

**Phytochemical Investigation, In- Vitro Antioxidant and
Anti-inflammatory activity of extract of *Cassia
Spectabilis***

A Thesis

Submitted towards the Requirements for the Award of Degree of

Doctor of Philosophy

In

**Pharmacy
Under the Faculty of Pharmacy**

By

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(Enrollment no. 16155817553)**

Under the Supervision of

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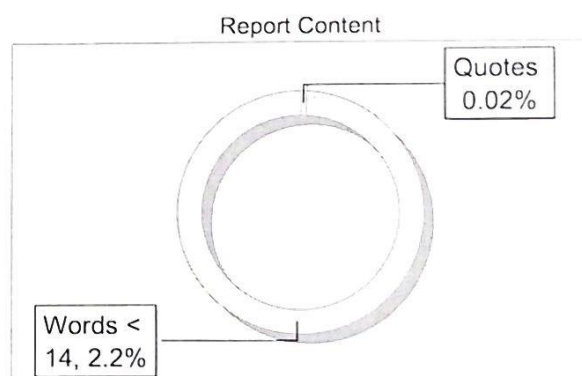
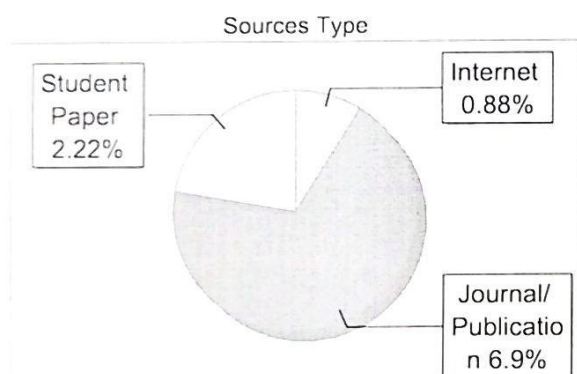
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Date:

(SEEMA WADHWANI)

Place:

1. INTRODUCTION

1.1 Human Health and Diseases

1.1.1 Inflammation

The body uses inflammation as a defense mechanism, a biological reaction of the immune system to damaging stimuli. Numerous pathogens (bacteria, viruses), poisons, toxic chemicals, and tissue damage can all cause inflammation (Medzhitov, 2010). These detrimental stimuli set off a chain of chemical signals that activate leukocytes, which in turn generate and release inflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1) and interleukin-6 (Jabbour *et al.*, 2009). TLR4, GM-CSFR, TNFR-1, TNFR-2, IL-6R, and other receptors are all impacted by and activated by these cytokines (Kaminska, 2005). Phosphorylation of several signaling molecules, including nuclear factor kappa-B (NF- κ B), mitogen-activated protein kinase (MAPK), and Janus kinase (Jak), is triggered by receptor activation. This phosphorylation in turn activates a number of transcription factors. Inflammatory mediator levels in resident tissue cells are controlled by this coordinated activation of signaling molecules, which also draws inflammatory cells from the bloodstream (Lawrence, 2009; Libby, 2007). Acute inflammation is therefore a defense mechanism that eliminates harmful stimuli, starts a healing process, and returns the body to equilibrium (Ferrero-Miliani *et al.*, 2007).

However, if acute inflammation is not controlled, it can develop into a chronic condition and serve as the basis for a variety of severe, long-term illnesses, including cancer, a number of neurodegenerative conditions such as Alzheimer's, Parkinson's, multiple sclerosis, lateral sclerosis, autoimmune diseases, diabetes, cardiovascular conditions, fibrosis, etc. (Nathan and Ding, 2010; Zhou *et al.*, 2016 & Chen *et al.*, 2018). Inflammatory mediators, regulatory circuits, and signaling pathways are common in most cases, despite the fact that the pathophysiology of various diseases varies. In every instance, 1) the cell membrane must have receptors that are internalized (to register and transmit signals through the plasma membrane into the cell interior); 2) the signaling cascade's members (transcription factors, various kinases) must come together in a platform known as "signaling organelles/endosomes" (Palfy *et al.*, 2012), where they are activated; and 3) activated transcription factors must move into the nucleus to control inflammatory genes, which leads to the synthesis of inflammatory cytokines and their release into the environment.

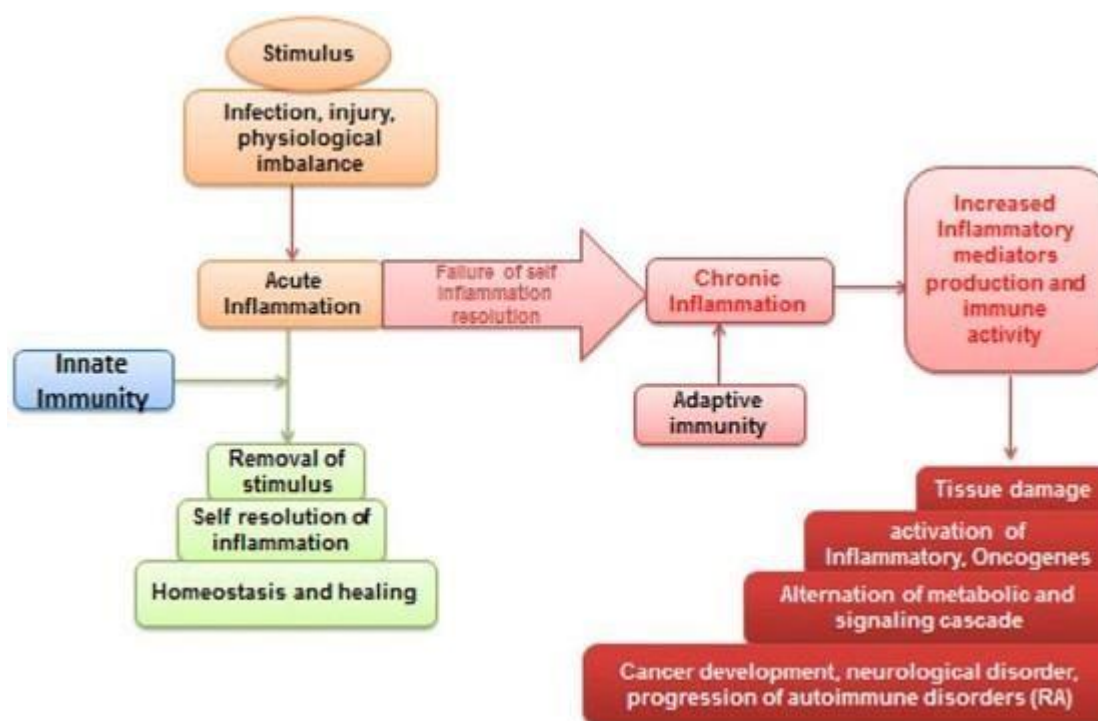


Fig 1.1: Overview of inflammation

1.1.2 Oxidation

Superoxide, hydroxyl, and nitric oxide radicals are examples of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that can harm DNA and cause lipid and protein oxidation in biological systems. However, excessive ROS and RNS are produced when exposed to cigarette smoke, alcohol, radiation, or environmental pollutants. This upsets the equilibrium between oxidation and anti-oxidation and leads to certain chronic and degenerative disorders. By preventing the start or spread of an oxidative chain reaction, scavenging free radicals, quenching singlet oxygen, and acting as reducing agents, increasing the consumption of exogenous antioxidants will lessen the harm brought on by oxidative stress. Antioxidants shield cells from the harm that free radicals can do (Betteridge, 2000; Pizzino *et al.*, 2017).

It has been demonstrated that antioxidants slow down or stop other molecules from oxidation. By removing radical intermediates and becoming oxidized themselves, they have the capacity to stop chain reactions and prevent oxidation reactions. The body is full of compounds that can reduce the harm caused by free radicals or prevent their development. Both internal and external sources are available for these antioxidants. Superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase are among the biological enzymes that produce antioxidants internally. On the other hand, they are obtained from

outside sources such as foods that contain minerals, polyphenols, vitamins A, E (alpha tocopherol), and C (ascorbic acid), which are mostly derived from plants (Clarkson and Thompson, 2000).

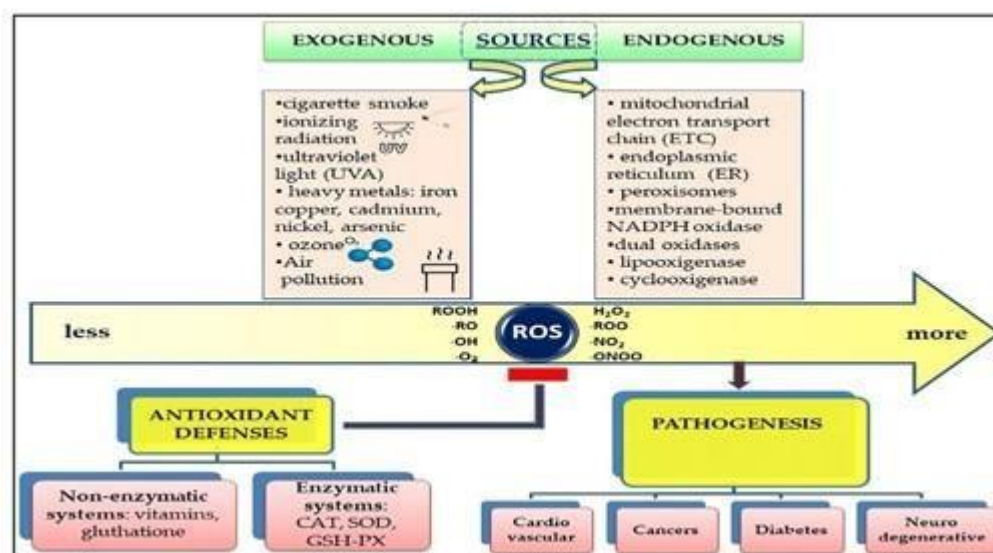


Figure 1.2: Pathophysiology of oxidative stress

Plants are rich in antioxidants that help protect against diseases linked to free radicals. The majority of the antioxidant compounds are made by plants as secondary metabolites. The term "plant-chemicals" can be used to actually describe phytochemicals. These are the chemical parts of plants that are not edible yet have many health advantages and the ability to prevent disease. They include non-essential nutrients, meaning the body does not need them to survive. In order to support life, plants create these compounds, which when consumed by humans have positive health effects. More than 1,000 phytochemicals are known to exist, and they are categorized as primary or secondary constituents according to how they function in plant metabolism (Kattappagari *et al.*, 2012).

Common sugars, amino acids, chlorophylls, purines and pyrimidines of proteins and nucleic acids, and other phytochemicals are categorized as primary ingredients. Alkaloids, flavonoids, terpenes, phenolics, lignans, plant steroids, curcurnines, saponins, and glucosides are among the other substances categorized as secondary ingredients. Phenolics are the most abundant of these secondary constituents, accounting for 45% of the secondary phytochemical contents of plants, followed by terpenoids and steroids (27%), alkaloids (18%), and miscellaneous substances (10%). Phytochemicals are important in nutraceuticals. They are bioactive components that preserve health and act as a link between

the pharmaceutical and food sectors. Phytochemicals serve a variety of purposes. Anti-inflammatory, antiparasitic, anti-allergic, antioxidant, antibacterial, antifungal, chemopreventive, neuroprotective, hypotensive, antiaging, and other special pharmacological actions are features of these substances. They boost the immune system, prevent carcinogens from forming, lower oxidation, slow the rate at which cancer cells grow, lower inflammation, induce apoptosis, stop DNA damage, and control hormones like estrogen and insulin, whose high levels are associated with a higher risk of breast and colon cancer (Lin, 1994).

The main dietary phenolics are polyphenols, which include flavonoids, phenolic acids (hydroxybenzoic and hydroxycinnamic acids), and polyphenols (hydrolyzable and condensed tannins). The most researched class of polyphenols is flavonoids. Legumes (beans and pulses), cereals (corn, barley, oats, sorghum, rice, and wheat), nuts, oilseeds (rapeseed, flaxseed, olive seeds, and canola), drinks (fruit juices, tea, coffee, beer, wine, and cocoa), and fruits and vegetables are the main food sources of polyphenols. Flavones, flavanols, and minor flavonoids (dihydroflavonols and flavanones) are subclasses of phenols.

By inhibiting the decomposition of hydroperoxide into free radicals and deactivating free radicals, they demonstrate their antioxidant capacity. Flavonoids have significant protective effects against oxidative stress-related illnesses. Inhibiting oxidases, reducing alpha tocopherol radicals, activating antioxidant enzymes, chelating metals, and transporting electrons to free radicals are some of its capabilities. The angiotensin converting enzyme (ACE), which causes blood pressure to rise, is blocked by them. They have also been shown to inhibit cyclooxygenase, which has been shown to create prostaglandins, and prevent enzymes that produce estrogen linked to breast cancer (Zhang *et al.*, 2015).

The antioxidant abilities and activities of different plants can be ascertained using a variety of techniques. In general, these techniques fall into two main groups: invitro and invivo. All experiments conducted outside of living organisms are included in the in-vitro methods. The ability of antioxidants to decrease iron from +3 to + 2 oxidation states (FRAP) or react with chromogenic radicals like 2,2'-azino-bis-3-ethylbenzothiazolin-6-sulfonic acid (ABTS⁺) or 2,2'-diphenyl-1-picrylhydrazyl (DPPH) is measured spectrophotometrically. Additionally, there are an increasing number of articles in the scientific literature evaluating

the effectiveness of medicinal plants, which have significantly contributed to the maintenance of health through a variety of processes (Shahidi and Zhong, 2015).

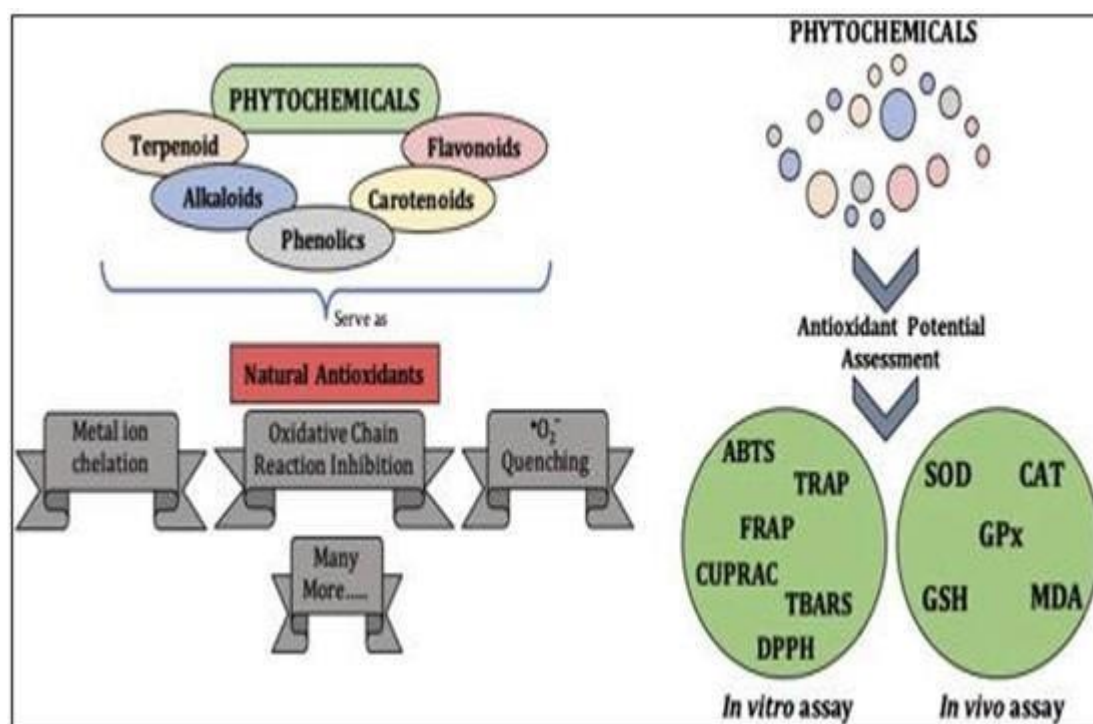


Figure 1.3: Phytochemicals as antioxidants

Reactive Oxygen Species and Inflammation

The endoplasmic reticulum, mitochondria, and peroxisomes are only a few of the cellular compartments that produce reactive oxygen species (ROS), which are byproducts of regular cell metabolism. Because of their potent oxidative properties, these reduced metabolites can seriously damage DNA in addition to oxidizing proteins, lipids, and other cellular components. When present in physiological concentrations, ROS serve as second messengers and signaling molecules involved in cell adhesion, differentiation, and growth. (Ticha *et al.*, 2017; Thannickai and Fanburg, 2000). ROS act as second messengers, oxidizing redox-sensitive cysteine residues to posttranslationally alter proteins (Chelomstiko, 2018). A growing body of research indicates that reactive oxygen species play a role in the development, course, and resolution of inflammatory reactions. Increased production of reactive oxygen species is a consequence of chronic inflammation. Thus, ROS control many kinds of transcription factors and kinases, including nuclear kappa B (NF- κ B), which are implicated in the activation of proinflammatory genes. (Flohe *et al.*, 1997; Ranneh *et al.*, 2017). Chronic inflammation causes an excess of reactive oxygen species (ROS), which damages cells and tissues and leads to severe illnesses (Henriquez-

Olguin *et al.*, 2015). Although neutrophil granulocytes are the immune system's main source of reactive oxygen species (ROS), other phagocytic cells, such as macrophages, can also produce ROS, which greatly raises the level of ROS. NADPH oxidase (NOX), which is attached to the phagosomal membrane, is activated and develops superoxide during post-bacterial phagocytosis (Leto *et al.*, 2006).

