella, Staphylococcus aureus and E. coli* were procured from MTCC, Chandigarh (Punjab). Bacterial cultures were maintained on the agar media as per given instruction of MTCC.

Collection of plant material

The fully mature *Catharanthus roseus* plants were collected from Botanical section of Jiwaji University (M.P.). Plant materials were washed separately under running tap water, followed by rinse using sterilized distilled water. Excess of water was removed from the plant material using filter paper before they were used for extraction. Separated Plant leaves were shed dried. Dried leaves were grinded and extracted with methanol.

Extract preparation

Solvent extract

10 gm of grinded plant leaf material was dissolved in 100 ml organic solvent Methanol using soxhelet apparatus. This methanoic extract was further dried using rotary evaporator.

Assessment of antimicrobial activity of plant extract against pathogenic microbes

The antimicrobial activities of plant extract of *Catharanthus roseus* were measured *against* the procured pathogenic microbes.

Agar well diffusion method (Perez *et al.,* 1993)

Muller Hinton Agar medium was prepared as per manufacturer's instructions. Sterilized media was poured into sterile Petri dishes each contains approximately 25 ml. This was allowed to solidify and spread the Bacterial culture and fungal spores on set petri plates. Five agar wells per plate were made in the set medium by using a sterile cork- borer of 9 mm diameter.

Thereafter, the wells were filled with the prepared extract solution at varying concentrations. This was done in duplicate and plates were incubated at suitable temperature for 24 hrs at 37ºC for Antibacterial activity. The plates were observed for zone of inhibition (mm) around the wells.

Phytochemical screening

These methods performed with slight modifications were used to test for the presence of the active ingredients in the test samples (Harborne, 1978).

Test for steroids

1 ml of plant leaf extracts was taken in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tubes. The upper layer turns red and sulphuric acid layer show yellow colour with green fluorescence. This indicated the presence of steroids.

Test for saponins

Take 20 ml of distilled water in a test tube and mixed plant leaf extract and vigorously shake that test tube for 10 minutes. The formation of foam showed the presence of saponins.

Test for tannins

Take 5 ml of the extract and add few drops of 1% lead acetate. Formation of yellow precipitate indicated the presence of tannins.

Test for triterpenes

Take 10 mg of extract in a test tube and add 1 ml of chloroform later 1 ml of acetic anhydride. In the last add 2 ml of conc. H_2SO_4. Reddish violet colour indicated the presence of triterpenoids.

Test for alkaloids

Take 300 mg of leaf extract in a test tube and digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature and examined the alcoholic layer for the pink colour, indicating the presence of alkaloids.

Test for flavonoids

Take 1 ml of extract in a test tube and add few drops of dilute sodium hydroxide. An intense yellow colour was produced in the plant extract, which became colourless on the addition of a few drops of dil. acid indicating the presence of flavonoids.

Test for anthraquinones

Take 5 ml of the extract in attest tube and hydrolysed with dil. Sulphuric acid, later extracted with benzene and add 1ml of dil. ammonia to it. Rose pink rings suggested the positive responses for Anthraquinones.

Test for phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete.

Results and Discussion

In the present study, Methanaolic and aquous extract from leaves of *Catharanthus roseus* were extraxted and analysed for presence of phytochemicals namely steroids, saponins, tannins, Triterpenes, flavonoids, anthraquinines, alkaloids. Phytochemicals Tannins, triterpenes and Anthraquinones were found absent in methanolic extract of *Catharanthus roseus*.

However, tannins was found present in aqueous extract. Similarly methanolic extract, anthraquinine was found absent in aqueous extract also. Steroids, saponins, flavonoids, and alkaloids were found present in both methenolic and aqueous extract as shown in table 1.

Chinnavenkataraman Govindasamy and Rajendran Srinivasan, (2012) investigated phytochemicals in medicinal plant *Catharanthus roseus*. The phytochemical analysis of organic solvent methanolic extracts of plant leaf revealed the presence varying degree of major phytoconstituents Saponins, terpenoids, alkaloids and tannin (Chinnavenkataraman Govindasamy and Rajendran Srinivasan, 2012).

The present study was carried out to evaluate the phytochemical screening & antimicrobial activity of Methanolic and aquous extracts of *Catharanthus roseus* leaves. Methanolic and aqueous extracts were prepared in 1mg/ml. Bacterial strains used in this study were *Staphylococcus aureus* (MTCC), *Salmonella* and *E.coli*.

In agar well diffusion assay performed against *Catharanthus roseus* methanolic extracts of leaves in different concentration 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml. Highest activity 17.0 mm was measured against *Staphylococcus* for aqueous extract when applied in a 100 mg/ml.

Minimum Zone of inhibition was measured for aqueous extract against *E.coli* (10.0 mm). Highest Zone diameter has been measured against Staphylococcus (15.0 mm) followed by *salmonella* (13 mm) for a methanolic extract when used in a concentration of 75 mg/ml. On the other hand lowest inhibition zone is recorded in aqueous extract against the *E.coli* (9.0 mm). aqueous and methanolic extract has not given any activity against *E.coli* when used in a concentration of 25 mg/ml and 50 mg/ml. Likewise *E.coli,* Both extracts methanolic and aqueous extracts not gave n used in a concentration of 25 mg/ml against *Staphylococcus* and *Salmonella*.

When a concentration of 50 mg/ml against all three bacteria Extarct showed no activity against *E.coli*, aqueous extract showed activity against staphylococcus (10 mm). Both extract showed antimicrobial activity against salmonella. The Inhibition zones were 11 mm for mathanolic extracts and 12 mm for aqueous extracts.

In another study, the antimicrobial activity was assessed for the leaf extract of C. against *Salmonella typhimurium* and *Staphylococcus aureus* was similar to the present study (Patil *et al.,* 2010).

Goyal *et al.,* (2008) studied antibacterial activity of *C. roseus* against *E. coli* along with 5 other bacterial strains and found that ethanolic extracts were most effective than methanol and which showed maximum inhibition against *Klebsiella pneumoniae* followed by *E.coli* (Goyal *et al.,* 2008). This study also supports the findings of Goyal *et al.,* (2008) for antibacterial activity of plant leave extract against inhibition of *E.coli.*

Figures 1 showing the antibacterial activity of methanolic extract of Leaves against (A) *Salmonella* (B) *E.coli* (C) *Staphylococcus aureus.*

Ramya *et al.,* (2008) observations stated that antibacterial activity of ethanolic extracts of flower, leaves, stem and roots were found to show antibacterial activity. However No antimicrobial activity was observed for methanolic and aqueous extracts of different plant parts of *C. roseus* using disc diffusion assay.

Similarly, the present study methanolic and aqueous extract of leaves is not showed antibacterial activity for some concentration against *E.coli, Staphylococcus* and *Salmonella*.

For both plant varieties best antibacterial activity was found in methanolic leaf extract at 70 µl concentrations. Methanolic stem extract of *C. roseus var. "rosea"* gave better zones at 100 µl conc. as compared to 70 µl conc. of *C. roseus var. "alba"*. The present study also showed the similar results for three varying concentrations 50 mg/ml, 75 mg/ml and 100 mg/ml of methanolic leaves extracts (Ramya *et al.,* 2008).