Development of ROS can also be induced by oxidative stress and altered metabolic activity. STAT3, a transcription factor that is translocated into the nucleus and controls the transcription of inflammatory genes, is activated by inflammatory cytokines (Walker and Smith, 2005). Inflammatory cytokines can directly promote development of ROS, as demonstrated by recent findings that a pool of Ser727 phosphorylated STAT3 translocates into the mitochondria and stimulates ROS production (Balic *et al.*, 2020; Wegrzyn *et al.*, 2009 & Gough *et al.*, 2009). The production of inflammatory cytokines and ROS are most likely coordinated, coordinated, and interactive during chronic inflammation, amplifying the effects of one another.

1.1.3 Microbial Infectious Disease

Humans are usually negatively impacted by infectious diseases since they are pathological conditions caused by specific infectious agents, which can be viruses, bacteria, fungi, or protozoa. These agents affect the part of the body or tissues and are characterized by a set of signs and symptoms. Although A.V. Leeuwenhoek is credited with discovering bacteria in 1675, a German physician named Robert Koch found that certain organisms are linked to specific diseases. The doctor then developed four criteria to prove that specific agents caused a particular disease, which he called Koch's Postulates. These criteria elevate medical diagnosis to a new level because they make it more accurate to determine the appropriate medication for various diseases (Baker and acharya, 2004).

Infection, host, pathogen, and pathogenicity are all associated with infectious disease. An infectious disease can spread through a variety of methods, and it can go through several stages. The secret to curing this infectious disease lies in modern microbiology, which is founded on molecular genetics. The pivotal moment in the history of medical study that has persisted to this day was the discovery that DNA was the material responsible for the transmission of hereditary features. In the current situation, advances in the detection and management of many diseases are driven by new and enhanced methods (HorneF *et al.*, 2002).

1.1.4 Diabetes mellitus

Due to either insufficient insulin, impaired insulin action, or both, diabetes mellitus is a combination of diverse conditions that frequently manifest as bouts of hyperglycemia and glucose intolerance (Sicree *et al.*, 2006). Defects in the regulatory mechanisms for the storage and mobilization of metabolic fuels, such as the catabolism and anabolism of proteins, lipids, and carbohydrates resulting from impaired insulin action, secretion, or both, cause these difficulties (Shillito, 1988 & Votey and Peters, 2004).

Diabetes mellitus is classified according to its clinical presentation and etiology. Type 1 diabetes, type 2 diabetes, gestational diabetes, and other specialized forms are the four types or classifications of diabetes mellitus. Despite being the most common form of diabetes in younger age groups in the majority of wealthy nations, type 1 diabetes is thought to represent a very small portion of the overall burden of diabetes in a population. Type 1 diabetes is becoming more common in both wealthy and developing nations. Additionally, there will soon be a movement toward type 1 diabetes in children at younger ages.

Type 2 diabetes accounted for 85-95% of all cases of diabetes in high-income nations, with an even greater prevalence in underdeveloped nations. It is closely linked to target cells and tissues' inappropriate use of insulin. It is currently a widespread and significant worldwide health issue.

According to WHO (1994), this issue has been made worse by a number of factors, including dietary changes, decreased physical activity, aging populations, urbanization, rapid cultural and social dynamics, and other undesirable behavioral and lifestyle trends. Diabetes mellitus and milder forms of glucose intolerance, especially impaired glucose tolerance, are now prevalent in nearly every population worldwide, and epidemiological data indicates that the prevalence of diabetes will probably keep rising in the absence of efficient prevention and control initiatives (World Health Organization, 1994).

It was estimated that 285 million persons aged 20 to 79 had diabetes globally in 2010, with around 70% of those individuals residing in developing countries. By 2030, this number is anticipated to rise to over 438 million. Furthermore, it is anticipated that 472 million people, or 8.4% of the adult population, will have IGT by 2030.

Numerous organ failures and escalating metabolic consequences such as retinopathy, nephropathy, and/or neuropathy are among the incapacitating effects of diabetes mellitus (Piero, 2006).

Diabetes increases the risk of peripheral vascular, cerebrovascular, and cardiovascular disorders. Diabetes is caused by a number of pathogenetic processes, such as the death of pancreatic P-cells, which reduces the sensitivity of insulin action (World Health Organization, 1999).

1.1.5 History of diabetes mellitus

Diabetes mellitus has been known since antiquity, its pathophysiology has mostly been clarified in the 20th century, and its therapies have been known since the Middle Ages. It was nearly impossible to diagnose non-progressing Type II diabetes (Patlak, 2002). In 1889, Oskar Minkowski and Joseph Von Mering discovered the pancreas's involvement in diabetes. When the pancreas was removed entirely from the canines, they discovered that the dogs showed every symptom of diabetes and passed away soon after. Edinburgh, Scotland's Sir Edward Albert Sharpey-Schafer proposed in 1910 that diabetics were deficient in a particular molecule that the pancreas normally developed. Later, the name insulin was suggested for this substance (Himsworth, 1936).

The work of Von Mering and Minkowski was best repeated in 1921 by Frederick Grant Banting and Charles Herbert, who went one step further and demonstrated that they could reverse the induced diabetes in dogs by administering an extract from the pancreatic islets of Langerhans of healthy dogs. This was a breakthrough in understanding the pancreas' endocrine function in insulin metabolism and existence (Banting *et al.*, 1922). At the University of Toronto in Canada, these researchers went on to separate insulin from the pancreases of cows, which made diabetes mellitus treatable. The first clinical patient was treated in 1922. Sir Harold Percival (Harry) Himsworth distinguished between what is now called type I and type II diabetes in 1935 (Himsworth, 1936).

1.2 Herbal medicine

It is not hyperbole to state that the usage of herbal remedies is as old as humanity itself, having been utilized for centuries to treat a wide range of illnesses. India has long used herbal remedies in its officially recognized alternative medical systems, including Ayurveda, Unani, Sidha, homeopathy, and naturopathy. Over 2500 different plant species are now employed as herbal medicines in India. Herbal remedies have been utilized as folk remedies for over 3,000 years, or they are employed indirectly in the preparation of modern pharmaceuticals. Approximately 80% of the world's population still receives their primary medical treatment from herbal medicine today, particularly in underdeveloped nations. In

affluent nations like France and Germany, the use of plants as prescription medications has also suddenly increased. There is worry, though, that not all herbal remedies are as safe as they are said to be. The practice of traditional medicine over the years has given us useful guidelines for choosing, preparing, and using herbal treatments. To be a competitive alternative to western medicine, the same rigorous clinical and scientific approach must be used to confirm the efficacy and safety of curative treatments (Thirumal *et al.*, 2012).

Eighty percent of people worldwide utilize herbal medications for their basic medical needs, according to the WHO. Since the beginning of civilization, herbal remedies have been used as a component of human culture to fight disease (Sudha and Mathanghi, 2012). Rheumatoid arthritis can often be effectively treated with a variety of historically used herbal medicinal plants that contain active substances such as flavonoids, terpenes, sesquiterpene lactones, phenols, and carotenoids, as well as anti-inflammatory and antioxidant properties.

While many allopathic drugs are effective in managing symptoms and slowing the progression of illnesses, synthetic drugs have been shown to have major negative effects on patients in clinical settings. In addition to evaluating the effects of plant extracts containing phytoconstituents such as flavonoids, terpenoids, alkaloids, and sterols that embrace antioxidant and anti-inflammatory activities, clinical and preclinical investigations have documented the mechanism of herbal plants. Studies that have been published have shown that using herbal plants to treat arthritis is more successful than using synthetic drugs (Singh *et al.*, 2014).

Traditional herbal medicines are defined by the World Health Organization as naturally occurring compounds derived from plants that have undergone little to no industrial processing and have been employed in local or regional healing practices to treat sickness. Because of its natural origin and fewer adverse effects, traditional herbal medicine and its preparations have been used extensively for thousands of years in both developed and developing nations. Originally, these remedies were unrefined herbal formulations including tinctures, teas, poultices, powders, and other concoctions. Many aspects of modern medicine have their roots in the ancient use of plants for therapeutic purposes. Aspirin (willow bark), digitoxin (foxglove), morphine (opium poppy), quinine (cinchona bark), and pilocarpine (Jaborandi) were among the early medicines that were based on clinical, pharmacological, and chemical studies of these traditional medicines, which were primarily derived from plants. For primary healthcare, between 75 and 80 percent of the

world's population still uses herbal medicine, mostly in underdeveloped nations. The usage of herbal therapies is two to three times more common worldwide than that of conventional drugs, according to the World Health Organization (WHO) (Pal and Shukla, 2003).

1.2.1 Advantages of Herbal medicine

- Low/Minimum cost
- potency and efficiency
- enhanced tolerance
- More protection
- fewer side-effects
- complete accessibility
- Recyclable

1.2.2 Future importance of Herbal Medicine

Of the approximately 350,000 species of plants that now exist (including ferns, bryophytes, and seed plants), 287,655 species have been identified as of 2004. Both humans and other animals consume only a small portion of these (1-10%). There may be many more that are utilized for therapeutic purposes. The World Health Organization (WHO) has expressed a strong interest in recording the medicinal plant usage of various tribes worldwide. In order to document the ethno-medical information on medicinal plants, many developing nations have stepped up their efforts. There is now more scientific data to support the claims made by tribal healers about Indian plants.

People will be more knowledgeable about effective drug therapy and better health status once these regional ethno-medical preparations have undergone scientific evaluation and are appropriately distributed (Verma and Singh, 2008).

Before it is permanently lost, the traditional knowledge system must be researched, recorded, conserved, and applied for the good of humanity. The market size of the herbal industry, which is currently valued at Rs. 7,500 crores (75 billion), is expected to double to Rs. 15,000 crores by 2015, according to projections made by the Associated Chambers of Commerce and Industry of India (ASSOCHAM). This is because the industry is expected to grow at a compound annual growth rate of more than 20% going forward. According to a 2015 ASSOCHAM report on the herbal industry and global market, India has a wealth of medicinal plants and traditional knowledge in this field, but its share is now regarded as

being extremely small. According to a crude assessment of the potential, India can easily produce value-added goods worth about Rs. 150 billion and generate raw materials worth about Rs. 300 billion. India is therefore hardly able to utilize half of its potential. It's interesting to note that there is a ready market for both herbal goods and raw materials (herbs) worldwide (Dhar *et al.*, 2002).

1.2.3 Research in Herbal Medicine

The process of isolating and determining the chemical structures of bioactive compounds from an extract might take months or even years in the past, making the drug discovery of bioactive compounds from plants a laborious task. These days, time is greatly reduced by new methods like robotics, higher magnetic field-strength nuclear magnetic resonance (NMR) equipment, high-performance liquid chromatography (HPLC) connected to mass spectrometry (MS)/MS (liquid chromatography, LCMS) (Salim *et al.*, 2008).

The demand for herbal medicine, which is mostly based on traditional knowledge, is growing, making it impossible to regulate the protection of natural product intellectual property rights. Access to basic lead resources, benefit sharing throughout the commercial phase, and other procedures have become increasingly complicated in many countries as more nations have ratified the Convention on Biological Diversity (CBD). Regardless of the issues behind these procedures, they frequently slow down the discovery process at different stages (Katiyar *et al.*, 2012).

Even though drug development from medicinal plants has clearly been successful, there are still many obstacles to overcome in future efforts. To stay up with other drug discovery initiatives, pharmacognosts, phytochemists, and other scientists studying natural products will need to constantly enhance the caliber and number of compounds that make it into the drug development stage.

It has been reported that the drug discovery process takes an average of 10 years or more and costs over 800 million dollars. A significant amount of effort and resources are devoted to the many leads that are rejected throughout the drug discovery process. Indeed, it has been calculated that just one out of every 5,000 lead compounds will make it through clinical trials and receive acceptance for use (Balunasa and Kinghorn, 2005).

1.2.4 Regulation of Herbal Medicine

Herbal treatments include everything from plants that people gather on their own and use for health purposes to authorized pharmaceuticals. Over 80% of herbal sales are believed to

be unlicensed preparations, placing many herbal goods in the far end of this regulatory range. According to European Union law, herbal products must be approved for sale if they are manufactured industrially and fall under the category of therapeutic products due to their presentation, function, or both. Sadly, it is challenging to define a distinct boundaries. Due to two factors, a large number of medicine-like products on the British herbal market are still unregistered: the hefty license charge and the possible lack of enough data on efficacy, safety, and quality (De Smet, 1995).

In Germany, where more than 300 monographs have established regulatory evaluations of medicinal herbs, and in France, where more than 200 herbs have been designated as permissible constituents of phytomedicines, special licensing procedures for herbal medicines are already in place. Australia created a comprehensive strategy for the herbal sector that includes a range of non-Western herbs (De Smet, 1995). The National Institute of Medical Herbalists, based in Exeter, UK, is the primary regulator and registration agency for Western herbal practitioners. Only those who have completed authorized courses are permitted to join the register, and a rigorous code of ethics is upheld. In an effort to promote better unity among herbalists, the European Herbal Practitioner Association was established. It currently has approximately 1000 members. Nevertheless, it does not yet have a published code of ethics or official membership screening standards (Vickers and Zollman, 1999).

1.2.5 Distribution of Herbal medicine

The medicinal plants are found in a variety of habitats and landscape features, according to the distribution analysis. Almost 70% of India's medicinal plants are found in tropical forests in the Himalayas, Aravalis, Vindhya, Chota Nagpur plateau, and Eastern and Western Ghats. The Kashmir region of the Himalayas is tucked away in the Northwestern folds of the Himalayas, which was recently named a global biodiversity hotspot (Mittermeier *et al.*, 2005). Geologically speaking, it is a younger yet important portion of the main Himalayan range. A good number of medicinal plants are among the region's many floral treasures.

Kashmir and Ladakh are locations to 111 of the medicinal plants mentioned by Kaul (Kaul, 1997). In addition, he discussed in his book therapeutic properties of 291 species of local medicinal plants. However, Kashmir's medicinal flora has not received enough study, and it may have at least twice as many as this. *Dioscorea deltoidea*, *Rheum Emodi*, *Arnebia*

benthamii, *Inula racemosa*, *Datura stramonium*, *Aconitum heterophyllum*, *Artemisia* spp., *Podophylum hexandrum*, *Juniperus macropoda*, *Hypericum perforatum*, *Hyoscyamus niger*, *Sassurea* spp., and *Picrorhiza kurroa* are some of the most significant medicinal plants in the Kashmir Himalaya. These plants grow in large quantities in places like Yusmarg, PirPanjal, Sonamarg, Gurez, Lolab valley, Gulmarg, Khilanmarg, Pahalgam, and Tilail valley. In addition, several fragrant and therapeutic plant species are cultivated in the Kashmir Valley's various high-altitude areas. Caraway (*C. cervi*), saffron (*Crocus sativus*), siya zira (*Bunium persicum*), garlic (*Allium sativa*), coriander (*Coriandrum sativum*), mint (*Mentha* spp.), fennel (*Foeniculum vulgare*), and hare's foot (*Trigonella foenum-graecum*) are among the significant fragrant plant species. Standardized plant extracts contain a lot of these plants.

1.2.6 Herbal Medicine as anti -inflammatory agent

Since there are numerous herbal plants that have been suggested to have anti-inflammatory properties and it would be impossible to cover them all in one piece, we have compiled papers that discuss the herbs for which there is available and therapeutically meaningful data. In the Indian traditional medical system, herbal therapy is commonly used to alleviate inflammation. It is also one of the most significant components of contemporary medicine. Because scientific clinical research has shown how significant herbs are for reducing inflammation. Herbs that have proven effective in anti-inflammatory clinical and experimental trials. Numerous mechanisms underlie the inflammatory process, and a range of drugs are used to treat them.

There are numerous cytokines and enzymes (such phospholipase A2) that have been implicated as inflammatory mediators, along with mediator release, vasodilation, cell migration, and tissue damage. The experimental animal investigations showed that pro-inflammatory molecules including cytokines and PG2 are inhibited by the possible role of herbal active compounds (Shazhni *et al.*, 2018; Cao *et al.*, 2017).

1.2.7 Mechanism of antimicrobial activity of Herbal Medicine

Overall, the natural products contain higher levels of scaffold variation, molecular complexity, stereo chemical abundance, diversity in the ring system, and carbohydrate contents, while having lower levels of nitrogen, sulfur, phosphorus, and halogens. Apoptosis, mitosis, immunological response, and signal transduction can all be effectively modulated by plant products because they can alter or suppress protein-protein interactions.

Plant extracts provide a variety of chemically complicated phytochemicals that bacteria cannot become resistant to.

Traditional medicine is a clear example of how biologically active compounds can kill germs and prevent the sickness from progressing further. New studies involving the separation and purification of plant active chemicals using various conventional methods and techniques started in the early 19th century. For instance, quinine from *Cinchona calisaya* Wedd, cocaine from *Erythroxylum coca* Lam., and codeine and morphine from the poppy plant (*Papaver somniferum* L.) were discovered and are currently in use as analgesics. There are various ways in which secondary metabolites might impact the microbial cell. These include:

- Modifications to the structure and function of cell membranes
- Interference with the metabolism of intermediaries
- The synthesis and function of DNA and RNA are disrupted
- Quorum sensing, or regular cell communication, is disrupted
- Cytoplasmic constituent coagulation is induced

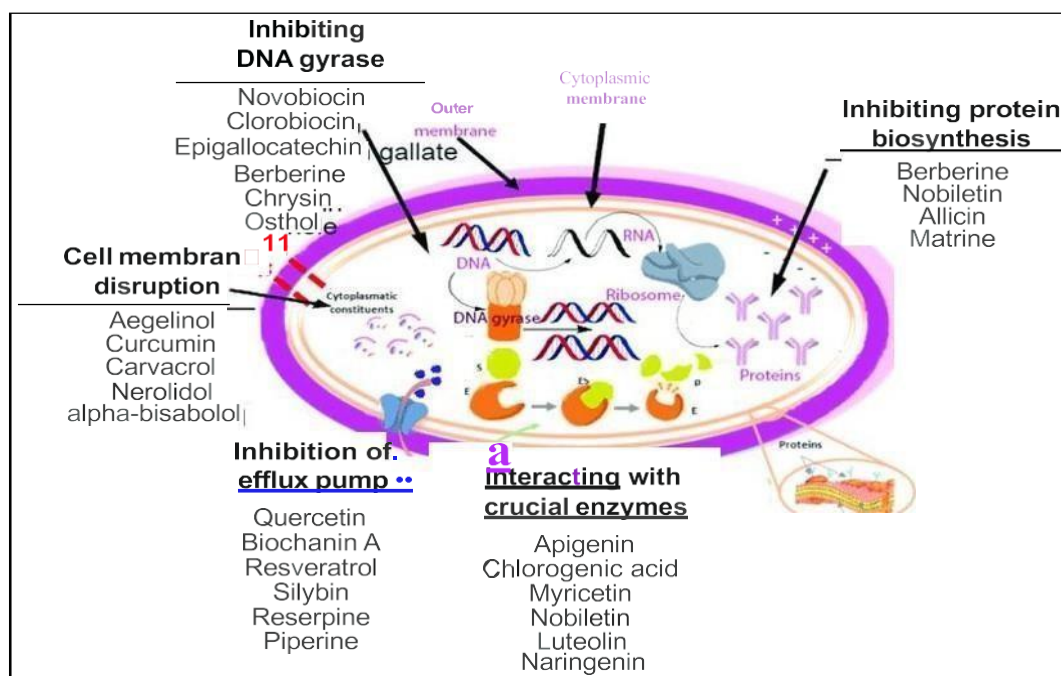


Figure 1.4: Antimicrobial activity of plant extract

In addition to being rich sources of new, physiologically active compounds with antibacterial properties, plants also contain thousands of elements. Ethnobotanical research on plants is crucial for contemporary medicine, but its value cannot be overstated if procedures are not standardized to produce comparable and repeatable outcomes. The

search for plant items with antibacterial properties is now underway. Standardization of extraction techniques and in vitro antimicrobial activity testing would be beneficial in order to improve the interpretation of results and make the search for novel biologically active plant products more methodical. To determine how effective they are at reducing the prevalence of disease in humans, plants, and crops, thousands of phytochemicals that have inhibitory effects on all kinds of microbes in vitro ought to undergo in vivo research. For an intriguing lead compound to be fully developed into a commercially viable product, effective partnerships between pharmacologists, physicians, plant pathologists, and microbiologists are essential.

1.2.8 Herbal medicine for diabetes

Due to growing research in traditional medicine, plant-based medications that are eco-friendly, bio-friendly, affordable, and generally safe have emerged from the fringe to the mainstream in recent decades. The review by Atta-ar-Rahman, who has included over 300 plant species recognized for their hypoglycemic microperties, is the most instructive of the many literature evaluations on anti-diabetic herbal compounds written by various writers. *Momordica charantia*, a member of the Cucurbitaceae family, is one such plant. The World Health Organization has classified 21,000 plants that are used medicinally worldwide. Of the 2500 species found in India, 150 are employed on a reasonably considerable scale for commercial purposes. Known as the world's botanical garden, India is the world's largest producer of medicinal plants (Modak *et al.*, 2007).

Some medicinal plants have recently been utilized empirically as antidiabetic and antihyperlipidemic treatments, with reports of their benefits in diabetes being made all around the world. Diabetes and its associated consequences remained a significant medical issue even with the availability of well-known antidiabetic medications on the pharmaceutical market. These plants' capacity to restore the function of pancreatic tissues by increasing insulin production, blocking intestinal glucose absorption, or facilitating metabolites in insulin-dependent processes is thought to be the cause of their antihyperglycemic actions. Glycosides, alkaloids, terpenoids, flavonoids, carotenoids, and other compounds found in most plants are commonly thought to have antidiabetic properties.

1.3 Phytochemistry

The study of phytochemicals produced by plants, including their structural makeup, biosynthesis pathways, roles, and mechanisms of action in living systems, is known as phytochemistry (Egbuna *et al.*, 2019). According to their structure and biosynthesis routes, the chemical compounds found in plants, commonly referred to as phytochemicals, are divided into primary and secondary metabolites (Velu *et al.*, 2018). Primary metabolites are extremely homologous in their structural characteristics. Additionally, they are essential for plant growth, metabolism, development, and survival. Among the primary metabolites are proteins, carbohydrates, lipids, and nucleic acids (Erb and Kliebenstein, 2020; Eastwood, 2001 & Bentley, 1997). Lipids are essential for the integrity of cells and organelles because they control cell metabolism (Kim, 2020). Similar to hormones, carbohydrates are signaling molecules that play a crucial role in energy production and storage (Minchin and Lodge, 2019). In addition to being components of genetic information storage and transmission, nucleic acids also stimulate the creation of proteins (Pagare *et al.*, 2015). Although secondary metabolites are not essential to plant survival, they do influence how plants interact with their environment and, consequently, how they survive in the ecosystem. Examples of secondary metabolites include anthocyanins, cardiac glycosides, polyphenols, flavanoids, alkaloids, and saponins. Specific plants and plant parts have unique phytochemicals. They protect plants from harmful elements like insects, bacteria, and diseases as well as from environmental conditions like high temperatures and salinity. They are also in charge of giving the plant its color (Martinez *et al.*, 2017 & Sharma *et al.*, 2019). Conventional plant-based remedies are chosen over synthetic ones because they are less likely to have adverse effects and are environmentally friendly. Plant compounds known as phytochemicals have a wide range of bioactivities and numerous health benefits. They are also used in traditional medicine to treat a number of illnesses and conditions (Konappa *et al.*, 2020 & Sharma *et al.*, 2019). A wide range of bioactivities, including antioxidant, anticancer, analgesic, anti-microbial, anti-diarrheal, and others, are possessed by plants that contain different bioactive phytochemicals (Ganesan and Xu, 2017).

1.3.1 Bioactive compounds

Foods contain phytochemicals known as bioactive compounds, which have the power to alter metabolic processes and improve health. Antioxidant activity, enzyme inhibition or induction, receptor activity inhibition, and gene expression induction or inhibition are some of its positive impacts. Each bioactive chemical has a very different bioaccessibility and

bioavailability, and the compounds that are most prevalent in the fruit that is consumed are not always the ones that result in the highest amounts of active metabolites in the target tissues (Manach *et al.*, 2005).

1.3.2 Classification of phytochemicals

Secondary plant metabolites known as phytochemicals can be categorized by their chemical makeup (whether or not they contain nitrogen), their structure (such as rings or sugar), their biosynthesis (such as phenylpropanoid, which produces tannins), or how soluble they are in different solvents.

Phenolic Compounds

The most important class of chemicals produced by plants is phenolic compounds, which are found in a wide variety of plants. They are vital defensive compounds that function as secondary metabolites. Phenolics have a variety of advantageous qualities for people, but their antioxidant properties are particularly important in defining their role as defenses against diseases brought on by free radicals. Flavonoids, polyphenols, and phenolic acids are the three primary types of phenolics that are present in diets.

A class of chemicals known as flavonoids is produced by joining two phenyl rings with phenolic hydroxyl groups through the middle three carbon atoms. Flavonoids are commonly found, low in toxicity, and safe to incorporate in diets. They have potent antioxidant and anti-inflammatory properties. Although flavonoids' antioxidant properties are well established, they can also have anti-inflammatory effects. In addition, they interfere with the nuclear factor- κ B transcription factor and the production of pro-inflammatory cytokines such as nitric oxide and eicosanoids. By regulating the connection between the gut and joints, inhibiting inflammatory responses, and modulating many targets linked to immune control, flavonoids reduce the symptoms of RA (Koche *et al.*, 2016; Dai J. and Mumper, 2010 & Li *et al.*, 2014).

Coumarin

Vascular plants are abundant in coumarins (2H-benzopyran-2-one), a class of simple phenolic compounds made up of a big phenolic substance created by the fusion of a benzene ring and a-pyrone rings. Originally discovered in the tonka bean (*Dipteryx odorata*), they have since been found in about 150 species across 30 families (Venugopala *et al.*, 2013). It has been determined that over 1300 coumarins are SMs in plants; these coumarins serve a number of purposes in numerous plants, mostly in defense (Iranshahi *et*

al., 2009). In addition to its protective effects on fungus and herbivores, coumarins also have antibacterial action because of a particular class of bioactive compounds. They are found in all plant sections, although seeds have the largest concentrations of them (Evans and W.C, 2009). Natural coumarins can be divided into six groups according to its chemical structure: simple (like fraxin), furano (like imperatorin), dihydrofurano (like anthogenol), linear-type (like grandivittin), phenyl (like inophyllum), and bicoumarins (like dicoumarol).

Lignin

Lignin, which makes up almost 30% of the organic carbon in the biosphere, is the most prevalent biopolymer on Earth after cellulose. One of the intricate racemic heteropolymer components of the cell wall is lignin, which is made up of three p-hydroxycinnamoyl alcohol monomers, also known as monolignols, and has a high molecular weight (Cesarino *et al.*, 2012). Lignin is a significant SM that generally supports plant growth, tissue and organ development, loading resistance, and tolerance to biotic and abiotic stress. Because lignification makes plant tissues physically tough, making them indigestible to insects and other herbivores, it is a defense mechanism used by plants against pathogens and herbivores.

Furanocoumarins

Known for their phytotoxicity, furanocoumarin compounds are mostly found in Apiaceae and Rutaceae species. Furanocoumarins are partially produced by combining dimethylallyl pyrophosphate with 7-hydroxycoumarin in the phenylpropanoid and mevalonate pathways. These compounds typically turn hazardous when exposed to UV-A rays, which causes a higher electron energy state to be activated.

Furanocoumarins penetrate the DNA double helix as a result of this activation and attach to pyrimidines, which results in transcription inhibition and cell death. Furanocoumarins also have mutagenic and carcinogenic qualities (Mazid and Khan, 2011). Numerous insects' eating habits and physiological development are also influenced by furanocoumarins (Gebreyesus, 1980). It is found largely in plant roots leaves and fruits and storage in the form of essential oil. It is highly effective at making cells more sensitive to UV and visible light from the sun (Del *et al.*, 2014).

Flavonoids

Approximately 8000 flavonoid molecules have been identified to far. Flavonoids are a varied group of low-molecular-weight phenolic compounds that are present in the plant

kingdom and in the human diet as phytonutrients. They make up one of the most distinctive groups of SMs found in higher plants. The typical chemical structure of flavonoids is a 15-carbon skeleton with a heterocyclic ring and a phenyl ring. Flavonoids are essential for the defense and pigmentation systems of plants. They are also an essential component of pharmaceutical, medical, and cosmetic products, and they offer a variety of health-promoting properties. For instance, they are effective inhibitors of several enzymes and have anti-inflammatory, antioxidative, anticarcinogenic, and antimutagenic properties that can change important cellular enzymatic processes (Walker *et al.*, 2000).

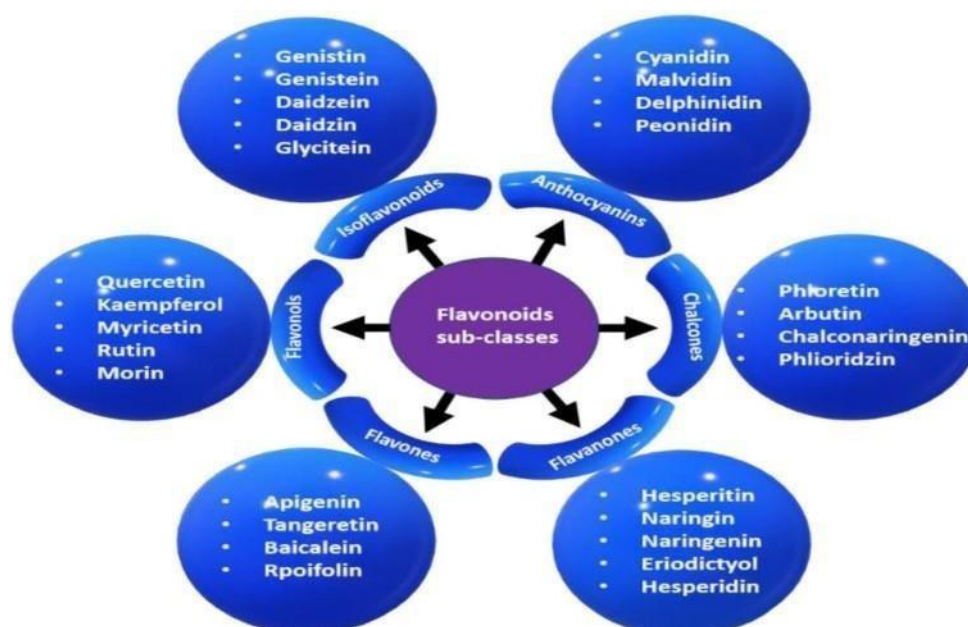


Figure 1.5: Classification of plant flavonoids

Isoflavonoids

Pterocarpan, isoflavones, and isoflavans are all members of the vast class of diphenolic compounds known as isoflavonoids, which are traditionally regarded as dietary antioxidants. In contrast to flavonoids, which have the B-ring at the C2 position, they have a phenyl ring joined with a heterocyclic C-ring and another B-ring fused at the C3 position (Han *et al.*, 2009). Only a small number of plants contain isoflavonoids, with leguminous species being the most common. The production of nitrogen-fixing nodules and, consequently, the encouragement of symbiotic rhizobia depend on these naringenin derivatives (Sreevidya *et al.*, 2006). Isoflavonoids might play a role in the formation of phytoalexins during plant-microbe interactions (Dixon *et al.*, 2002). Microbes have been reported to contain some isoflavonoids.