Table.1 Qualitative analysis of phytochemical present in methanolic and aqueous extracts of *Catharanthus roseus.*

Phytochemicals	Leaves of *Catharanthus roseus*	
	Methanol	Aqueous
Steroids	++	+
Saponins	++	++
Tannins	_	+
Triterpenes	_	+
Flavonoids	++	++
Anthraquinines	_	_
Alkaloids	+++	+++

Table.2 Antimicrobial activities of *C. roseus* against different pathogenic microbes

Bacteria	Zone of inhibition (mm)					
	***E.coli* (MTCC 1687)**		***S .aureus*(MTCC 96)**		***Salmonella* (MTCC 98)**	
Concentration (mg/ml)	**ME**	**Aq E**	**ME**	**Aq E**	**ME**	**Aq E**
25	0	0	0	0	0	0
50	0	0	0	10±0.2	11±1.5	12±0.3
75	10±1.25	9±1.65	15±1.1	12±1.25	12±1.5	13±1.15
100	12±1.3	10±0.65	17±1.9	13±1.5	13±1.35	14±0.85
Streptomycin Standard (50µl)	22±0.3	19±0.65	17±1.9	15±0.5	29±0.4	25±0.55

Abbreviations: Aq E-Aqueous Extract; ME-Methanolic Extract

Figure.1 Antibacterial activity of Methanolic extract of *Catharanthus roseus* leaves

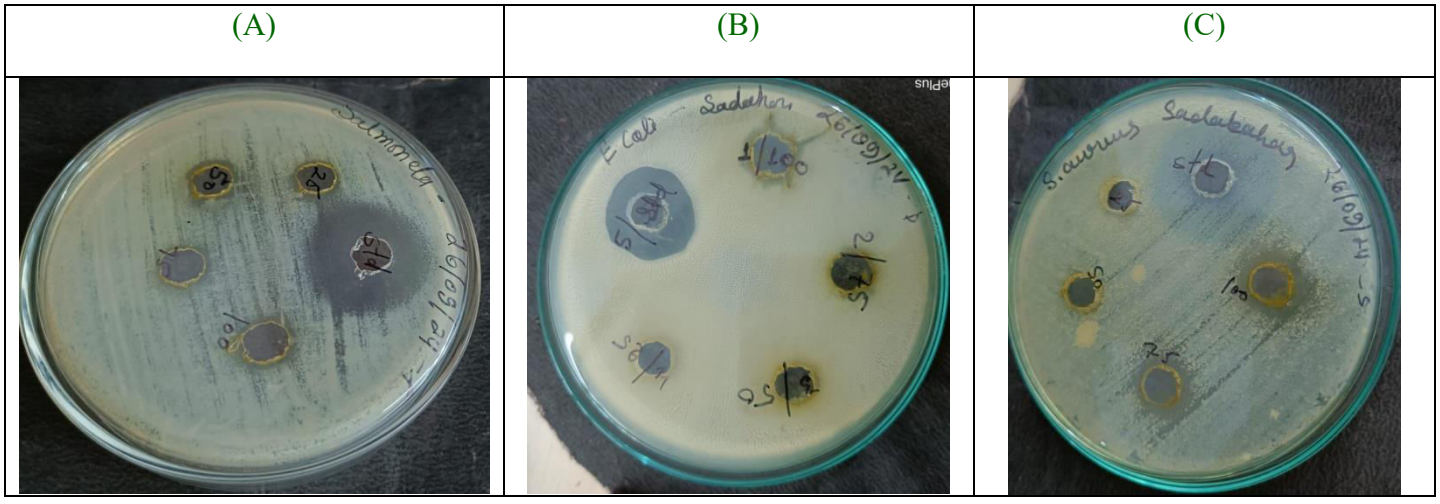
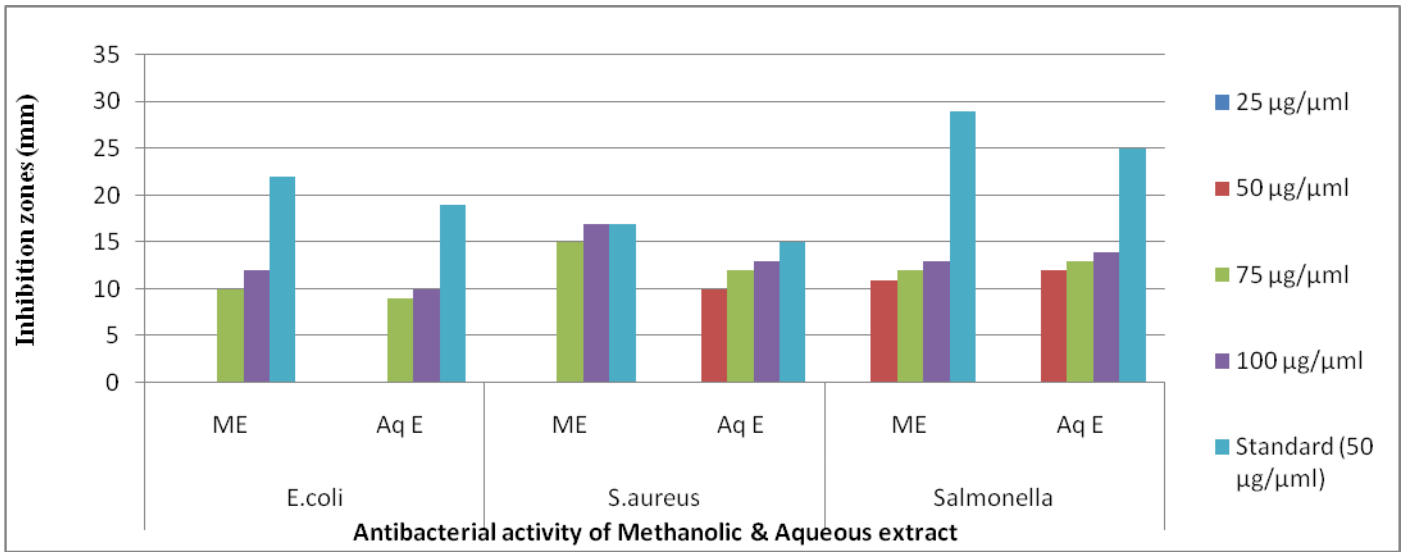


Figure.2 Graphical representation of Antibacterial activity of Methanolic & Aqueous extract



In the present study widely grown *catharanthus roseus* was collected from the Jiwaji University, Gwalior (M.P.). The plant leaf were segregated, shed dried and extracted with methanol and double distilled water for *in vitro* phytochemical and antibacterial studies. The qualitative analysis of phytochemicals revealed the presence of steroid, saponins, alkaloids and Flavonoids in methanolic extract and steroids, saponins, tannins, Triterpenes, flavonoids, and alkaloids in aqueous extract.

The Highest and lowest antibacterial activity of *Catharanthus* plant leaves extract were observed against *Staphylococcus aureus* and *E.coli* respectively in varying concentrations.

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Author Contributions

A Pooja Sahu: Conceived the original idea and designed the model and wrote the manuscript.; B Manish Kumar: Designed the model and the computational framework and analysed the data.; C Arvind Sharma: Review the Manuscript

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Allamsetty, J., Pedada, S. P., Pedada, N., Dhanunjayarao, K. A basic review on *Vinca rosea*. *Int J Pharmacogn Chem.* 2020; 1(1):31-6.

Almagro, L., Fernandez Perez, F., Pedreno, M. A. Indole alkaloids from *Catharanthus roseus*: bioproduction and their effect on human health. *Molecules.* 2015; 20(2):2973-3000. https://doi.org/10.3390/molecules20022973

Arulvendhan, V., Saravana, B. P. and Rajaganesh, R. Molecular identification and phytochemical analysis and bioactivity assessment of *Catharanthus roseus* leaf extract: Exploring antioxidant potential and antimicrobial activities, *Appl Biochem Biotechnol*,

2024, 1-28, https://doi.org/10.1007/s12010-024-04902-w.

Bhambhani,S., Kirtikumar, R., Kond Hare, Ashok, P. Diversity in chemical structures and biological properties of plant alkaloids. *Molecules.* 2021; 26(11):3374. https://doi.org/10.3390/molecules26113374

Chandrashekhar, R., Pagadala, J. C., Bhavani, N. L. Vinca alkaloids from Madagascar periwinkle (*Catharanthus roseus* (L.) G. Don): an overview. *Everymans Sci.* 2021; LV (5):187-92.