Tannins

The third essential class of phenolic compounds is tannins. They can form compounds with proteins, minerals, cellulose, and starch and are abundant in oligos and polymers. Tannins with molecular masses between 600 and 3000 can be separated into two subgroups: condensed tannins and hydrolyzable tannins. Generally speaking, tannins are poisons that might potentially impact the development and survival of the majority of herbivores; as a result, they serve as deterrents to a variety of grazing animals. Plants' ability to withstand a variety of illnesses is also greatly influenced by tannins like protocatechuic acid and chlorogenic acid. Environmental elements that can drastically change how tannins accumulate in plants include high temperatures, light levels, nutritional fluctuations, and an abundance of CO₂ in the environment (Fine *et al.*, 2006).

Terpenes

The primary class of secondary metabolites (SMs) found in plants, terpenes or terpenoids, are derived from acetyl CoA or glycolytic intermediates. They exhibit a great deal of structural diversity more than 50,000 distinct structures and are lipid-soluble (Vranova *et al.*, 2012). Terpenes are the primary constituents that give plants their scents and physiological effects. They are also the fundamental building blocks of many complex phytohormones, sterols, and pigments. The majority of terpenes function as defensive poisons and herbivore deterrents, while others have a pleasant scent and attract pollinators.

Monoterpenes

A significant class of terpenes, monoterpenes are linked to the isoprenoids that contain ten carbons and are widely utilized in food and medicine. The antibacterial, antifungal, antiviral, antiaggregating, anti-inflammatory, antihistaminic, antispasmodic, antioxidant, and local anesthetic properties of natural monoterpenes and their derivatives are important medicinal characteristics (Moniczewski *et al.*, 2011). Derivatives of monoterpenes, including as p-pinene, a-pinene, myrcene, and limonene, build up in the resin ducts of twigs, needles, and trunks and are poisonous to insects. Additionally, they may have a role in heat tolerance and growth regulation. The monoterpenes in plants give them a distinct identity in environments.

Examples include thymol, which contributes to the flavor of mandarin oranges, and citral, a monoterpene found in lemons that give them their distinct scent. While some monoterpenes

are found in flower scents and can draw pollinators to plants, others act as predator repellents (Boncan *et al.*, 2020).

Sesquiterpenes

Another important family of terpenes are sesquiterpenes, which come in both cyclic and acyclic forms and include three units of isoprene. They can exist as hydrocarbons like alcohols, lactones, aldehydes, and ketones, or in oxygenated forms. The Asteraceae family of plants contains the majority of the more than 5000 sesquiterpene compounds that have been discovered (Canales *et al.*, 2005) such as α -caryophyllen and α -bisabolene. A five-membered lactone ring is a characteristic of sesquiterpenes, such as costunolides. They are renowned for their capacity to fend off herbivores and deter insects and mammals from feeding on them (Kessler *et al.*, 2001). Additionally, sesquiterpenes may act as transcriptional activators and regulate the onset and maintenance of bud and seed dormancy (Schnall and Quatrano, 1992).

Diterpenes

Geranylgeranyl pyrophosphate is used in the isoprenoid pathway (also known as the mevalonate pathway) by plants, animals, and fungi to biosynthesize diterpenes, which are made up of two terpene units. Linear, bicyclic, tricyclic, tetracyclic, penta, and macrocyclic forms can be distinguished based on their core skeleton. Retinal, retinal, and phytol are biologically necessary metabolites with anti-inflammatory and antibacterial properties that are derived from diterpenes. They exhibit strong biological activity in a variety of medicinal plants; paclitaxel and ingenol-3-angelate, for instance, are effective against cancer. Forskolin, triptolide, carnosic acid, salvinorin A, and ginkgolide B are a few other diterpenes that may have analgesic, cardioprotective, antioxidant, and anti-inflammatory properties (Mafu and Zerbe, 2018). Gibberellins, also known as phytohormones, are significant diterpenes that play a crucial role in CO₂ fixation, biomass synthesis, leaf growth, flower and fruit setting, stomatal conductance, and seed germination.

Triterpenes

More than 200 skeletons have been found to contain triterpenes, a class of terpenes made up of six isoprene units; to far, about 20,000 distinct terpenes have been identified (Hill *et al.*, 2013). Triterpenes are mostly produced from squalene and come in linear, dicyclic, tricyclic, tetracyclic, and pentacyclic forms. They have 30 carbons (Breitmaier, 2006). According to Chudzik *et al.* (2015), the well-known members of this group exhibit antiviral,

antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory properties. In addition to being found in culinary, cosmetic, and pharmaceutical items, they are crucial for controlling pests and diseases and ensuring the quality of agricultural plants (Thimmappa *et al.*, 2014).

Polyterpenes

Since they are made up of several hundred isoprene units, polyterpenes, including tetraterpenes, have large molecular weights. A polyterpene polymer, rubber is made up of several repeating isoprene units. Laticifers typically contain rubber, which acts as a defensive mechanism by allowing wound healing and offering protection against herbivores (Eisner and Meinwald, 1995). As co-tackifiers and viscosity diluents, polyterpenes function similarly to waxes (Benedek, 2004).

Sulfur-Containing secondary metabolites (SMs)

About 200 compounds make up the comparatively little group of secondary metabolites found in plants that include sulfur. The group includes the well-known glucosinolates and the byproducts of their breakdown, including oxazolidinethiones, thiocyanates, isothiocyanates, and epithionitriles (Venditti and Bianco, 2020). Protein synthesis depends on sulfur-containing SMs; protein synthesis is inhibited in the absence of sulfur. Only the young leaves of plants exhibit the symptoms of sulfur deficit, which are comparable to those of nitrogen shortage (Schnug and Haneklaus, 2005). Because plants cannot effectively use nitrogen if they do not have enough sulfur, sulfur-containing SMs have a major impact on plant health (Bloem *et al.*, 2007). Research has shown that the supply of sulfur and nitrogen affects SMs that include these elements, meaning that the right quantity of each improves the plant's resistance to a variety of environmental stressors. The plant defense system is either directly or indirectly associated with sulfur-containing SMs, such as glutathione, glycosphingolipid, phytoalexins, allinin, thionins, and defensins.

Nitrogen-Containing secondary metabolites (SMs)

Nitrogen is included in the skeleton of several plant SMs. Alkaloids, cyanogenic glycosides, glucosinolates, and nonprotein amino acids are the four primary groups into which nitrogen-containing SMs fall. These are produced from common amino acids.

Alkaloid

While very few alkaloids have been identified in lower plants, they are typically found in higher plants, especially dicots. Either the entire plant or a particular organ may contain alkaloids. Alkaloids are generated from amino acids and typically have one or more carbon rings, most of which contain nitrogen. Alkaloids are vital to plants because they prevent herbivores from feeding, shield them from harmful effects, and suppress rivals. Alkaloids' pharmacological significance includes antihypertensive (various indole alkaloids), antiarrhythmic (quinidine, sparteine), antimalarial (quinine), and anticancer (dimeric indoles, vincristine, vinblastine) properties (Saxena *et al.*, 2013).

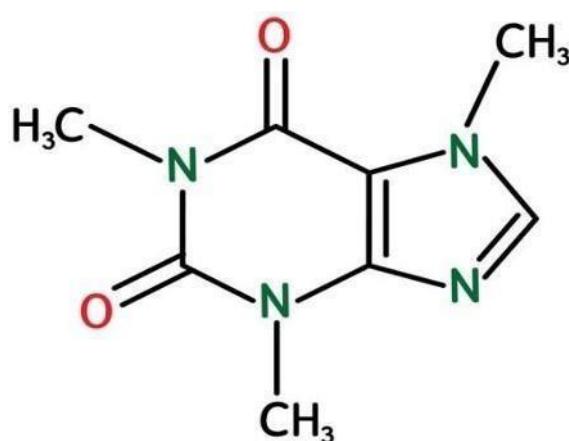


Figure 1.6: Structure of caffeine alkaloid

Glycoside

Glycosides are a family of natural chemicals made up of two molecules: aglycone, which is made of flavonoids or terpenes, and sugar, which is mostly D-glucose but can also occasionally contain L-rhamnose and L-fructose. Aglycone is a highly poisonous component of cardiac glycosides, which are present in many plants. Cardenolide and bufadienolide are the two chemical families into which the aglycone of cardiac glycosides falls (Akinmoladun *et al.*, 2014). Na⁺-, K⁺-ATPase is essential for the most significant phenomenon of cell transport and neural signaling; accumulation of Na⁺ and K⁺ gradients, which is hindered by glycosides, results in cardiac and respiratory arrest and death (Wink, 2015).

1.3.3 Natural antioxidants from medicinal plants

1.3.3.1 Free radicals and Reactive Oxygen Species (ROS)

The oxidation process is the primary cause of free radical production in food, drugs, and biological systems. One definition of a free radical is any atom or molecule with unpaired

electrons. ROS and free radicals interact with a number of biomolecules to start a chain reaction. Only when the free radicals are removed do these interactions cease; the produced free radical then combines with another free radical or with a main antioxidant or chain-breaking agent.

Superoxide anion ($O_2^\bullet$), hydroxyl ($OH^\bullet$), hydroperoxyl ($OOH^\bullet$), peroxy ($ROO^\bullet$), and alkoxy ($RO^\bullet$) are the main free radicals produced from oxygen. Hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl$), ozone (O_3), and singlet oxygen ($^1O_2$) are examples of radicals and non-free radicals. Reactive oxygen species (ROS) are the collective term for these reactive intermediates. Similarly, nitrogen dioxide (NO_2), peroxyxynitrite ($ONOO^\bullet$), and nitric oxide ($NO^\bullet$) make up the majority of reactive nitrogen species (RNS).

As metabolic byproducts of vital aerobic metabolism, free radicals and other reactive oxygen species are continuously released. These can harm biomolecules like membrane lipids, cellular proteins, and DNA, resulting in cell death and a number of diseases (Antolovich *et al.*, 2002). In addition to aging, the most prevalent diseases associated with radicals are atherosclerosis, diabetes, cancer, and neurological conditions like Parkinson's, Alzheimer's, and Huntington's disease (Pourmorad *et al.*, 2006).

Humans have utilized medicinal plants for their healing properties since the dawn of civilization. For thousands of years, therapeutic compounds have been found in nature, and a remarkable number of contemporary medications have been derived from natural sources. The use of the agents in conventional medicine served as the basis for many of these isolations. Ayurveda and Unani are two of India's many traditional medicinal systems that have endured for over 3,000 years, mostly via the use of plant-based drugs. These systems' "Materia Medica" includes a wealth of traditional herbal remedies that have supported the health of the majority of India's rural residents. Many plants are used as medicines, according to ancient Indian literature in Sanskrit, including the Atharva Veda and the Rig Veda (4500-1600 BC). The usage of about 700 herbs is mentioned in ayurvedic medicine classics like Susruta Samhita and Charaka Samhita (Narayanaswamy, 1981). The Ayurvedic method places a great emphasis on promoting good health and preventative medicine. Rasayanas, which are Ayurvedic remedies, are used to enhance health.

1.3.4 Antioxidant activity of Phytochemicals

Medicinal plants have natural active compounds that are used to treat illnesses or ease pain. It is commonly known that traditional medicines and medicinal plants are used as

therapeutic agents to maintain good health in the majority of underdeveloped nations (UNESCO, 1997). Eighty percent of the 120 active compounds that have been identified from higher plants and are being utilized extensively in contemporary medicine have a positive association between their traditional use and their current therapeutic use. In the current pharmacopoeia, plants are the source of at least 7,000 medicinal compounds.

At least 25% of drugs in modern pharmacopoeia are still sourced from plants, and many more are synthetic counterparts based on plant-derived prototype chemicals. Growing trust in herbal therapy is one of the factors contributing to the continued and increasing acknowledgment of medicinal herbs.

Additionally, the process of extracting and developing drugs and chemotherapeutics from these plants, as well as from traditionally used herbal treatments, has led to an increased dependence on the usage of medicinal plants in industrialized cultures (UNESCO, 1998). The World Health Organization claims that the greatest source of a wide range of drugs is medicinal plants. Consequently, more research should be done on these plants to learn more about their characteristics, effectiveness, and safety.

The natural origin of bioflavonoids and their significance as effective scavengers of free radicals give them significant antioxidant activity among phenolic compounds. Supplements known as dietary antioxidants have the potential to postpone or lessen the effects of oxidative stress, and phenolic compounds are phytochemicals found in many plants that have a variety of bioactivities. They fall under the category of natural antioxidants, which are essential components of our diet because they absorb and neutralize free radicals by donating a hydrogen atom from their hydroxyl groups (Boskou *et al.*, 2006).

Researchers are very interested in antioxidants because of their potential to protect biological systems. Even if every living thing has its own antioxidant defense system, it is impossible to undervalue the significance of dietary or exogenous antioxidants because they play a significant role in preventing a number of illnesses. In addition to the numerous classic natural antioxidants found in tea, wine, fruits, vegetables, and spices, which are used commercially as nutritional supplements, numerous more species have been studied in an effort to find new antioxidants. Therefore, it is necessary to find novel and potent radical scavengers from natural sources. The use of an antioxidant-rich diet, particularly from natural sources, is becoming a more compelling strategy to prevent oxidative stress-related damage (Vertuani *et al.*, 2004).

1.3.5 Phytocompounds as antimicrobial agent

Random selection following one or more biological tests; random selection following chemical screening; reports on biological activity and the use of plants in ethnomedicine are some of the strategies for drug development that can be distinguished from one another. The latter strategy makes use of databases, herbalism, and plants employed in traditional medical systems. Particularly, the goal is to separate new bioactive phytocompounds. Once an active extract has been identified, the first step is to identify the bioactive phytocompounds.

Either a full identification of a bioactive phytocompound after purification or a partial identification up to the level of a recognized chemical family may be required. Among the most significant sources of therapeutic drugs are plants. In the past, ancient societies viewed plants as the primary source of novel ideas for pharmacological and antibacterial treatment development (Mcchesney *et al.*, 2007).

The earliest known written records date to approximately 2600 BC and are found in Mesopotamia (Iraq) on hundreds of Sumerian clay tablets in cuneiform. While Egyptian medicine dates back to approximately 2900 BC, the 1500 BC "Ebers Papyrus," a pharmacological record, included over 700 drugs, the majority of which were plant-based. About 45,000 different species of medicinal plants have therapeutic properties (Grover *et al.*, 2002). Information on herbal drugs was gathered and recorded in herbal pharmacopoeias by people in ancient cultures. According to current pharmacopoeias, almost 25% of drugs are derived from plants, some of which are synthetic equivalents. Approximately 80% of all treatments were made from plant roots, bark, and leaves until the 1990s; many of these had been tested for antibacterial properties.

As a source of full-time and part-time jobs, the harvesting and processing of medicinal plants and plant-derived products plays a significant role in India's annual economic output. The two main categories of health management that make substantial use of medicinal plants worldwide are traditional medicine and modern medicine Mohd *et al.*, (2012). The active compounds used in several new drugs have been extracted from plants for thousands of years. Prior to the development of synthetic drugs, humans relied entirely on plant-based remedies for their essential medical needs (Singh *et al.*, 2008). Numerous medicinal herbs have been demonstrated by the Nigerian traditional medical system to be effective

antibacterial agents against clinically manifested pathogens as well as multi-drug resistant pathogens such *Candida albican*, *E. Coli*, and vancomycin-resistant enterococci (VRE).

Antimicrobials developed from plants have vast medicinal potential. In addition to effectively treating infectious infections, they also lessen a number of the negative consequences that are frequently connected to synthetic antimicrobial drugs. They are delicate but effective. Tropisms to particular body organs or systems are seen in many plants. Typically, phytomedicines affect the body in a number of ways. They frequently do more than only relieve the symptoms of illness.

1.3.6 Impact of Phytochemicals on Managing Diabetes

Vegetables are essential parts of many foods and human diets because they contain energy, proteins, lipids, and carbohydrates (Offor *et al.*, 2015). Phytochemicals and dietary fiber, two of their non-nutritive components, have been demonstrated to lower the incidence of type II diabetes mellitus (Nwali *et al.*, 2015). Evidence from laboratory, epidemiological, and animal research shows that certain phytochemicals, such as those found in palm, tuber, and pulse, are essential for successfully lowering the risk and complications of type II diabetes in adults (Orji *et al.*, 2016). For those with type II diabetes, these items are also crucial parts of dietary weight loss plans. The amount of calories consumed, the percentage of fat, fiber, and carbs in the host's pre-prandial index, and the liver's capacity to release glucose all play a part (Chukwuemeka *et al.*, 2013). The blood glucose level for the first insulin dose needs to be lowered if the glucose is present in the food for an extended period of time. Non-nutritive plant compounds with anti-inflammatory or disease-preventive properties are called phytochemicals. Although some may have an impact on bodily systems or cell signaling, they are known to have antioxidative properties (Udeozo *et al.*, 2014). Food plants include phytochemicals that give them their color, flavor, and inherent resistance to disease (Ikechukwu *et al.*, 2015). These can be classified as either necessary, meaning they must be consumed on a daily basis by humans, or non-essential, meaning they are not addictive and do not improve human health but contribute to physiologically active plant statements. Carotenoids, flavonoids (plant pigments) including flavones and flavonones, phytoestrogens (lignans, saponins, isoflavonoids), and ellagic acid are a few common examples of phytochemicals. Good health depends on these phytochemicals being present in low amounts (Ude *et al.*, 2022).