Chaudhary, K., Parihar, S., Sharma, D. A. Critical Review on Nanoscience Advancement: In Treatment of Viral Infection. *Journal of Drug Delivery and Therapeutics*. 2021 Nov; 11(6):225-237.

Chinnavenkataraman Govindasamy and Rajendran Srinivasan. 2012. In vitro antibacterial activity and phytochemical analysis of Catharanthus roseus (Linn.) G. Don. Asian Pacific Journal of Ttopical Biomedicine, S155-158

Das, S, Sharangi, A. B. Madagascar periwinkle (*Catharanthus roseus* L.): diverse medicinal and therapeutic benefits to humankind. *J Pharmacogn Phytochem*. 2017; 6(5):1695-701.

Dubey, A., Kushwaha, R. A discussion on vinca plant. *Journal of Pharmacognosy and Phytochemistry.* 2020; 9 (5):27-31.

Ferreres, F.,Pereira, D. M., Valentao, P., Andrade, P. B., Seabra, R. M., Sottomayor, M. New phenolic compounds and antioxidant potential of *Catharanthus roseus. J Agric Food Chem.* 2008; 56(21):9967-74. https://doi.org/10.1021/jf8022723.

Goyal, P., Khanna, A., Chauhan, A., Goyal, G. In vitro evaluation of crude extract of *Catharanthus roseus* for potential antibacterial activity, *IJGP*, 2(2008) 176-181. https://doi.org/10.22377/ijgp.v2i3.27

Harborne, J. B. *Phytochemical Methods: A guide to modern Techniques of Plant analysis,* Chapman and Hall, London., 1978, pp:75.

Jamal, Q. M. S. and Ahmad, V. Identification of metabolites from *Catharanthus roseus* leaves and stem extract, and *In vitro* and *In silico* antibacterial activity against food pathogens, *J Pharm*, 2024, 17(4), 450, https://doi.org/10.3390/ph17040450

Leveque, D., Jehl, F. Molecular pharmacokinetics of catharanthus (vinca) alkaloids. *J Clin Pharmacol.* 2007; 47(5):579-88. https://doi.org/10.1177/0091270007299430.

Malar, Retna, A., Ethalsha, P. A review of the taxonomy ethnobotany chemistry and pharmacology of *Catharanthus roseus* (Apocyanaceae). *Int J Eng Res Technol.* 2013; 2(10):3899-912.

Parihar S and Sharma D. Navagraha (nine planets) plants: the traditional uses and the therapeutic potential of nine sacred plants of india that symbolises nine planets. IJRAR 2021; 8(4):96-108.

Parihar, S. Sharma, D. A brief overview on *Asparagus racemous. IJRAR*. 2021; 8(4):96-108.

Patil, P. J. and Ghosh, J. S. (2010). Antimicrobial activity of *Catharanthus roseus*–a detailed study. *Br. J. Pharmacol.*2021; 1: 40-44.

Perez, C., Pauli, M., Bazerque, P. An Antibiotic assay by Agar Well Diffusion method, *Acta Biologi. Medecine Experimentalis*, 15(1993)113-115.

Pham, H. N., Vuong, Q. V., Bowyer, M. C., Scarlett, C. J. Phytochemicals derived from *Catharanthus roseus and* their health benefits. *Technologies.* 2020; 8(4):80. https://doi.org/10.3390/technologies8040080

Prabha Lahare, R., Yadav, H. S., Dashahre, A. K., Kumar Bisen, Y. An updated review on phytochemical and pharmacological properties of *Catharanthus roseus*. *SJMPS*. 2020; 6(12):759-66.

Prajapati N D, Purohit, S S, Sharma A K, Kumar T. "*A hand book of medicinal plants*". 1st ed. India: Agrobios, 2003. IDMA. *Indian Herbal Pharmacopoeia.* Mumbai: 2002. 14.

Rai, S. P., Rai, S. K. Periwinkle M. Horticultural and therapeutic potential of *Catharanthus roseus*: a model plant for terpenoid indole alkaloid synthesis. *Agric. Food.* 2021; 3(8):375-8.

Ramya, S., Govindaraji, R., Navaneetha, K., Kannan, R. J. In vitro evaluation of antibacterial activity using crude extracts of *Catharanthus roseus.L.(G) Don. Eth. Leaflets,* 12(2008)1067-72.

Van Der Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. 2004. The Catharanthus alkaloids: pharmacognosy and biotechnology. Curr Med Chem. 2004 Mar;11(5):607-28. doi: https://doi.org/10.2174/0929867043455846

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PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL POTENTIALITY OF *SOLANUM NIGRUM* LEAF EXTRACT AGAINST SOME PATHOGENS

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ABSTRACT

Present study has been focussed on the phytochemical screening and antimicrobial study of methanolic extract of leaves of *Solanum nigrum* in Gwalior region (M.P.). The phytochemical compounds like tannins, phenols, alkaloids, flavonoides, terpenoids, anthrocyanin, steroids and saponins were studied qualitatively. The Phytochemical screening results revealed that the alkaloids tannins are abundantly found. Other phytochemical compounds such as saponins, flavonoides were more or less presence in *Solanum nigrum*. Antimicrobial activity suggested that leaf extract has great impact on *klebsiella pneumoniae*, and also found effective against *E.coli*. Leaf extract also have significant antifungal activity against *Candida albicans*. Thus the present investigation suggested that *Solanum nigrum* composed of antimicrobial compounds that can be utilized as futuristic antimicrobial drug.

KEYWORDS: *Solanum nigrum*, Antibacterial activity, Phytochemical activity, Secondary metabolites.

INTRODUCTION

Plants are regarded as significant tools in the drug formulations. Anciently plants are being used as traditional medicines and also considered as the base of Homeopathy, Ayurveda. In present time, several research studies establish the plants as potential remedies

against severe diseases with low side effects comparatively to synthetic drugs. A large population approximately 85% peoples in developing countries depends on traditional medicines obtained from plant resources.[1]

Therefore, medicinally important plants contain significant amount of various bioactive metabolites in different chemical composition.[2] Bioactive metabolites present in Medicinal plants the key raw materials for pharmaceuticals formulations.[3] Currently focus towards the phytochemicals research has been intensified and results the findings of new anti-infective compounds from higher plants which are physiologically significant.[4]

The investigations of new effective bioactive compounds comprised of antimicrobial as well as antifungal activity is the most promising effort in present time against the developing resistant to pathogen against synthetic antibiotics.[5-8]

Bioactive compounds investigations and familiarity to present technically progressive population worldwide, is significant relative to extensively usage of commercially synthetic preparations. Sources of Natural compounds from microorganism as well as the plant world are limitless. The prevalent antibiotics as well as pesticides are used with natural plant's derived bioactive compounds in a combination, which reduces the risk of resistance development in pathogens without affecting the effectiveness of plant produced products. Plants extract and essential oils from plants impart antimicrobial activity have significant application in pharmaceuticals, as alternatives of medicine and natural therapies, in food processing industries.[9,10]

Furthermore, Plant synthesizes a variety of Secondary bio metabolite compounds which are significant for antimicrobial, antioxidant and antitumor activity.[11,12] flavonoids, a major Bioactive compounds exhibits a strong chemopreventive effect.[13]

Moreover the epidemiological investigations state that partly consumption of flavonoids rich diets have effect on life expectancy,[14,15] due presence of anticancer activity.[16,17] Therefore, scientists are focussing on the significant medicinal activities of natural bio compounds synthesized by plants which includes the effect of flavonoids against various kind of lung cancer, breast cancer, cervical, tumourogenic cells as well as neoblastic cells.[18-20] Secondary metabolites such as essential oils constituents, phenolic compounds,

saponosides and alkaloids remain the object of much in vivo as well as in vitro research.[21-24]