2. REVIEW OF LITERATURE

2.1 Review of Literature

2.1.1 Plant *Cassia spp.* with other activity

Mahesh et al., (2025) created a topical plant-based gel with ethanolic extract from *C. accidentalis* leaves, and evaluated its phytochemical makeup, physical and chemical characteristics, anti-Candida activity, and other quality control metrics. Even though there is a vast arsenal for treating fungal infections, research into new herbal substances is still ongoing. Kasw1da, or *Cassia occidentalis*, is a member of the Fabaceae family and is prized for its therapeutic qualities. The presence of pharmacologically active ingredients is linked to these therapeutic benefits.

Suciati et al., (2024) examined *C. spectabilis* water and ethanolic extracts as cholinesterase inhibitors as potential therapies for Alzheimer's disease. Numerous metabolites found in the *Cassia spectabilis* plant have the potential to have pharmacological effects. To ascertain which elements are in charge of the bioactivity, the extracts' chemical makeup was also examined. The modified Ellman's method was used to perform the cholinesterase inhibitory activity assay against butyrylcholinesterase (BChE) and acetylcholinesterase (AChE). The extracts' chemical profiles were examined using LC-MS/MS analysis, and then GNPS conducted a molecular networking investigation. The ethanolic and water extracts of *C. spectabilis* flowers showed promise in treating Alzheimer's disease. The extract's ability to inhibit cholinesterase may be due to the presence of piperidine alkaloids.

Brahmaiah et al., (2024) developed, tested, and optimized herbal films containing *Cassia auriculata* ethanol leaf extract to treat chronic constipation. The rapidly dissolving herbal films of *C. auriculata* leaf extract were made using a solvent-casting technique, varying ratios of hydroxy propyl methyl cellulose as polymer, and propylene glycol as a film softener. Weight fluctuation, thickness variation, surface pH, percentage moisture uptake, percentage moisture loss, disintegration time, and folding durability were among the quality control metrics evaluated. The results showed that all of the films had good folding endurance, a smooth texture, no particle matter, and a respectable look.

Suciati et al., (2023) examined *C. spectabilis* water and ethanolic extracts as cholinesterase inhibitors as potential therapies for Alzheimer's disease. The flowering plant *Cassia spectabilis* has a number of metabolites that may have therapeutic effects. To ascertain which elements are in charge of the bioactivity, the extracts' chemical makeup was also examined. The modified Ellman's method was used to perform the cholinesterase inhibitory activity assay against butyrylcholinesterase (BChE) and acetylcholinesterase (AChE). The extracts' chemical profiles were examined using LC-MS/MS analysis, and then GNPS conducted a molecular networking investigation.

Amah et al., (2023) assessed the hepatoprotective properties of *Cassia spectabilis* stem bark aqueous extract against oxidative stress-induced hepatic damage mediated by thioacetamide. *Cassia spectabilis* stem bark was freshly cut and then dried. A fine powder was made from dried stem bark. Cold water was used to remove precisely 500 grams of powdered plant material. Five sets of five adult male albino rats each were created from the twenty-five rats. Group V received 50 mg/kg of silymarin for 10 days, after which the animals were killed and a blood sample was taken for analysis using standard protocols. Group I served as the normal control and received 2 ml of distilled water. Groups III and IV received extract only and 400 mg/kg of extract before thioacetamide (TAA) was administered.

Anjali et al., (2023) examined the plant *Cassia auriculata*'s pharmacological characteristics, safety/toxicity studies, pharmacognostic studies, and phytochemical analysis. Anti-cancer, anti-fungal, antibacterial, antimicrobial, antiviral, anti-obese, and anti-diabetic properties are among its wild pharmacological homes. One of the common plants in Asia, *Cassia auriculata* Linn is used in Ayurveda. According to a literature review, this plant contains precursory phytochemical constituents like alkaloids, phenolics, glycosides, flavonoids, tannins, saponins, proteins, carbohydrates, and anthroquinone derivatives, all of which contribute to its potent pharmacological effects. Around the world, some acute and chronic illnesses are treated with the antimicrobial qualities of medicinal plants and plant components such flowers, roots, fruits, seeds, and oils.

Rathod *et al.*, (2023) created and assessed a herbal remedy for sporia utilizing the leaves of *Cassia tora*. Using a UV-B-induced photodermatitis model, the ant psoriatic activity of three flavonoids-luteolin-7-O-glucopyranoside, quercetin-3-O-D-glucuronide, and formononetin-7-O-D-glucoside-isolated from the ethanol extract of *C. tora* leaves was assessed. The flavonoids present in the ethanol extract were also identified using HPLC by comparing their retention durations with those of recognized standards such as luteolin, quercetin, and formononetin. Histological analysis of the slice in the UV-induced photodermatitis model revealed that the standard group, isolated substance, and ethanol extract (400 mg/kg) did not display dilated capillary loops, elongated rete ridges, or Munro's microabscess.

Ogbe *et al.*, (2022) examined the effects of a hydroethanolic extract of *Cassia spectabilis* leaves on oxidative stress and hepatorenal injury in Wistar rats caused by diclofenac. This study was started to assess the impact of *Cassia spectabilis* leaves extract (CSE) on diclofenac-induced oxidative stress and hepatorenal injury in Wistar rats since the rich plant resources have not been thoroughly examined. The first group of rats received an intramuscular injection of normal saline, while the second group received an intramuscular injection of diclofenac sodium (DF). Rats in the third through fifth groups received injections of DF and oral gavages of varying dosages of CSE. Biochemical assays were used to determine the blood indicators of oxidative stress and hepatorenal injury in rats.

Ekasari *et al.*, (2021) examined the antiplasmodial activity and mechanism of *C. spectabilis* DC leaf extract as an antimalarial in vitro and in vivo. The pure chemical was obtained by fractionating, sub-fractionating, and isolating the extract. To determine the active ingredient, perform an in vitro antiplasmodial activity test against *Plasmodium falciparum*. Prophylactic and antiplasmodial activity, either by itself or in conjunction with artesunate, were assessed in an in vivo test against mice infected with *P. berghei* ANKA. The Basilica method was used to suppress the heme detoxification test, which is one of the antimalarial processes. The leaf of *C. spectabilis* DC exhibited strong antiplasmodial activity and could be a promising *Candidate* for a cost-effective and efficient antimalarial phytomedicine.

Raphael et al., (2020) examined and contrasted the effects of leaf extracts from *Cassia spectabilis* (CSE) and *Alchornea cordifolia* (ACE) on rats' hepatorenal injuries caused by diclofenac. Four groups of five adult rats each were chosen for the study. For five days each, the rats in groups 1 and 2 were given normal saline and 11.0 mg/kg diclofenac sodium (DF) intramuscularly. Following DF exposure as in group 2, rats in groups 3 and 4 received oral gavages of 342 mg/kg ACE and CSE, respectively, for six days. Rats' blood was drawn around 24 hours after treatment, and the serum was isolated and estimated biochemical parameters. Rats' liver and kidney were extracted and prepared using histology methods. Rats exposed to DF had significantly higher levels of sodium ions, urea, creatinine, ALT, AST, and total bilirubin ($p < 0.05$) than normal control.

Singh et al., (2013) examined the traditional, therapeutic, and pharmacological uses of the *Cassia species* (caesalpinaceae) plant or plants in underdeveloped nations. A well-known plant that grows widely in India and other tropical nations is the *Cassia species*. It grows in untamed wasteland and is an annual under shrub. The plant's leaves, seeds, and roots are all thought to have therapeutic properties. This plant contains a number of chemical compounds that have been isolated, including anthraquinone glycosides, naphthopyrone glycosides, phenolic compounds, flavonoids, and others. These compounds are used in traditional medicine as laxatives and to treat ringworm infection, leprosy, ocular, skin, and liver diseases. According to estimates from the World Health Organization (WHO), 80% of people in underdeveloped nations get their primary medical care from traditional medicines. As more individuals look for treatments and health strategies free from the negative effects of synthetic pharmaceuticals, medicinal herbs are becoming more widely employed.

Subramanion et al., (2012) The 600 species in the genus *Cassia* are found all over the world and are well-known for their vast range of biological and pharmacological characteristics. In tropical and subtropical regions, *Cassia spectabilis* (sin *Senna spectabilis*) (DC) Irwin et Barn (Fabaceae) is commonly grown as an attractive plant. For many years, *C. spectabilis* has been widely utilized in traditional medicine. According to information in the biomedical literature, *C. spectabilis* has a number of chemical compounds that are useful for medicine. Numerous research teams' pharmacological investigations have demonstrated that *C. spectabilis* has strong biological activity, including antibacterial, antibiofilm, antifungal, and antioxidant qualities. In addition, research on the plant's toxicity have shown no harmful effects on mice.

Torey & Sasidharan, (2011) assessed the ability of *Cassia spectabilis* leaf methanol extract to suppress *C. albicans* biofilm formation. Due to phenotypic adaptability inside the biofilm or the development of resistance to the commonly used antibiotic, *Candida* (*C. albicans*) infection in its biofilm mode of growth has gained attention as its involvement in human infections becomes more widely recognized. The broth dilution method and the disc diffusion assay were used to conduct anti-yeast activities against *C. albicans*, which forms biofilms. The XTT (2, 3-bis [2-Methoxy-4-nitro-5- sulphophenyl]-2H-tetrazolium-5-carboxanilide) reduction test was used to evaluate the extract of *C. spectabilis* leaves in order to quantify the biofilm using fluconazole as a positive control. Further research using confocal scanning laser microscopy (CLSM) and scanning electron microscopy (SEM) showed that the extract from *C. spectabilis* leaves reduced the amount of *C. albicans* biofilm.

Torey et al., (2010) standardized *Cassia spectabilis* leaf in terms of chemical constituent analysis, assay, and authenticity. Several factors were used in the authentication process, including as functional group analysis using Fourier Transform Infrared (FTIR) spectroscopy, leaf microscopy, and gross morphology. Finding the extract's minimal inhibitory concentration (MIC), which might aid in evaluating the chemical effects and establishing curative values, was part of the test portion of standardization. The Broth Dilution Method was used to determine the MIC of the *C. spectabilis* leaf extracts. The extracts, regardless of extraction duration, displayed a MIC value of 6.25 mg/mL. The primary chemical components of *C. spectabilis* are quantified as part of the standardization process for chemical constituents.

Singh and Singh, (2010) assessed the common herb *Cassia fistula* is a member of the Caesalpiniaceae family. Since plant seeds contain gluco-mannose, an effort is made to determine whether the seeds are suitable as tablet binder. The current study reports on the isolation of *Cassia fistula* seed mucilage. There was no chemical interaction shown by the medication and mucilage's DSC and FTIR thermograms. Mucilage's phytochemical and physiochemical properties were examined, confirming its nature. Diltiazem HCl was used as a model drug to assess the mucilage's granulating and binding qualities in compressed tablets. The mucilage-prepared granules were contrasted with xanthan gum, a common binder. Over 85% of the medication was released within three hours, and the pills exhibited good physiochemical characteristics.

Krishnan et al., (2010) evaluated the ability of several crude extracts of *Cassia spectabilis* leaves to inhibit the growth of bacteria and yeast. Extracts of *C. spectabilis* leaves in acetone, n-hexane, dichloromethane, ethyl acetate, and methanol were tested in vitro against yeast (*Candida albicans*), Gram negative bacteria (*Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*), and Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). Using the micro dilution technique, the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC) were ascertained. The greatest yield was 14.12% for methanol, followed by 8.37% for dichloromethane, 6.66% for acetone, 4.76% for ethyl acetate, and 1.80% for n-hexane. With MIC values ranging from 0.625 to 2.5 mg mL⁻¹ and MBC or MFC values ranging from 1.25 to 5 mg mL⁻¹, acetone and methanol crude extracts demonstrated strong antibacterial activity. These extracts' MIC, MFC, and MBC values were 10-80 times lower than those of amoxilin and miconazole nitrate, two common antibacterial medications.

2.1.2 Anti-inflammatory activity of other plants

Patel et al., (2025) assessed the ethanolic extract of *Trichosanthes palmata* leaves' anti-inflammatory properties in vitro against protein denaturation. The anti-inflammatory properties of the protein denaturation inhibition technique were assessed. Using the protein denaturation method, the ethanolic extract of *Trichosanthes palmata* demonstrated anti-inflammatory properties.

Bhakta et al., (2024) investigated how natural medicines for rheumatoid arthritis can reduce inflammation. The body's reaction to inflammation includes pain, redness, swelling, and irritation. When acute inflammation turns into chronic inflammation and begins to harm internal processes, it can sometimes get worse and result in the death of internal organs or even cancer. One of the inflammatory conditions that results in joint pain is rheumatoid arthritis (RA). Other ligaments and tendons weaken, and the bones and cartilage of the joints are damaged. Approximately seven million individuals, or 1% of the population, in India reported having RA. While there are a number of potential therapies and medications to treat RA and other inflammation-related conditions, they are primarily chemical in nature and may have negative side effects that are detrimental to humans. As a result, more people are turning to herbal or ayurvedic remedies for RA, which include medicinal plants with anti-inflammatory qualities and the ability to treat RA more safely.

Lebeloane et al., (2024) examined the anti-inflammatory properties of seven plant species that may be added to animal feed. The cytotoxicity tests and suppression of the 15-lipoxygenase (15-LOX) enzyme and nitric oxide (NO) release in LPS-induced RAW 264.7 macrophages were used to assess the anti-inflammatory potential of plant extracts. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to assess how plant extracts affected the expression of pro-inflammatory mediators such as cyclooxygenase (COX), inducible nitric oxide synthase (iNOS), and the cytokine interleukin-6 (IL-6). Using chemical tests, in vitro antioxidant activity was examined. Potentially beneficial phytochemicals and essential element concentrations were found using ULPC-MS and ICP-MS, respectively.

Dubey et al., (2024) sought to develop a polyherbal remedy using extracts of *Soymida febrifuga* (SF), *Glinus oppositifolius* (GO), *Curcuma longa* (CL), *Centella asiatica* (CA), and *Anogeissus latifolia* (AL). These selected medicinal herbs are used by the people of Chhattisgarh state to treat wounds. This study evaluated the extracts' and their polyherbal combination's capacity to cure wounds using spectrophotometric techniques. Aluminum chloride and Folin's ciocalteu methods were used to optimize the total polyphenols. The DPPH and FRAP tests were used to evaluate the antioxidant capability. Protein denaturation tests were used to assess anti-inflammatory action, whereas phenol-protein precipitation experiments were used to assess the potential for wound healing.

Chirumarnilla and Taduri, (2023) conducted to identify antidiabetic, antioxidant, and anti-inflammatory properties in various *S. khasianum* plant sections. *S. khasianum* leaf, fruit, and root methanolic extracts were tested for their in vitro anti-inflammatory properties using the Human Red Blood Cell (HRBC) membrane stabilization method; antioxidant activity was assessed using **DPPH** (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, H₂O₂ radical scavenging, and reducing power assays. Additionally, the α -amylase and α -glucosidase inhibition assay was used to determine the antidiabetic activity. According to our findings, *S. khasianum* root methanolic extract shown strong anti-inflammatory, antioxidant, and antidiabetic properties in a dose-dependent manner.

Gonfa *et al.*, (2023) examined the state of research on medicinal plant phytochemicals and their nanoparticles as anti-inflammatory agents as documented by numerous publications. About 50 medicinal plants were found to contain phytochemicals that were used to treat inflammatory illnesses. These phytochemicals included flavonoids, terpenoids, polyphenols, saponins, tannins, alkaloids, anthraquinones, and some of its M/MONPs. These plants' natural compounds showed encouraging anti-inflammatory properties to treat inflammation-related conditions of the skin, liver, heart, joints, gastrointestinal tract, nervous system, and lungs. Additionally, a brief synopsis of the mechanism of action of phytochemicals and their M/MONPs agents against inflammation is provided. Numerous investigations into the phytochemistry, M/MONPs, and anti-inflammatory properties of phytochemicals derived from medicinal plants have produced new, safe medication with fewer adverse effects.