Plant *Solanum nigrum* belongs to the family *Solanaceae. Solanum nigrum i*s known with others name as Makoi or black nightshade. Traditionally *S. nigrum* has been broadly used in healing of various illnesses such as fever, pain and inflammation. The plant *Solanum nigrum is* also comprised of antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic properties.[39] *Solanum nigrum fruits* berries have been traditionally used in the cure of stomach ulcers as folk medicine.[40]

Solanum nigrum is gown as locally agricultural weed worldwide.[28,29] Investigated bioactive compounds in *Solanum nigrum are* alkaloids (solanine, soladulcine, chaconine, solasodine), steroid, resins and essential oils with constituents, among others, including dillapiol, α-cadinol, p-cymene, α-phellandrene, β-pinene and dyes: brown, green as well as blue and saponins,. Even so, presence of solaine content in higher amount makes the black nightshade as a poisonous plant regardless of all those remarkable features.[26]

Solanum nigrum L is an annual herbaceous, semiclimber plant which comes under the family Solanaceae. S. nigrum is known as Black nightshade in English., whereas, Kachcipandu, Munatakali, Piludi, Kamuni and Makoi are the Gujrati, Telugu, Tamil, Marathi and Hindi, names for the plant.[30]

Average length of *Solanum nigrum* plant is usually 10 to 15 cm, with a semi-climbing, green, smooth herbaceous stem with uniformly oval shaped & slightly clogged opposite leaves. This plant is grown in the wet woods, waste land, quagmire, old field, ditches road side, in wet cultivated land and river bund.

MATERIALS AND METHODS

Solanum nigrum leaves were collected from local areas of Gwalior (M.P.) and shade dried at room temperature (24 ± 2˚C). The 10 g of dried and powdered leaves were extracted under the soxhelet apparatus with 150 mL each of methanol and water, respectively. The methanol was removed by using a rotary evaporator. Methanolic and aqueous extracts were stored at 8˚C for further use.

Qualitative preliminary Phytochemical analysis

Qualitative Phytochemical screening was performed by using Harborne method with slight modifications.[31]

Test for steroids: 1 ml of extracts was taken in 10ml of chloroform in test tube and equal volume of concentrated sulphuric acid was added by sides of the test tubes. Red colouration of upper layer & yellow colouration of lower sulphuric acid layer shows presence of steroids. ***Test for saponins:*** Extract was taken in a test tube with 20 ml of distilled water and vigorously agitated. The formation of layer of foam showed the presence of saponins.

Test for tannins: In 5ml of the extract on addition of few drops of 1% lead acetate formed a yellow precipitate indicated the presence of tannins.

Test for triterpenes: 10 mg of extract was mixed with 1 ml of chloroform later 1 ml of acetic anhydride was added by following the addition of 2 ml of conc. H_2SO_4. Development of Reddish violet colour indicated the presence of triterpenoids.

Test for alkaloids: 300 mg of extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature and examined the alcoholic layer for the pink colour, indicating the presence of alkaloids.

Test for flavonoids: Few drops of dilute sodium hydroxide were mixed in 1 ml of extract. A sharp yellow colour developed in the leaf extract, on addition of few drops of diluted acid, disappearing of colour indicating the presence of flavonoids.

Test for glycosides: 2 ml of glacial acetic acid with one drop of ferric chloride solution was added to 5ml of the mushroom extract. Addition of 1ml of concentrated sulphuric acid in reaction mixture formed a brown ring at the interface indicated the presence of cardiac glycosides.

Test for phenols: Plant Extract dissolve in 5 ml of distilled water and few drops of $FeCl_3$ were added, development of dark green/Bluish black confirmed the presence of Phenols.

Estimation of total phenolic content

The total phenolic amount of samples was detected by using the Folin-Ciocalteu test.[25] Gallic acid (20, 40, 60, 80, 100 mg/ml) was used as a standard in this work. Shortly, 400 µL of 0.5 N Folin-Ciocalteu tests, 20 µl methanolic plants (1 mg/ml), 680 µL of distilled water, and 20 µL of different concentrations of gallic acid were mixed and the mixture was vortexes. 400 µL of Na_2CO_3 (10%) solution was added after 3-minute incubation and again votexed· Then the mixture was incubated for 2 hours. Following the incubation time at room temperature, absorbance of the mixtures were determined at 760 nm. The concentrations of total phenolic compounds were measured as mg of gallic acid equivalents per g of the dry weight of the sample.

Estimation of total flavonoid content

The total flavonoid amount was determined by using the aluminum chloride test.[27] Quercetin was used as a standard. 4.3 ml methanol, 0.1 ml 1ml NH_4CH_3COO and 0.5 ml of Quercetin (20, 40, 60, 80, 100 mg/ml and 0.1 ml 10% $Al(NO)_3$ were kept in tubes and the they were mixed. Mixtures were incubated for 40 minutes. Following incubation, absorbance was determined at 415 nm. The total flavonoid contents of plants were defined as mg quercetin equivalents per g of dry Weigh of sample they were mixed. Mixtures were incubated for 40 minutes. Following incubation, absorbance was they were mixed. Mixtures were incubated for 40 minutes. Following incubation, absorbance was determined at 415 nm. The total flavonoid contents of plants were defined as mg quercetin equivalents per g of dry weight sample.

Scavenging effect on 1, 1-Diphenly-2- picrylhydrazyl (DPPH)

The Scavenging effect of mushroom extracts on 1, 1-Diphenly-2- picrylhydrazyl are calculated by using the method of Burits and Bucar, 2000.[38] The hydrogen atom or electron donating capability of the mushroom extract was measured from the discoloration of the purple-coloured methanolic solution of 1, 1- Diphenly-2-picrylhydrazyl (DPPH). This Spectrophotometric assay used the stable radical DPPH as a reagent.

A range of concentration from 100 µg to 500 µg/ml of methanol and aqueous extract of mushroom and 0.004 % methanol solution of DPPH were prepared.1000 µl extract was taken from every concentration and add 4.0 ml of DPPH.A blank was also prepared by adding 1000 µl methanol and 4.0 ml of DPPH. After an incubation period of 20-30 min Absorbance was

taken at λ = 517 nm (UV Vis spectrophotometer Make–systronics, Model no: 2701). Inhibition of free radical by DPPH in percent (%) was calculated by the given formula.

$$\% \text{ inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{(A_{\text{blank}})} \times 100$$

Where "A_{blank}" is taken as the absorbance of the control (Solvent) and "A_{sample}" is the absorbance of the test sample. Extract concentration provided 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration. Tests were carried out in triplicate.

Antimicrobial activity

Muller-Hinton Agar (MHA) plates were inoculated with 24 hours old bacterial and fungal culture. The ME & AqE were dissolved in dimethylsulfoxide (DMSO). Strptomycin and Fluconazole standard were prepared in conc. of 1mg/ml while the sample were prepared in 10 mg/ml or 10 µg/µl. Wells were bored using sterilized syringe of pore size 8 mm. various concentrations of the extracts (25µl, 50µl, 75µl and 100µl) were added into the sterile 8 mm diameter well. Incubation was made at 37º C for 24 hrs. Antibacterial & antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well using standard (Hi-Media) scale. The used bacterial and fungal strains were ***Escherichia coli*** **(MTCC 1687)**, ***Klebsiella pneumonia*****(MTCC 39),** ***Salmonella typhi*** **(MTCC 98)** ***& Candida albicans*** **(MTCC 227) respectively.** The experiment was repeated three times and the average values were calculated for antimicrobial activity. [32]

RESULTS

Qualitative preliminary screening of present Photochemicals

In the present study, phytochemicals were assessed qualitatively for Methanolic and Aqueous Extract of leaves from *solanum nigrum* plant as table 1: given below.