Madake and Kadbhane, (2023) examined the anti-inflammatory qualities of herbal herbs. Inflammation is one of the vascular tissue's several natural reactions to an irritant or injured cell. It is characterized by redness, joint pain, difficulty swallowing, and even loss of function. Conventional drugs for inflammation have a number of adverse effects, including the potential for blood clots to cause heart attacks and strokes. To overcome this, a drug with fewer side effects, or none at all, is needed. Because medicinal plants can help treat many inflammation-related disorders, natural goods are an excellent method to find new medications. Many herbs have been utilized in traditional medicine to reduce inflammation.

Harun and Mohamad (2023) Study on *Z. officinale* had a variety of pharmacological qualities, including immunomodulatory, antibacterial, anticancer, anti-inflammatory, and antioxidant. Finding out how *Z. officinale* affects antibacterial activity from a selection of prior investigations (2000-2020) is the aim of this study. Ten randomized controlled trials (RCTs) were used in this investigation to ascertain *Z. officinale*'s antibacterial properties. The systematic analysis's findings demonstrated that *Z. officinale* demonstrates antibacterial activity in both in vitro and in vivo tests. A significant difference between this plant and controls was revealed by the meta-analysis of relevant data from four sources. According to the overall result and inhibition zone, there is no discernible difference between *Z. officinale* and positive controls for the antimicrobial analysis.

Radovanovic et al., (2023) investigated the anti-inflammatory qualities of Serbian traditional medicine plants. Historically, using medicinal herbs has been regarded as safe, affordable, and generally accepted. The most common kind of treatment in Serbia is traditional medicine, which is founded on a strong belief in the therapeutic properties of plants. Serbia's designation as one of 158 global centers of biodiversity lends credence to this, demonstrating the nation's abundance of therapeutic herbs. Yarrow, common agrimony, couch grass, onion, garlic, marshmallow, common birch, calendula, licorice, walnut, St. John's wort, chamomile, peppermint, white willow, sage, and many more are among the most commonly used herbs in Serbian tradition for the treatment of inflammations of various causes.

Riaz et al., (2023) Many ailments are gradually treated with studied medicinal plants, including their extract, seeds, oils, and fruit. A number of cytological and chemical processes can produce inflammation, a pathological condition that is typified by tissue damage or destruction. By inhibiting the inflammatory process, the metabolism of arachidonic acid, the enzymes, and the inflammatory cells, the medicinal herbs diminish inflammation. This study discusses the anti-inflammatory mechanisms of several plants, including *Curcuma long*, *Zingiber officinale*, *Rosa canina*, *Cassia fistula*, *Salvia officinale*, *Borago officinale*, *Rosmarinus officinale*, *Evening Primrose*, *Ribesnigrum*, *Oleaeuropea*, *Herpagophytum*, and *Elaeagnusangustifolia*. We attempted to bring a multifaceted medical approach to treat inflammation because there is no one-size-fits-all solution. This was accomplished with the aid of anti-inflammatory botanicals and lifestyle modifications such as food and exercise.

Bouyahya et al., (2022) demonstrated that Moroccan medicinal plants block many inflammatory-related enzymes, and in vivo studies using a variety of experimental paradigms demonstrated significant analgesic and anti-inflammatory benefits. In vitro and in vivo studies have demonstrated the anti-inflammatory and analgesic effects of a variety of bioactive compounds with a variety of mechanisms of action. According to clinical research, some plants' secondary metabolites and bioactive ingredients have strong anti-inflammatory effects.

Karrat et al., (2022) evaluated the analgesic and anti-inflammatory properties of the leaves of *Pinus brutia* and *Cedrus libani*. Using sodium diclofenac as a positive reference, the anti-inflammatory properties were assessed using the albumin denaturation assay and the Human Red Blood Cells (HRBC) membrane stabilization assay. Ethanolic extracts at a dose of 30 mg/kg and gel containing 2% (w/v) of ethanolic extract of each plant were used in the formalin and tail flick tests to assess the analgesic properties. Positive controls included lidocaine gel 2%, diclofenac sodium, and diclofenac gel 1%. At dosages of 2.5-12.5 µg/ml for *P. brutia* and 2.5-25 µg/ml for *C. libani*, the impact of hemolysis inhibition was noted. By calculating the maximum potential effect (**MPE%**) for both extracts and Diclofenac, it was found that both extracts could significantly reduce paw licking time in the Formalin test, and that each plant extract gel was more effective than the Diclofenac gel in the tail flick test.

Nigussie et al., (2021) investigated Ethiopian medicinal plants that have anti-inflammatory or wound-healing properties in an effort to gather data for future research on their possible use in lymphoedema treatment. When it comes to their anti-inflammatory properties, *Lawsonia inermis*, *Azadirachta indica*, *Achyranthes aspera*, and *Cuminum cyminum* are the most researched plant species; when it comes to wound healing, *Lawsonia inermis* and *Azadirachta indica* are the most researched. Carrageenan-induced paw oedema and excision and incision wound models were the most widely utilized in vivo methods for the anti-inflammatory and wound healing assays, respectively.

Sarni et al., (2021) discussed how anti-inflammatory medications can interfere with the pathophysiology of inflammation to lessen tissue damage and enhance patient comfort. The successful development of novel naturally occurring anti-inflammatory drugs mostly depends on a multidisciplinary strategy to discovering novel chemicals due to the vast number of species available for study. Even though there have been a lot of review papers done in this field, the great majority of them have only examined the topic from a regional standpoint. Numerous non-steroidal anti-inflammatory drugs have been shown to reduce pain and inflammation by preventing the cyclooxygenase enzyme's isoform from breaking down arachidonic acid, which in turn reduces the production of prostaglandins.

Shingala *et al.*, (2021) Since ancient times, plants have been utilized to cure a variety of illnesses, making them one of the most significant sources of medicinal materials. Many medications are now made from traditional medicinal herbs. The body's immunological reaction to any type of injury is inflammation. Redness, heat or warmth, pain, and swelling are the four main signs of inflammation. Both in vitro and in vivo study models are used to evaluate the anti-inflammatory properties of medicinal plants, plant extracts, or pure compounds. These include the constituents of various medicinal plants and their marker chemical.

Jagessar, (2021) investigated plant extracts' anti-inflammatory properties. Humanity has turned to traditional or phytomedicine in response to the rise in inflammatory disorders worldwide. Tropical nations have a major edge in this area due to their enormous biodiversity, as their distinctive flora and fauna provide these medicinal anti-inflammatory medications. Plants can be used to treat inflammatory illnesses by administering their crude extracts or by isolating and using natural anti-inflammatory compounds. Among the natural compounds extracted from plants with notable anti-inflammatory properties are ursolic acid, oleanolic acid, boswellic acid, betulinic acid, curcumin, resveratrol, and baicalein.

Barbhuiya *et al.*, (2021) sought to develop a scientifically assessed pharmacognostic profile and screen six plants for anti-inflammatory properties in vitro. Each plant's leaf sample was assessed using macroscopic, microscopic, and physicochemical characteristics (in accordance with the standardization guidelines suggested by the WHO). Using diclofenac sodium as a reference, the anti-inflammatory activity screening of methanolic extracts was conducted using the inhibition of protein denaturation method. The kind of stomata in the leaves, as well as the existence of parenchyma cells, collenchyma cells, upper epidermis, lower epidermis, vascular bundle, palisade cells, and trichomes in the transverse section of leaves, are revealed by the macroscopic characteristics and microscopical examination. Total ash, acid-insoluble ash, water-soluble, methanol-soluble extractives, and moisture content are all revealed by physicochemical parameters.

Borquaye *et al.*, (2020) examined the anti-inflammatory and antioxidant properties of extracts from three medicinal plants that are often used in Ghanaian folk medicine: *Reissantia indica*, *Grosseria vignei*, and *Cissus cornifolia*. Using accepted techniques, the extracts' phytochemical makeup and total phenolic content (TPC) were ascertained. The ferric reducing antioxidant activity, nitric oxide and hydrogen peroxide scavenging, and phosphomolybdenum tests were used to screen for antioxidant potential. To assess the anti-inflammatory properties, an *in vivo* experiment was used. Extracts of all three plants contained coumarins, glycosides, terpenoids, alkaloids, and flavonoids. While *Cissus cornifolia* had the lowest TPC (7.59 µgig GAE), *Reissantia indica* had double the TPC (38.44 µgig GAE) of *Grosseria vignei* (19.12 µgig GAE).

Uwaya *et al.*, (2020) intended to assess the aqueous extracts of *Lactuca virosa* and *Euphorbia hirta* plants' anti-inflammatory properties. *In-vivo* anti-inflammatory investigations were conducted using xylene-induced ear edema and albumin- and histamine-induced inflammation in mice. *In vitro* anti-inflammatory experiments were conducted using erythrocyte membrane stability and suppression of protein denaturation assays. In the albumin and histamine-induced inflammatory test, mice's inflamed paw was significantly reduced ($P < 0.05$) by the combined doses of 100 mg/kg *Euphorbia hirta* and 50 mg/kg *Lactuca virosa*, 100 mg/kg *Euphorbia hirta* and 100 mg/kg *Lactuca virosa*, 100 mg/kg *Lactuca virosa* only, and 10 mg/kg diclofenac.

Lisa *et al.*, (2020) A wealth of potential medication *Candidates* can be found in reviews of medicinal plants with possible therapeutic properties. Opioids, NSAIDs, and other analgesics and anti-inflammatory drugs have a number of undesirable side effects. Therefore, the creation of novel active substances with negligible side effects is required. Comprehensive list of plant species and phytoconstituents that have been shown to have anti-inflammatory and analgesic properties. In this research, we have included 87 species from 52 plant groups with known components and activities. Future development of new active agents with improved therapeutic activity and selectivity may result from extensive research on the screening of novel analgesic and anti-inflammatory agents from natural sources, followed by an examination of their pharmacological characteristics and clinical uses.

Nunes et al., (2020) investigated plants for their anti-inflammatory properties. Since plants are the primary source of molecules used to create new medications, transnational corporations are more interested in finding compounds derived from plants, especially since the great majority of species have not yet been thoroughly investigated chemically or biologically, especially with regard to their anti-inflammatory properties. Anti-inflammatory medications can reduce tissue damage and increase patient comfort by interfering with the pathophysiological mechanism of inflammation. Therefore, it is crucial to remember that the effective creation of novel naturally occurring anti-inflammatory medications primarily rely on a multidisciplinary effort to uncover new compounds because there are many species available for investigation.

Fyad et al., (2020) examined the analgesic and anti-inflammatory properties of *Bubonium graveolens* aqueous extract in male NMRI Albinos strain mice weighing 22-38 g. The mice were made to writhe and develop paw edema by applying acetic acid. An assessment of the plant's analgesic activity revealed that the aqueous extract, at 150 mg/kg, reduced the frequency of cramping in the abdomen brought on by 1% acetic acid. The plant's aqueous extract showed an analgesic effect that was nearly identical to that of Diclofenac; in fact, at a concentration of 150 mg/kg, *Bubonium graveolens* caused a pain inhibition of $49.6 \pm 2.1\%$, whereas Diclofenac caused a pain inhibition of $49 \pm 1.1\%$.

Reddy et al., (2020) examined the anti-inflammatory properties of plants. The body's natural reaction to disturbances or illnesses is inflammation. The development of novel medications with fewer side effects greatly benefits from the use of traditional herbs. Since ancient times, plants have been used extensively in human health care. New medications are therefore desperately needed to solve this issue. Plants include a variety of phytochemical elements, including lignans, polyphenols, anthraquinones, flavonoids, alkaloids, terpenoids, saponins, polysaccharides, and peptides. which have fewer adverse effects and are beneficial in reducing inflammation.

Sundaram, (2020) centered on rural medicinal plants utilized for their anti-inflammatory properties in the Azhagar hills of Tamil Nadu, India's Madurai region. It has been demonstrated that 24 medicinal plants from 20 families have anti-inflammatory properties. Rural residents have been using traditional medicine to treat even unknown illnesses with symptoms. In rural regions, traditional healers are adept at identifying the therapeutic qualities of plants that can cure illnesses.

Komakech *et al.*, (2019) revealed that *T. indica*'s diverse sections have analgesic and anti-inflammatory properties. These effects may be ascribed to the plant's numerous bioactive substances, which include steroids, alkaloids, flavonoids, tannins, phenols, and saponins. The extracts from the various parts of *T. indica* may have analgesic and anti-inflammatory effects because they can inhibit several biological processes, such as the expression of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), 5-lipoxygenase biosynthesis, and tumor necrosis factor- α .

Eze *et al.*, (2019) examined on the process of studying anti-inflammatory action entails creating a model that either simulates or stimulates the synthesis or release of the biochemical mediators of inflammation and tracking how these biochemicals react to the test medications. The *in vitro* and *in vivo* experimental paradigms for evaluating the anti-inflammatory properties of synthetic medications and plant extracts have been updated in this article. Each method's advantages and disadvantages have also been examined. A comparison of *in vitro* enzyme models and *in vivo* animal models has been developed to help researchers and scientists choose an assay method based on factors like cost, ethical considerations, test time, sensitivity, and reliability.

Modi *et al.*, (2019) 25 distinct medicinal plants that grow in the Junagadh region of Gujarat were tested for their *in vitro* anti-inflammatory properties. For every plant extract, a phytochemical screening was conducted. To assess anti-inflammatory activity, two distinct techniques were used: 1. Albumin denaturation inhibition; 2. Protease inhibition test. Water extracts of the leaves of *Adansonia digitata* L., *Flueggea leucopyrus* Willd., and the aerial portion of *Solanum xanthocarpum* Schrad. & H. Wendl. demonstrated inhibitions of 87.54, 80.23, and 80.38 percent, respectively, in the albumin denaturation assay.

Virshette *et al.*, (2019) examined the anti-inflammatory properties of medicinal herbs. The ancient Indian mythologies, Rigveda and Ayurveda, reference a number of plants with different qualities; hence, the use of medicinal herbs in India dates back to 3500- 1800 B.C. A series of actions that take place in reaction to harmful stimuli, infections, or trauma might be referred to as the inflammatory process. Instead of using non-steroidal anti-inflammatory medicines (NSAIDS), people employ medicinal plants. The gastrointestinal toxicity of NSAIDs is the primary factor limiting their use.

Anyasor *et al.*, (2019) created to assess the methanol extract of *J. secunda* leaves' (MEJSL) anti-inflammatory properties utilizing both in vitro and in vivo inflammation models. In accordance with regular protocol, 70% MEJSL was prepared. Heat-induced denaturation of bovine serum albumin (BSA) and erythrocyte membrane stabilization assays were used in in vitro anti-inflammatory tests. To assess MEJSL's anti-inflammatory properties in vivo, formaldehyde and carrageenan-induced inflammation in rat models were employed. The reference medication was diclofenac sodium. In addition, liver and kidney function testing and hematological analysis were carried out. Comparing different concentrations of MEJSL to diclofenac sodium, the results showed that they significantly ($P < 0.05$) prevented heat-induced BSA denaturation and stabilized the erythrocyte membrane against hypotonicity-induced hemolysis in a concentration-dependent manner.

Oguntibeju, (2018) Identified medicinal plants from a few African nations and regions that have anti-inflammatory properties. By preventing the isoform of the cyclooxygenase enzyme from metabolizing arachidonic acid, a variety of nonsteroidal anti-inflammatory medications have been demonstrated to lessen pain and inflammation by lowering prostaglandin synthesis. Unfortunately, the use of nonsteroidal anti-inflammatory medications is linked to a number of adverse consequences. Nonetheless, there are therapeutic herbs that have anti-inflammatory properties and little to no adverse effects. Numerous medicinal plants with anti-inflammatory properties that have been proven to be successful in treating inflammatory diseases in traditional medicine are abundant on the African continent. It's interesting to note that researchers have studied several of these African medicinal plants and recorded their biological and medical properties.

Amri *et al.*, (2018) intended to evaluate *P. atlantica* leaf extract's anti-inflammatory and antioxidant properties in light of flavonoid phytochemical research. Leaf powder was sonicated in 80% methanol to create the extract. Thin-layer chromatography (TLC) was used to analyze the phenolic chemicals. The decrease in carrageenan-induced hind paw edema in mice served as a measure of the anti-inflammatory effect. Findings: Three glycosylated flavonoids and derivatives of gallic acid were detected by TLC. Rutin, quercetrin, and other heterosides of quercetin, kaempferol, and myricetin were the flavonoids that were found. With the exception of the antioxidant activity measured by the FRAP assay, which was noticeably higher for the extract of male tree leaves, there was no discernible difference between the two sexes.

Bernstein *et al.*, (2018) examined that Natural compounds from plants have been utilized in traditional medicine all across the world since ancient times and can yield potent and promising medications. There is still much to learn about the potential of higher plants as a source of novel medications, and only a small portion of the estimated 250,000-500,000 plant species have undergone pharmacological or biological screening. The therapeutic potential of traditionally used plants and their constituents is currently being investigated in the search for new anti-inflammatory medications. Medicinal plants and their isolated compounds are used in traditional medicine all over the world to treat a variety of inflammatory conditions.

Abu Bakar *et al.*, (2018) discovered that at a concentration of 250 µg/ml, *Melicope ptelefolia* (Tenggek burung) and *Portulaca oleracea* (Gelang pasir) had the greatest anti-inflammatory effect, with over 90% efficacy. The presence of various naturally occurring phytochemicals from various categories, including flavonoids, coumarins, alkaloids, steroids, benzophenone, triterpenoids, curcuminoids, and cinnamic acid, was demonstrated to be the primary cause of the activity.

Oppong *et al.*, (2018) examined matured, ripe, and dried seeds of *Scaphium affine* (Mast.) Pierre, also called Pangdahai (PDH) teas (water decoctions), which are used in traditional and ethnomedical medicine to treat constipation, pharyngitis, and pain. Pharmacological studies on the polysaccharides and aqueous extracts have supported the use of these teas. Furthermore, despite their varied pharmacological actions (anti-inflammatory, anti-ulcer, anti-pyretic, anti-microbial, anti-obesity, and analgesic), the organic (ethanolic and methanolic) extracts of PDH have not gotten much attention, according to this review. There is a significant scientific vacuum in our understanding of PDH because the majority of research on the disease has been on polysaccharides, or big molecular weight metabolites.

Alonso *et al.*, (2018) Inflammation is the study of a complex set of defensive and restorative reactions to tissue damage brought on by infection, autoimmune, or mechanical stressors. Acute and chronic inflammation are also possible. Despite its preventive function, inflammation is the etiological cause of many diseases, including ischemic heart disease, cancer, arthritis, and atherosclerosis. Pro-inflammatory mediators are synthesized and secreted via a variety of mechanisms. This chapter examines the various intracellular signaling pathways associated with inflammation. The two main categories of anti-inflammatory medications are nonsteroidal anti-inflammatory drugs, which reduce damage

by inhibiting cyclooxygenase enzymes, and steroidal anti-inflammatory drugs, which reduce inflammation by binding to cortisol receptors.

Romana et al., (2018) investigated phytochemical substances with anti-inflammatory properties as secondary metabolites. In India, herbal medicines provide significant therapeutic solutions for a range of illnesses. Ayurveda is one of the oldest therapeutic systems and the traditional health care system of India. India has enormous potential and an edge in the developing field of herbal medicines because of its high biodiversity. About 17,000 species of higher flowering plants are included among the roughly 7500 species that make up the group of medicinal plants. Even when synthetic chemistry surpasses expectations, the utilization of natural products in the production of medications utilized in modern medicine remains unparalleled. In contrast to synthetic chemicals, natural drugs heal many ailments completely rather than just alleviating their symptoms.

Neevashnhi et al., (2017) sought to assess the roots and stems of *Impatiens balsamina*'s anti-inflammatory properties. Using the cold maceration method, separate ethanol and water extracts of *I. balsamina* roots and stems were made. The carrageenan-induced paw edema method was used to assess the anti-inflammatory activity in wistar albino rats. The experimental rats were given extracts orally at a concentration of 50 mg/kg 30 minutes before carrageenan (0.1 ml of 0.1%w/v) was given. A digital plethysmometer was used to measure the paw volume at predetermined intervals. Significant anti-inflammatory efficacy was demonstrated by all three extracts. At the second hour, the water extract of *I. balsamina* stems demonstrated the highest significant ($p < 0.001$) anti-inflammatory effect, followed by the ethanol extract of *I. balsamina* roots and stems.

Swandari et al., (2017) sought to identify substitute anti-inflammatory medications, particularly those derived from natural sources. This study used three medicinal plants- *Arnomum xanthophlebium* Baker, *Clerodendrum buchananii* (Roxb.) Walp., and *Donax canniformis* (G.Forst.) K. Schum. that were documented from Dayak Abai in North Kalimantan, Indonesia, for health issues brought on by the inflammatory process. Rat paw edema caused by carrageenan was used in the experiment, and a plethysmometer was used to measure the results. The findings demonstrated that there were significant variations in the AUC (area under the curve) between the treatment group with plant medicinal extracts, the positive control, and the negative control, with $p = 0.001$ ($p < 0.05$). The *C. buchananii* leaf ethanol extract's AUC demonstrated the most potent anti-inflammatory properties.

Shaikh et al., (2016) The COX-1 and COX-2 inhibitory and antioxidant properties of plant samples that were progressively extracted, such as *Cissus quadrangularis*, *Plumbago zeylanica*, *Terminalia bellarica*, and *Terminalia chebula*, in water, ethanol, and hexane, were assessed in vitro. Carrageenan and Phorbol Myristate Acetate (PMA)-induced mouse edema were used to evaluate the in vivo anti-inflammatory efficacy of certain samples exhibiting encouraging COX-2 inhibition. According to the results, the majority of the plants were shown to suppress COX-2 activity in contrast to COX-1. When compared to other samples, it was shown that the extracts of *T. bellarica* (73.34%) and *T. chebula* (74.81%) demonstrated considerable COX-2 selective inhibition. The chosen plants' ethanol extract showed strong DPPH, OH, and superoxide radical scavenging properties.

Singh et al., (2016) assessed in male Wistar rats the analgesic and anti-inflammatory properties of an aqueous extract of dried *M. koenigii* Linn. leaves. The plethysmometer and hot plate method were used to assess the acute anti-inflammatory and analgesic effects of *M. koenigii* Linn. aqueous extract administered orally to healthy albino rats at doses of 100, 300, and 500 mg/kg body weight. When compared to the control and standard medication, the aqueous extract demonstrated dose-dependent anti-inflammatory effectiveness in acute trials by significantly reducing the volume of paw edema. In the hot plate method, *M. koenigii* Linn. aqueous extract significantly and dose-dependently increased the delay of paw licking and decreased the quantity of acetic acid-induced writhing.

Verma, (2016) examined medicinal plants that have anti-inflammatory properties. One aspect of the body's immunological reaction is inflammation. Pain, redness, heat or warmth, and swelling are the four main signs of inflammation. Numerous phytochemical substances with anti-inflammatory properties can be synthesized by plants as secondary metabolites. An attempt has been made to examine the anti-inflammatory properties of a few medicinal plants in this review.

Nworu and Akah, (2015) studied the molecular mechanisms of action of a few anti-inflammatory plants. Herbal extracts and compounds derived from herbs have been shown in numerous studies to have anti-inflammatory properties primarily because they inhibit the metabolism of arachidonic acid (AA), cyclo-oxygenase (COX), lipo-oxygenase (LOX), pro-inflammatory cytokines, inducible nitric oxide, and transcription activation factor (NF- κ B). It has been found that certain anti-inflammatory medicinal herbs stabilize the lysosomal membrane, while others induce the intracellular signaling molecules' oxidative

phosphorylation to uncouple. It has also been demonstrated that several of them have potent oxygen radical scavenging properties.

Adebayo *et al.*, (2015) measured the extracts' inhibitory effect on the pro-inflammatory enzymes lipoxygenase and inducible nitric oxide synthase in order to ascertain their anti-inflammatory properties. Using the 2,2-diphenyl-1-picrylhydrazyl (**DPPH**) scavenging and 2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) methods, extracts of *Peltophorum africanum* demonstrated good antioxidant activity with IC₅₀ values of $4.67 \pm 0.31 \mu\text{g/mL}$ and $7.71 \pm 0.36 \mu\text{g/mL}$ as compared to the positive control ascorbic acid ($2.92 \pm 0.14 \mu\text{g/mL}$ and $13.57 \pm 0.44 \mu\text{g/mL}$, respectively).

Bhagyasri *et al.*, (2015) examined the anti-inflammatory properties of medicinal herbs from India. The intricate biological reaction of vascular tissues to pathogens, irritants, or injured cells is known as inflammation. NSAIDs are mostly useful in treating pain. The gastrointestinal toxicity of NSAIDs is the reason that restricts their use. Consideration is mostly focused on the creation of powerful anti-inflammatory medications and natural products. The chemical diversity of herbal items makes them an excellent source for the development of novel medications. Herbal remedies made from medicinal plants are helping to treat a number of inflammatory disorders. Inflammation can be treated with traditional medications, which have a number of negative effects.

Okemy *et al.*, (2015) examined Wistar rats' volume edema caused by 2% carrageenan was significantly reduced by an aqueous extract of a preparation made of three medicinal plants, while the implantation of cotton pellets beneath the aponevrose of the posterior leg of the rats increased the formation of granulomas. This preparation's extract, which has a dose-independent vascular permeability of 100 mg/kg, also inhibits leukocyte migration to the site of inflammation and exhibits strong antioxidant properties. According to a phytochemical analysis, this extract contains flavonoids, tannins, and saponins that may enhance its anti-inflammatory properties. This aqueous extract is also effective in treating acute inflammation in the suggested formulation.

Susanna et al., (2015) examined the *Murraya* leaves' anti-inflammatory properties. In India, Bangladesh, and Sri Lanka, the Ayurvedic, Unani, and Siddha medical systems have utilized *Aegle marmelos* as a natural remedy to treat diabetes mellitus. Separate methanolic extracts from *Aegle marmelos* and *Murraya koenigii* were made. Carrageenan-induced inflammation and paw edema in albino wistar rats were used to test the individual and combined anti-inflammatory properties of both plants. The two extracts mentioned above had anti-inflammatory effects when taken separately. However, the two extracts mentioned above demonstrated strong synergistic anti-inflammatory activity when taken in combination. Arachidonate cyclooxygenase (COX) inhibition successfully controls the carrageenan-induced paw edema test, indicating that MEMK and MEAB may have arachidonate COX inhibitory properties.

Sagnia et al., (2014) examined the anti-inflammatory and antioxidant properties of ethanol extracts of *Polyscias fulva* stem bark, *Eleusine indica*, *Carica papaya*, *Eremomastax speciosa*, and *Cassia alata* leaves that were gathered in Cameroon. The protection against antioxidant-induced DNA damage in white blood cells following hydrogen peroxide treatment was examined using comet assays. Flow cytometry was utilized to measure $\gamma\delta$ T cells proliferation and anti-inflammatory activity of $\gamma\delta$ T cells and of immature dendritic cells (imDC) in the presence of different amounts of plant extracts. Strong antioxidant qualities against the superoxide anion and hydrogen peroxide were demonstrated by ethanol extracts. The maximum antioxidant activity was found in *Cassia alata*. These findings unequivocally demonstrate the plant extracts' anti-inflammatory and antioxidant properties that were gathered in Cameroon.

Mohammed et al., (2014) worked on There are two types of inflammation: acute and chronic. Inflammation is a complicated pathophysiological process that is mediated by numerous signaling molecules. Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are the two main groups under which anti-inflammatory medications fall. Some of these medications are no longer used because of their serious side effects. For thousands of years, people have used potent anti-inflammatory plants as part of their diet and medicine collection. Secondary chemicals made from these plants may provide significant supplies of anti-inflammatory medicines.

Mulaudzi et al., (2013) Twelve medicinal plants utilized by the Venda people to treat venereal and related disorders were investigated for their mutagenic qualities and anti-inflammatory effects. The Ames test was used to assess the plants' genotoxicity against *Salmonella typhimurium* tester strain TA98, both with and without S9 (metabolic activation), as well as their anti-inflammatory efficacy against the cyclooxygenase (COX-1 and -2) enzymes. At 250 µg/ml, the best anti-inflammatory activity was demonstrated by DCM and PE extracts of the bark of *Adansonia digitata*, *Bolusanthus speciosus*, *Pterocarpus angolensis*, and *Pappea capensis*, as well as by EtOH and water extracts of the stem of *Bolusanthus speciosus* and the bark of *Ekebergia capensis*. To ascertain ICS₀ values, these were subsequently assessed at three further doses (31.25, 62.5, and 125 µg/ml).

Agyare et al., (2013) Selected species and the compounds, extracts, and fractions that have demonstrated anti-inflammatory or analgesic effect in clinical or experimental settings, along with the potential mechanism of action and therapeutic utility. 50 African medicinal plants from 33 families that have been shown to have analgesic and anti-inflammatory effects in the literature over the past 20 years are included in the current review. Two pure compounds with anti-inflammatory qualities have been identified: leonotinin, which was extracted from the aerial portions of *Leonotis nepetaefolia* (L.) W.T. Aiton, and pseudoakumarnigine, which was obtained from the seeds of *Picralima nitida* (Stapf) T. Durand & H. Durand.

Kumar et al., (2013) examined the bioactive ingredients, properties, chemical makeup, mechanism of action, function, adverse effects, ongoing research, and possible uses of Zinger in contemporary medicine. The current study shows that ginger has a wide range of effects, including anti-inflammatory ones. Accordingly, the current study comes to the conclusion that ginger and its bioactive ingredients may soon be used in modern medicine to treat a variety of illnesses, including anemia.

Lalitha et al., (2013) investigated the anti-inflammatory effects of several solvent extracts of *Eichhornia crassipes* (Mart.) Solms on male Swiss Albino mice's paw oedema caused by formaldehyde. The petroleum ether, ethyl acetate, and aqueous extracts of *Eichhornia crassipes* were tested for their in vivo anti-inflammatory activity because the phytochemical screening findings indicated that these extracts contained a wide variety of phytochemical ingredients. All of the studied extracts of *Eichhornia crassipes*' leaves and shoot part were shown to have strong anti-inflammatory properties. The extracts' potent ability to prevent pain has been demonstrated by the percent inhibition of anti-inflammatory activity, which

offers compelling scientific support for the traditional usage of this herb to treat animal inflammation.

Hassan et al., (2013) conducted to examine the ethanol extract of leaves from *Andrographis paniculata*, *Mangifera indica*, and *Desmodium pauciflorum* for its analgesic and anti-inflammatory properties. Swiss albino mice and Wistar albino rats were utilized in the acetic acid-induced writhing response model and the carrageenan-induced paw edema model, respectively, to evaluate the analgesic and anti-inflammatory effects. The effects of the leaf extract (2 grams per kilogram of body weight) were compared to those of the commercially available analgesic and anti-inflammatory medication Dclofenac sodium (40 mg per kilogram of body weight) in both circumstances. The study's control was distilled water (2 milliliters per kilogram of body weight).

Stankov, (2012) The present definition of inflammation based on its cardinal signs is out of date and inappropriate for directing effective treatment plans. The amount of the osmotic pressure and temperature fluctuations within the inflamed tissue, in particular, cannot be explained by the current understanding of the inflammatory process, which views vascular phenomena as crucial for the production of cardinal symptoms. Localized edema is the only distinct macroscopic indicator of inflammation among the five cardinal symptoms. While the natural anti-inflammatory response that follows acute inflammation attempts to return tissue homeostasis to normal, the inflammatory process itself is a pathological process. As such, it should be considered a true defense reaction of the damaged tissue.

Apurba et al., (2012) investigated the anti-inflammatory properties of Bangladeshi natural medicinal herbs. The symptoms of inflammation include pain, swelling, and redness. medicines that lower inflammation are known as anti-inflammatory medicines. It has been discovered that the osteoarthritis process is advanced by traditional synthetic NSAIDs, which hasten joint cartilage deterioration and erosion. Long-term usage of these NSAIDs has also been linked to liver and renal damage. According to experimental studies, using herbal remedies that have been proved to be natural anti-inflammatory has not been linked to digestive tract erosion, increased cartilage degradation, or the development of liver and kidney toxicities. This makes it possible for practitioners to utilize these drugs responsibly and safely.

Vikrant and Arya (2011) highlighted the research on the anti-inflammatory properties of medicinal plant barks. The chemical components and pharmacological profile of several plant barks are discussed in this research, with an emphasis on the dose given and the bioactive extract's role in the anti-inflammatory process. This study encourages more investigation into the possible applications of medicinal plant barks with anti-inflammatory qualities.