Table 1: Qualitative preliminary screening of present Phytochemicals.

Plant Extract	Saponin	Steroid	Glycosides	Terpenoids	Flavonoids	Phenol	Tannin	Alkaloids
ME	ND	ND	+	+	+	+	+	+
AqE	ND	ND	ND	+	+	+	+	+

Note: (+) indicated the presence of phytochemicals, (ND) indicated the no detection. Abbreviation: ME (Methanolic extract) and AqE (Aqueous extract).

Present study showed the presence of all the phytochemicals as given in table 1, except saponin and steroids for methanolic extract. However in methanol extract also gave the same finding as methanolic extract with no detection of saponin, steroid and glycosides. Thus the presence of phytochemicals indicated the presence of bioactive compounds.

Quantitative estimation of total phenolic content and total flavonoids contents

In the present study methanolic and aqueous extract were quantitatively analysed for total phenolic content & total flavonoid content.

Estimation of Total phenolic content

Phenolic compounds have an important role in radical scavenging activities against various free radicals. Total phenolic contents were estimated by using FC reagents in relation to Gallic acid equivalent as standard. Standard calibration curve was plotted against gallic acid with regression equation $y = 0.00112x–0.0067$ with R^2 of 0.994. Calibration curve was shown below.

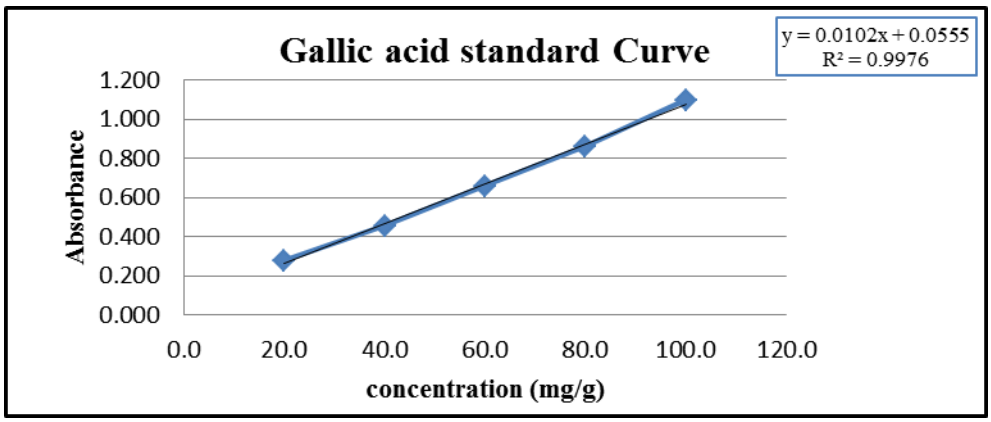


Figure 1: Gallic acid Calibration curve.

Total phenolic content were estimated for both methanolic and aqueous extractby of leaves of *Solanum nigrum* using Gallic acid standard curve. The estimated total phenolic compounds in methanolic (ME) and aqueous extract (AqE) of plant leaves were 161.4±1.2mg GAE/g and 208.5±1.7mg GAE/g respectively as shown in table 2 and Fig. 2.

Table 2: Total Phenolic content in plant leaves extracts of *S. nigrum*.

Plant leaf extract	Total phenolic content (mg GAE/g)
ME	161.4±1.2
AqE	208.5±1.7

Note: ME: methanolic extract, AqE: Aqueous extract & GAE: Gallic acid equivalent.

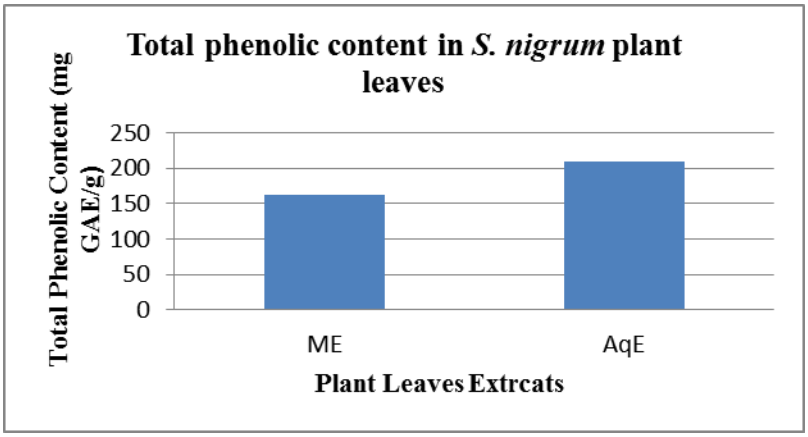


Figure 2: Total Phenolic contents in plant leaves extracts of *S.nigrum*.

Note: ME: methanolic extract, AqE: Aqueous extract & GAE: Gallic acid equivalent.

Total phenolic content in aqueous extract of leaves of *S. nigrum* was found higher comparatively to methanolic extract of leaves.

Estimation of Total flavonoids content

Flavonoids belong to secondary plant metabolites with important antioxidant properties. flavonoids play an important role in inhibition of lipid peroxidation and attenuation of processes involves in reactive oxygen species. Total flavonod contents were estimated by quercetin equivalent as standard. Thus standard curve was plotted against Quercetin with regression equation $y = 0.001x–0.076$ with R^2 of 0.994. Standard curve was shown in fig. 3 below.

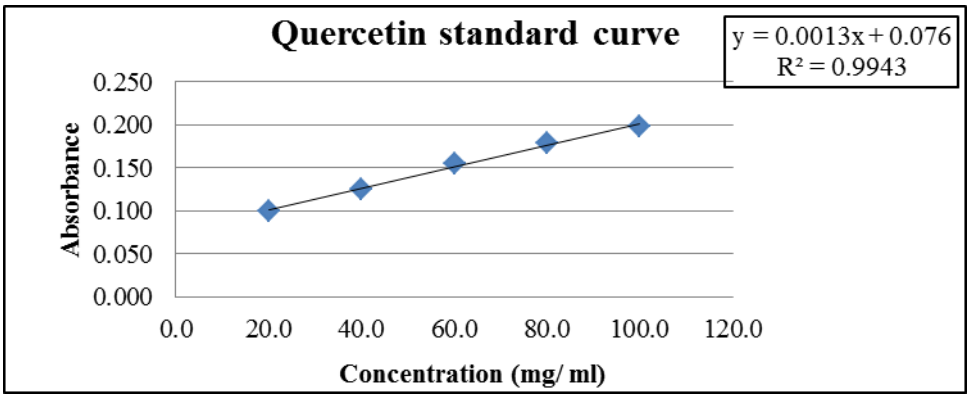


Figure 3: Quercetin standard curve.

Note: ME: methanolic extract, AqE: Aqueous extract & GAE: Gallic acid equivalent.

Table 3: Total flavonoid content in plant leaves extracts of *S. nigrum*.

Plant leaf extract	Total flavonoids content (mg QE/g)
ME	2.9±0.3
AqE	5.4±0.8

Note: ME: methanolic extract, AqE: Aqueous extract & QE: Quercetin equivalent.

Plant leaves of *S. nigrum* were extracted in methanol and aqueous solvents and flavoniids wer estimated in both extracts. Estimated total flavonoid contents (TFC) were 2.9±0.3 & 5.4±0.8 as shown in table 3. Aqueous extract was found with high total flavonoid content in comparison to methanolic extract for *S.nigrum* as shown in fig.4.

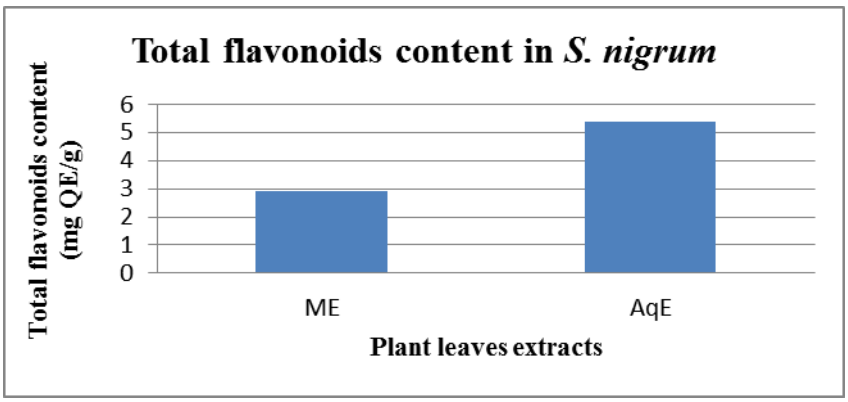


Figure 4: Total Flavonoid content in plant leaves extracts of *S. nigrum*.

Note: ME: methanolic extract, AqE: Aqueous extract & GAE: Gallic acid equivalent.

DPPH scavenging activity

Methanolic and Aqueous extracts of *S. nigrum* plant leaves were estimated for DPPH scavenging activity. Standard Ascorbic acids and extracts of *S. nigrum* were found effective against DPPH radicals as shown in fig.5. Ascorbic acid scavenge the DPPH radicals efficiently (37.89% - 80.08%) when used in a series of concentration (100µg/ml – 500 µg/ml). Moreover about the extracts, aqueous extract gave 25.24% - 62.43% inhibition as well as methanolic extract 15.48% - 54.49. Thus aqueous extract was showed the high DPPH scavenging activity comperative to methanolic extract.

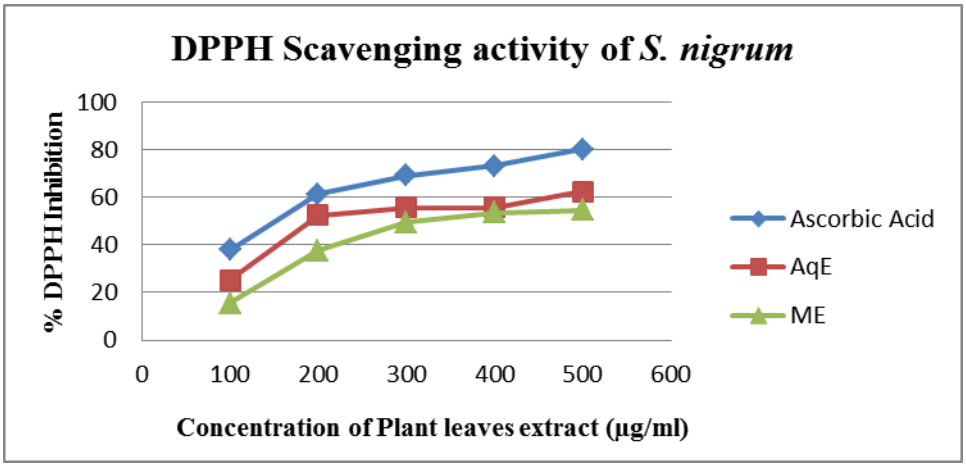


Figure 5: DPPH scavenging activity of ascorbic acid & plant leaves extracts of *S. nigrum*.

Note: ME: methanolic extract, AqE: Aqueous extract & GAE: Gallic acid equivalent.

IC_{50} value for Ascorbic acid and both methanolic and aqueous extracts were estimate from inhibition percentage. IC_{50} value is reduced as the inhibition percentage is increased. So the IC_{50} value is the reciprocal of inhibition percentage. The estimated lowest IC_{50} value was observed for Ascorbic acid (151.68±0.64 µg/ml). Estimated IC50 values for aqueous and metholic extracts were 298.21±0.8 µg/ml and 384.57±2.43 µg/ml respectively.

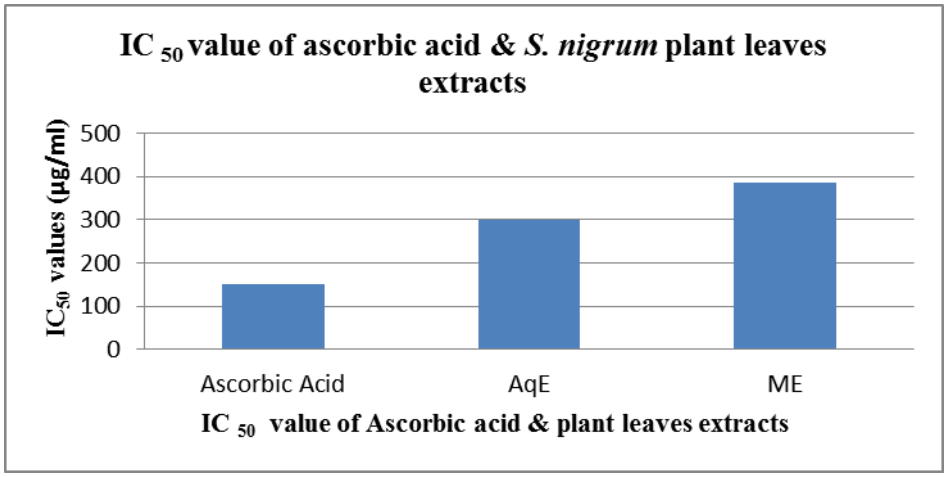


Figure 6: IC_{50} values of ascorbic acid and plant leaves extracts of *S. nigrum*.

Note: ME: methanolic extract, AqE: Aqueous extract & GAE: Gallic acid equivalent

Antimicrobial activity

Antimicrobial of Methanolic and Aqueous extract from the leaf part of *S. nigrum* were performed against some pathogens. Both extracts showed the antibacterial as well as antifungal activity at different concentrations as shown in table 4.

Table 4: Zones of inhibition of crude extracts of *S. nigrum* against pathogens.

mean zone of inhibition ±S.D. (mm)								
Bacteria /Fungi	***Escherichia coli***		***Klebsiella pneumonia***		***Salmonella typhi***		***Candida albicans***	
Volume (µl)	**AqE**	**ME**	**AqE**	**ME**	**AqE**	**ME**	**AqE**	**ME**
25	0	0	0	0	0	0	0	0
50	15±0.6	0	15±1.1	10±0.2	14±1.5	12±0.4	13±0.3	11±0.32
75	16±1.25	9±1.65	16±0.5	12±1.25	15±2.25	13±1.2	15±1.15	13±1.17
100	18±1.3	10±0.65	17±1.7	13±1.5	17±1.35	14±0.85	16±0.85	15±1.75
Streptomycin Standard (50µl)	20±0.3	19±0.65	18±1.9	16±0.5	19±0.4	17±0.35	NA	NA
Fluconazole	NA	NA	NA	NA	NA	NA	19±0.55	18±1.64

Note: ME: methanolic extract, AqE: Aqueous extract & GAE: Gallic acid equivalent.

In the present study aqueous extract (AqE) gave higher antimicrobial activity comparatively to methanolic extract. Aqueous extract gave highest inhibition zone 18±1.3 mm against *E.coli* at a concentration of 1000 µg/µl. However, methanolic extract observed with a minimum inhibition zone 10±0.65 mm against *E.coli.* AqE were ranged from 15±0.6 mm to 18±1.3 mm against *E.coli*, from 15±1.1 mm to 17±1.7 mm against *Klebsiella pneumonia*, from 14±1.5 mm to 19±0.4 mm against *salmonella typhi* and from 13±0.3 mm to 15±1.75 mm against *C. albicans.*

DISCUSSION

According to Ashrafudoulla et al., Tannin and alkaloids were found abundantly in the leaf extract of S. nigrum comparatively to flavonoids and saponin. Present study also exhibited the presence of Tannin, alkaloids, flavonoid, phenols and terpenoids.[41]

Aryal et al., 2019 estimated flavonoid contents 16.42 mg QE/g, and *Gę barowska et al., reported* 15.73 mg QE/g. Estimated flavonoid content,[35] in present study were 5.4±0.8 & 2.9±0.3 mg QE/g for aqueous and methonolic extract respectively. Gbadamosi and Afolayan reported the flavonoid content 3.63 ±0.44 mg QE/g and 2.31 ± 0.37 mg/ QE/g,[36] which supports the present study. Gbadamosi and Afolayan also

reported 233.14±2.42 mg TAE/g and 218.79±1.40 mg TAE/g in ethanolic and aqueous extract *S. nigrum.*[36] In aqueous extract also gave the 208.5±1.7 mg GAE/g.

Rohilla *et al.*, found the total phenolic content in ME, and AqE were 141.28 ± 0.66 mg GAE/g and 73.77 ± 0.24 mg GAE/g respectively.[33] Likewise, total flavonoid content (TFC) was found in ME and AqE were 48.73 ± 0.34 mg QE/g and 8.77 ± 0.32 mg QE/g. Thus, Rohilla *et al.*, findings varied from the present study.

Antioxidant capacity of plant extracts was measured against standard ascorbic acid and The results stated that AqE has highest antioxidant activity with % inhibition values ranging from 25.24 to 62.43%. While Rohilla *et al*., reported the % inhibition from 22.95±0.99 to 93.66±0.36% for ME.[33] Inhibition % of standard ascorbic acid was 37.89 to 80.08 % in the present study. Inhibition % of ME was 15.48 to 54.49 % in the present study. Which were different from findings of Rohilla *et al*. Rohilla *et al*., reported the IC_{50} 50.67±0.82 µg /ml,[33] which was lower than the present result 384.57 µg/ml for Methanolic extract.

Gę barowska et al., 2022 reported 4.8 mm Zone of inhibition against *E.coli* PCM 2057 through disc diffusion method at a concentration of 100 µg per Disc and also no zone were reported against *C.albicans* ATCC 10231.[34] In contrast, presently studied Aqueous and methanolic both extracts gave antifungal activity against *C. albicans* as shown in table 4. Rajathi D Modilal studied the aqueous extract of aerial part of S. nigrum and observed the inhibition zone of 10 mm against *K.pneuumia* at a conc. of 100 mg/ml.[37] 17 mm zone of inhibition was observed against the same bacteria in present study.

CONCLUSION

The main aim of this study was to highlight the phytochemicals status, antioxidant activity as well as antimicrobial study of plant leave extract of *S.nigrum*. In this study, preliminary screening of phytochecmicals exposed the presence of tannin, alkaloids, phenols, flavonoids and terpanoids. Quantitatively estimated total phenolic compounds and total flavonoid content were found in higher amount in AqE comparatively to ME of leaf. Similarly TPC & TFC, AqE also recorded for high DPPH scavenging activity as well as antimicrobial activity. Thus AqE of *S. nigrum* leaf gave the excellent results. Therefore further investigations are required to evaluate the presence of bioactive compounds in phytochemicals which leads these activities and their therapeutic mode of action.

SUMMARY

S. nigrum is a significant herbal plant, therapeutically important as Ayurvedic medicine in treatment of respiratory and gastric problems. The present study aimed to evaluate the AqE and ME of leaf of *S. nigrum* for qualitative preliminary screening of phytochemicals, quantitative estmation of total phenolic (TPC) and flavonoids contents (TFC), DPPH scavenging activity and Antimicrobial activity. AqE was recorded for higher TPC, TFC, a good DPPH scavenger and as potential antibiotic.

REFERENCES

1. Farnsworth NR, Aereele O, Bingel AS. Medicinal Plants in therapy, Bulletin of the World Health Organization, 1985; 63: 965-981.
2. Karthikeyan A, Shanthi V, Nagasathaya A. Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda Vasica* L. Int J Green Pharm., 2009; 3: 78-80.
3. Augusti KT. Therapeutic values of onion and garlic, Indian J Exp Biol., 1996; 34(3): 634-640.
4. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial Activity of Some Ethnomedical Plants used by Paliyar Tribe from Tamil Nadu, India. BMC Complementary and alternative medicine, 2006; 365.
5. Singh KBM, Sarkar PK and Chaturvedi OP: Improved livelihood, and Ecosystem Singh R, Dev I, Tewari RK, Rizvi RH, Garg KK, Singh AK, Dwivedi RP, Sridhar Services through Agroforestry based Watershed intervention in Bundelkhand region of Central India. In Agroforestry for Climatic Resilience and Rural Livelihood, Scientific Publishers, 2019; 242-250.
6. Yadav RB, Kumar A, Verma SK and Singh LJ: An Ethno botanical Survey of Medicinal Plants Used for the Treatment of Diabetes in Bundelkhand Region of India. Journal of Emerging Technologies and Innovative Research, 2021; 8(11): 203-209.
7. Nath V and Khatri PK: Traditional knowledge on ethno- medicinal uses prevailing in tribal pockets of Chhindwara and Betul Districts, Madhya Pradesh, India. Afr J Pharm & Pharmacol., 2010; 4(9): 662-670.
8. Maurya SK and Nigam G: Some Ethnomedicinal weed plants of Bundelkhand region, India. European Journal of Biomedical and Pharmaceutical Sciences, 2018; 5(2): 724-727.

9. Shankar R and Rawat MS: Conservation and cultivation of threatened and high value medicinal plants in North East India. International Journal Biodiversity Conservation, 2013; 5(9): 572-579.
10. Shankar R, Tripathi AK, Neyaz S and Anku G: Distribution and conservation of medicinal plants in Kohima, Mokokchung, Tuensang and Zunheboto districts of Nagaland. World Journal of Pharmceutical Research, 2016; 3(5): 1225-1237.
11. Mishra SK and Shankar R: Diversity and Conservation Strategies of Pharmaceutically important Medicinal plants of Bundelkhand region, India. American Journal of Pharmatech Research, 2019; 9(04): 35-48.
12. Rana P, Kumar A, Choudhary A, Kaur H and Singh R: The wisdom of prevention: Holistic, preventive herb approach for healing of the globe, The Pharma Innovation Journal, 2021; 10(1): 29-46.
13. Kumar S, Mittal A, Babu D and Mittal A: Herbal Medicines for Diabetes Management and its Secondary Complications. Curr Diabetes Rev., 2021; 17(4): 437-456.
14. Kikuchi, H.; Yuan, B.; Hu, X.; Okazaki, M. Chemo preventive and anticancer activity of flavonoids and its possibility for clinical use by combining with conventional chemotherapeutic agents. Am. J. Cancer Res., 2019; 9: 1517–1535.
15. Grosso, G.; Micek, A.; Godos, J.; Pajak, A.; Sciacca, S.; Galvano, F.; Giovannucci, E.L. Dietary flavonoid and lignan intake and mortality in prospective cohort studies: Systematic review and dose-response meta-analysis. Am. J. Epidemiol., 2017; 185: 1304–1316.
16. Godos, J.; Castellano, S.; Ray, S.; Grosso, G.; Galvano, F. Dietary polyphenol intake and depression: Results from the mediterranean healthy eating, lifestyle and aging (MEAL) study. Molecules, 2018; 23: 999.
17. Sak K. Intake of individual flavonoids and risk of carcinogenesis: Overview of epidemiological evidence. Nutr. Cancer, 2017; 69.
18. Imran M, Rauf A, Shah Z A. Saeed, F.; Imran A, Arshad M U. Ahmad B, Bawazeer S, Atif M, Peter DG, *et al*. Chemopreventive and therapeutic effect of the dietary flavonoid kaempferol: A comprehensive review. Phytother. Res., 2019; 33: 263–275.
19. Zanoaga O, Braicu C, Jurj A, Rusu A, Buiga R, Berindan-Neagoe I. Progress in Research on the Role of Flavonoids in Lung Cancer. Int. J. Mol. Sci., 2019; 20: 4291.

20. Yao M, Yuan B, Wang X, Sato A, Sakuma K, Kaneko K, Komuro H, Okazaki A, Hayashi H, Toyoda H, *et al.* Synergistic cytotoxic effects of arsenite and tetrandrine in human breast cancer cell line MCF-7. Int. J. Oncol., 2017; 51: 587–598.
21. Moga M A, Dimienescu O G, Arvatescu C A, Mironescu A, Dracea L, Ples L. The Role of Natural Polyphenols in the Prevention and Treatment of Cervical Cancer—An Overview. Molecules, 2016; 21: 1055.
22. Górniak, I.; Bartoszewski, R.; Króliczewski, J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem. Rev., 2019; 18: 241–272.
23. Djeussi, D.E.; Noumedem, J.A.; Seukep, J.A.; Fankam, A.G.; Voukeng, I.K.; Tankeo, S.B.; Nkuete, A.H.; Kuete, V. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complement. Altern. Med., 2013; 13: 164.
24. Subramani, R.; Narayanasamy, M.; Feussner, K.D. Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. Biotech, 2017; 7: 172.
25. Slinkard, K., & Singleton, V. L. Total phenol analysis: automation and comparison with anual methods. *American Journal of Enology and Viticulture*, 1977; *28:* 49-55.
26. Dhellot, J.R.; Matouba, E.; Maloumbi, M.G.; Nzikou, J.M.; Dzondo, M.G.; Nzikou, J.M.; Dzondo, M.G.; Linder, M.; Parmentier, M.; Desobry, S. Extraction and nutritional properties of *Solanum nigrum* L. seed oil. Afr. J. Biotechnol., 2006; 5: 987–991.
27. Chang, C C., Yang, M. H., Wen, H. M., & Chern, J. C. Estimation of total flavonoid content in propolis by two complementary colometric methods. *Yao Wu Shi Pin Fen Xi*, 2002; *10*(3): 178-182.
28. Subramani, R.; Narayanasamy, M.; Feussner, K.D. Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. Biotech, 2017; 7: 172.
29. Edmonds, J.M.; Chweya, J.A. Black Nightshades—*Solanum nigrum* L. and Related Species; International Plant Genetics Research Institute: Rome, Italy, 1997.
30. Guo T, He D, Liu Y, Li J, Wang F. Lanthanum promotes Solanum nigrum L. growth and phytoremediation of cadmium and lead through endocytosis: Physiological and biochemical response, heavy metal uptake and visualization. Sci Total Environ, 2024.
31. J.B. Harborne, *Phytochemical Methods: A guide to modern Techniques of Plant analysis,* Chapman and Hall, London., 1978; 75(4). L.A. Marcaurelle, C.W. Johannes, Application of.

32. Sridhar, T., Josthna, P. and Naidu, C., In Vitro Antibacterial activity and phytochemical analysis of *Solanum nigrum* (Linn.). An important antiulcer medicinal plant. J. Exper. Sci., 2011; 2(8): 24-29.
33. Rohilla P., Chhikara A., Dahiya P., Phytochemical Screening, in vitro Anti-bacterial, and Antioxidant Efficacy of Solanum virginianum L. Aerial Vegetative Parts Extracted in Four Solvents, Pharmacogn. Res., 2023; 15(4): 796-805.
34. Gębarowska, E.; Łyczko, J.; Rdzanek, M.; Wiatrak, B.; Pląskowska, E.; Gołębiowska, H.; Kuźniarski, A.; Gębarowski, T. Evaluation of Antimicrobial and Chemopreventive Properties and Phytochemical Analysis of *Solanum nigrum* L. Aerial Parts and Root Extracts. Appl. Sci., 2022; 12: 6845.
35. Aryal, S.; Baniya, M.K.; Danekhu, K.; Kunwar, P.; Gurung, R.; Koirala, N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants, 2019; 8: 96.
36. Gbadamosi and Afolayan, In vitro anti-radical activities of extracts of Solanum nigrum (L.) from South Africa Journal of Applied Biosciences, 2016; 98: 9240–9251.
37. Rajathi D., Modilal M., Anandan R., Sindhu R. & Logeshwari M.N., Screening Of *Solanum Nigrum* For Its Phytochemical And Antimicrobial Activity Against Respiratory Tract Pathogens. International Journal of Pure and Applied Zoology, 2015; 3(3): 210-215.
38. Burits, M. and Bucar, F. Antioxidant activity of *Nigella sativa* essential oil. *Phytother. Res.,* 2000; 14: 323–328.
39. Ayesha, M., Khan, Z., Ahmed, M. and Kashmiri, M., Chemotaxonomic value of Alkaloids in *Solanum nigrum* complex. *Pak.J.Bot.*, 2010; 42(1): 653-660.
40. Singh, K., Aali, N., Khan, M. and Ahirwar, V., Effect of *Solanum nigrum* on protein content of liver and kidney of albino rats. *Pharmacie Globale, IJCP*, 2011; 4(8): 1-3.
41. Ashrafudoulla, Md., Faysal Bellah, SM., Feroz Alam, Sayed Sakib Faisal, Hil Kafi, Md. Abdullah., Fuad Farhan. Phytochemical screening of *Solanum nigrum* L, *S. myriacanthus Dunal, Solanum melongena* and *Averrhoa bilimbi* in Bangladesh. Journal of Medicinal Plants Studies, 2016; 4(1): 35-38.

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PLANT AUTHENTICATION LETTR

Ref. No.: AETRL/MICRO/2023/007
Date: 17/10/2023

This is to certify that the plant specimens submitted for authentication have been examined and identified based on their morphological characteristics, taxonomic keys, and standard botanical references.

The details of the authenticated plant materials are as follows:

S. No.	Botanical Name	Common Name	Family	Part Examined	Authentication Status
1	*Solanum nigrum* L.	Black Nightshade	Solanaceae	Whole plant / Leaves	Authenticated
2	*Catharanthus roseus* (L.) G. Don	Periwinkle	Apocynaceae	Whole plant / Leaves	Authenticated
3	*Murraya paniculata* (L.) Jack	Orange Jasmine	Rutaceae	Leaves / Whole plant	Authenticated

Method of Identification:
The plant samples were authenticated using standard taxonomic procedures including macroscopic examination, morphological comparison with herbarium specimens, and reference to authenticated botanical literature.

Conclusion:
All submitted plant specimens have been correctly identified and authenticated as per the above-mentioned botanical names.

Enclosure: Plant sample for authentication.

Research Scholar: Mrs. Pooja Sahoo,D/O Ramkumar Sahu, Ph.D. Scholar of P.K. University, has submitted a plant sample which requires authentication for her research work.

Place: Gwalior

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Dr. R.K.Khare
Name & Signature of Botanist/Expert
Designation: Head of Department in Botany
Institution/Organization: SMS Govt. Model Science College Gwalior (M.P.)

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This is to certify that the microbial culture listed below has been issued from the Microbiology Laboratory of **Advanced Environmental Testing and Research Lab (P) Ltd.** to **Ms. Pooja Sahu**, Ph.D. Scholar, **P.K. University**, for research purposes.

Details of the culture are as follows:

Name of Culture: *E.coli, Salmonella Typhi, Staphylococcus aureus, Klebisiella pneumonia and Candida albican*s
Source: MTCC
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