Perez et al., (2011) stated that three species of the genus *Origanum*, three species of the *Citrus* genus, and three species of the *Pimpinella* genus were the sources of the essential oils. In many cases, the composition of the essential oils has been identified, and in other situations, the anti-inflammatory properties of the primary constituents of these oils-such as isoeugenol and carvacrol have been assessed.

Leelaprakash and Mohan Dass, (2011) used the albumin denaturation assay, proteinase inhibitory activity, membrane stabilization, and anti-lipoxygenase activity at varying doses to assess the methanol extract *Enicostemma Axillare*'s in vitro anti-inflammatory properties. Standard medications included aspirin, diclofenac sodium, and indomethacin. The findings demonstrated considerable ($p < 0.05$) activity of *Enicostemma axillare* Methanol Extract (EAME) at concentrations ranging from 100 to 500 $\mu\text{g/ml}$. Heat-induced protein denaturation was considerably ($p < 0.01$) protected by heat-induced erythrocyte hemolysis. EAME demonstrated significant ($p < 0.01$) inhibition of 42 and 53% of proteinase inhibitory action at 400 and 500 $\mu\text{g/ml}$, but no significant ($p > 0.05$) activity was observed at 100 and 200 $\mu\text{g/ml}$. Between 400 and 500 $\mu\text{g/ml}$, there was a substantial ($p < 0.05$) inhibition of heat-induced erythrocyte hemolysis.

Deshpande et al., (2011) examined *Coccinia grandis* L. Voigt leaves and stem aqueous extracts were studied in a mouse model of chemically induced inflammation. Rats' paw edema caused by formaldehyde was prevented by the extracts. When compared to the control, these inhibitions were statistically significant (p 0.05, 0.01, 0.001). The maximum activity was found in the leaf aqueous extract.

Rahman et al., (2010) examined the potential antidiarrheal and anti-inflammatory properties of *Murraya paniculata* (L.) Jack (Family: Rutaceae) ethanol extract in animal models. At an oral dosage of 600 mg/kg of body weight, the extract demonstrated antidiarrheal activity against mice with castor oil-induced diarrhea; it increased the mean latent period and significantly reduced the frequency of defecation ($P < 0.001$, $P < 0.01$), which was comparable to the standard medication Loperamide at a dose of 50 mg/kg of body weight.

Fawole et al., (2009) assessed how well the plant extracts inhibited the cyclooxygenase enzymes. Phytochemical analysis was also carried out in the search to find any plant metabolites that may be responsible for the reported anti-inflammatory action. The majority of the assessed plant extracts exhibited dose-dependent action against the enzymes COX-1 and/or COX-2. At 62.5 µg/ml, the root dichloromethane extract of *Agapanthus campanulatus* exhibited the highest COX-2 inhibitory activity (83.7%). Different plant parts and species had different levels of phenolics, condensed tannins, gallotannins, flavonoids, alkaloids, and saponins. The findings back up the usage of the plant under investigation to relieve cramps and pain associated with gastrointestinal tract illnesses. The different plant secondary metabolites found in the plant materials may be partially responsible for the reported anti-inflammatory activity.

Babu et al., (2009) examined the scientific underpinnings of the anti-inflammatory properties of various *Albizia lebbeck* organic solvent extracts. The carrageen an, dextran, cotton pellet, and Freund's complete adjuvant-induced rat models were used to investigate *Albizia lebbeck's* anti-inflammatory properties. At doses of 100, 200, and 400 mg/kg body weight, the extracts made with petroleum ether, chloroform, and ethanol were given. Maximum inhibition of inflammation caused by carrageen a (petroleum ether 48.6%; ethanol 59.57%), dextran (petroleum ether 45.99%; ethanol 52.93%), cotton pellet (petroleum ether 34.46%; ethanol 53.57%), and Freund's adjuvant (petroleum ether 64.97%; ethanol 68.57%) was demonstrated by the petroleum ether and ethanol extracts at 400 mg/kg. The plant's traditional use to treat a variety of inflammatory conditions is supported by the notable inhibitory effect on paw edema, which demonstrates *Albizia lebbeck's* exceptional anti-inflammatory action.

Talhounk et al., (2007) examined how plant bioactive water extracts or decoctions were commonly used in Eastern Mediterranean folk medicine traditions to treat a variety of inflammation-related illnesses. Research has indicated that phenols, alkaloids, glycosides, and carbohydrates are among the numerous natural compounds that are active anti-inflammatory components in water extracts. Plant extracts from various plant genera that have anti-inflammatory bioactivities; and second, to assess local folk medicines that are utilized by traditional healers to treat inflammatory conditions in this part of the world.

2.1.3 Anti-oxidant activity of other plants

Pereira et al., (2023) seeks to assess the anticancer, antimigration, and antioxidant potential of Clear Belongs Plus extract (CBL-P), which is made up of five medicinal plants: *Citrus aurantifolia*, *Tiliacora triandra*, *Alpinia galanga*, and *Piper nigrum*, on human colon cancer cells SW620 and HCT116 cell lines, as well as human non-small cell lung cancer cells A549 and NCI-H460 cell lines. After 48 hours of incubation, CBL-P showed antimigration activity on four distinct cancer cell types (A549, NCI-H460, HCT116, and SW620). The highest concentration tested (15 µg/mL) had the greatest effect on A549 cells (10.23% of wound closure) and NCI-H460 cells (9.16% of wound closure).

Khan et al., (2023) assessed the photocatalytic, antioxidant, and antibacterial properties of zinc oxide nanoparticles (ZnONPs) made with *Parmotrema perlatum*. A time kill analysis indicated that produced ZnONPs successfully inhibited the development of bacteria and demonstrated a broad spectrum of antimicrobial action. When ZnONPs and antibiotics are combined, their antibacterial effectiveness against microorganisms is improved. ZnONPs exhibit strong antioxidant action against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The ability of ZnONPs to photocatalytically degrade the dyes methylene blue, malachite green, and Congo red under UV and solar irradiation at varying intervals was assessed. When exposed to UV light instead of sunlight, ZnONPs demonstrated strong photocatalytic dye degradation.

Jafri et al., (2023) The antioxidant activity of extracts from various sections of traditionally used medicinal herbs was assessed in vitro. The ethanolic extracts of *Euphrasia stricta* (E. stricta, 58.19 GAE µg/mg) and *Euphorbia platyphyllos* L. (E. platyphyllos L., 46.05 GAE µg/mg) and (43.39 QE µg/mg), *Epimedium brevicomum* Maxim. (E. brevicomum Maxim., 51.93 GAE µg/mg), and (39.21 QE µg/mg) all exhibited high levels of total phenolic (expressed as gallic acid equivalent, GAE) and flavonoid (expressed as quercetin

equivalent, QE). Free radicals are scavenged by the hydroxyl groups in the phenolic and flavonoid chemicals that are abundant in plants.

Murugeswari et al., (2023) examined the antimicrobial and antioxidant properties of *Klenia grandiflora* leaf hydroalcoholic extract. By monitoring the Minimum Inhibitory Concentration zone of inhibition against gram-positive and gram-negative bacterial strains, such as *Staphylococcus aureus*, *Bacillus subtilis*, and *E. coli*, a hydroalcoholic extract of *Klenia grandiflora* (leaves) was evaluated for antimicrobial activity. Antibacterial activity was investigated using agar cup plates, while antioxidant activity was evaluated using hydrogen peroxide free radical scavenging. The hydroalcoholic extract of *Klenia grandiflora* showed inhibitory zones against both gram positive and gram negative bacteria. At very low concentrations, the antimicrobial properties of Gentamicin standard demonstrated an inhibitory effect on *E. coli*, *Staphylococcus aureus*, and *Bacillus subtilis*.

Dwarakanath et al., (2022) highlights the recovery of secondary metabolites with antibacterial and antioxidant properties from the foliose lichen *P. perlatum*. Gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS/MS) analyses of the secondary metabolites extracted from *P. perlatum* using hexane, chloroform, and methanol reveal that the extracts of the plant contain 2,4-dihydroxy-3,6-dimethyl-methyl ester, 1,4-benzenediol, atraric acid, orcinol, benzoic acid, 2,5-dimethyl-resorcylic acid, savinin, tetrahydroterotri-L- glutamate, primidolol, ustiloxin, mallotinic acid, and mecambaine. Three lichen solvent extracts were examined for their ability to inhibit the growth of Gram-positive *Lactobacillus plantarum*, Gram-negative *Pseudomonas aeruginosa*, and Gram-negative *Chromobacterium violaceum*. **When** compared to the other two solvent extracts, the methanol extract of *P. perlatum* exhibits the strongest antibacterial activity against the tested bacteria at a concentration of 10 µg/ml.

Silva et al., (2022) assessed the antiacne, antiglycation, photoprotective, and antioxidant properties of *Sapindus saponaria*'s crude extract (CESs) and triterpene saponin fraction (TSSs). Fruit extracts from *Sapindus saponaria* exhibit high antioxidant capability and anti-glycation activity against the development of AGE and glycation of bovine serum albumin. Additionally, they demonstrated photoprotective effects against UV-A and UV-B rays as well as antibacterial activity against *Cutibacterium acnes*.

Ravimannan and Nisansala, (2017) investigated the antioxidant content in fruits and vegetables. Antioxidants can be found in abundance in fruits and vegetables because they include a variety of antioxidant components. Fruits and vegetables can differ in their antioxidant activity. Different types of antioxidants can be found in different fruits and vegetables, and they might vary depending on the species and region. The antioxidant activity of fruits and vegetables can be assessed in vitro using a variety of techniques.

Phlicharoenphon et al., (2017) Fruit gel powder was tested for acute toxicity in an animal model, glucose absorption inhibitory impact, and in vitro antioxidant activity. Low antioxidant activity, low acute toxicity, and an inhibitory effect on glucose absorption were all demonstrated by amrong fruit gel powder, which was qualitatively controlled for both physical and chemical properties. With minimal acute toxicity, the quality-controlled Samrong fruit gel powder enhanced weight-control-related activities.

Shaikh et al., (2016) The COX-1 and COX-2 inhibitory and antioxidant properties of plant samples that were progressively extracted, such as *Cissus quadrangularis*, *Plumbago zeylanica*, *Terminalia bellarica*, and *Terminalia chebula*, in water, ethanol, and hexane, were assessed in vitro. Carrageenan and Phorbol Myristate Acetate (PMA)-induced mouse edema were used to evaluate the in vivo anti-inflammatory efficacy of certain samples exhibiting encouraging COX-2 inhibition. According to the results, the majority of the plants were shown to suppress COX-2 activity in contrast to COX-1. When compared to other samples, it was shown that the extracts of *T. bellarica* (73.34%) and *T. chebula* (74.81%) demonstrated considerable COX-2 selective inhibition. The chosen plants' ethanol extract showed strong DPPH, OH, and superoxide radical scavenging properties.

Adebayo et al., (2015) concluded that traditional medicine used *P. africanum*, *Z. capense*, and *C. anisata* as anti-inflammatory and antioxidant medicines. The safety in animal tests must be verified before they may be used as herbal products. These plant species most likely contain compounds with great activities because of the good inhibitory activity of crude extracts comprising numerous other compounds on 15-LOX inhibition. To isolate, identify, and describe the bioactive chemicals causing the actions, more work is needed. The mechanism of action can be investigated when the active chemicals have been separated.

Sagnia et al., (2014) examined the anti-inflammatory and antioxidant properties of ethanol extracts of *Polyscias fulva* stem bark, *Eleusine indica*, *Carica papaya*, *Eremomastax speciosa*, and *Cassia alata* leaves that were gathered in Cameroon. The protection against antioxidant-induced DNA damage in white blood cells following hydrogen peroxide treatment was examined using comet assays. The proliferation of yo T cells and the anti-inflammatory activity of immature dendritic cells (imDC) and yo T cells were measured using flow cytometry in the presence of varying plant extract concentrations. Strong antioxidant qualities against the superoxide anion and hydrogen peroxide were demonstrated by ethanol extracts. The maximum antioxidant activity was found in *Cassia alata*.

Khan et al., (2013) To establish a statistical relationship between phenolic contents and antioxidant potential, researchers examined the antioxidant qualities and phenolic contents (total phenols, flavonoids, flavonols, and proanthocyanidins) of methanolic extracts from the stem barks (TSB), root barks (TRB), leaves (TL), and fruits (TF) of *Marus alba* (locally known as Tut and commonly known as white mulberry). Using a spectrophotometer and an in vitro standard procedure, the antioxidant properties and phenolic contents of methanolic extractives were assessed. Total antioxidant capacity, ferrous reducing antioxidant capacity, hydroxyl radical scavenging test, DPPH (1,1-diphenyl-2-picrylhydrazine) radical scavenging assay, and lipid peroxidation inhibition assay methods were used to measure the antioxidant activities.

Sehwag and Das, (2013) investigated antioxidant properties, and as awareness of health issues grows, so is the use of natural antioxidants. These substances are in charge of lowering oxidative stress in the human body and impeding autoxidation reactions in the food system.

2.1.4 Anti diabetic activity of other plants

Das et al., (2023) aimed at demonstrating the strong antidiabetic effects of *Coscinium fenestratum* (CF) ethanol seed extract following validation by an in silica molecular docking investigation. The antidiabetic action was attributed to berberine, which was discovered and detected by TLC with an R_f of 0.37. When AutoDock4.2 was used for the in silica analysis, the binding energy of berberine was -7.84, indicating that the ethanol extract from CF seeds had important antidiabetic effects. Because berberine is one of the compounds in the ethanol seed extract of CF, it may have an anti-diabetic effect.

Mitra *et al.*, (2022) examined the methanolic extract of *A. alba* leaves' anti-inflammatory, anti-diarrheal, antidiabetic, and analgesic properties using a Swiss albino mouse model. Alloxan-monohydrate, carrageenan-induced paw edema, acetic acid-induced writhing test and the hot plate method, and castor oil-induced method were used to investigate the leaf extract's antidiabetic, anti-inflammatory, analgesic, and antidiarrheal properties, respectively. The study was carried out using dosages of the extract that ranged from 200 to 500 mg/kg. In mice with diabetes produced by alloxan, leaf extract at 400 and 500 mg/kg demonstrated strong antidiabetic effects. To regulate blood glucose levels and the anti-inflammatory effects of carrageenan paw edema, more research is required. The results of both experiments were dose-dependent and statistically significant.

Pottathil *et al.*,(2020) investigated the antidiabetic impact of MEPGL using an oral glucose tolerance test, blood glucose, plasma insulin, and glycated hemoglobin (HbA1c) measurements. By examining hepatic and renal antioxidant indicators, including lipid peroxidation, glutathione peroxidase (GPx), reduced glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), the antioxidant impact of MEPGL was ascertained. Along with total cholesterol, triglycerides, and high-density lipoprotein (HDL), other biochemical markers such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), urea, and creatinine were also examined. These measures were dramatically changed by type 2 diabetes, but they were greatly improved by oral MEPGL treatment.

Oguntibeju (2019) There are a few African medicinal plants that are used in traditional medicine to treat and manage diabetes mellitus. These plants have hypoglycemic and antidiabetic properties. Documenting the knowledge of medicinal plants that are primarily used in different parts of Africa to treat and manage diabetes mellitus is urgently needed. Many of the medicinal plants that are abundant in Africa are used to treat diabetes mellitus. Because of their affordability, availability, perceived lack of adverse effects, and understanding of their potential for illness therapy, these plants are significant in the field of alternative medicine.

Barapatre et al., (2015) demonstrated a relationship with their reducing power and antioxidant scavenging activities. The α -amylase inhibition assay and in vitro glucose movement inhibition were used to assess the antidiabetic qualities. When compared to their control samples, the modified samples showed 1.16-1.18 times greater α -amylase inhibition and lower glucose diffusion (55.5-76.3%), indicating increased glucose binding efficiency. The findings show that lignin's structure and functional alterations have a major impact on how effective it is as an antioxidant and antidiabetic. Biomodified alkali lignin from the deciduous plant *Acacia nilotica* was tested in vitro for its antidiabetic and antioxidant properties. The ligninolytic fungi *Aspergillus flavus* and *Emmericella nidulans* underwent microbial biotransformation on the extracted alkali lignin. These changes were made while the ratio of carbon to nitrogen sources varied.

Parasuraman et al., (2015) determined that *O. tenuiflorum*'s hydroalcoholic extract exhibited strong anti-diabetic and anti-hyperlipidemic properties against diabetes mellitus caused by STZ + nicotinamide. The anti-diabetic and anti-hyperlipidemic properties of each of *O. tenuiflorum*'s phytoconstituents need to be further investigated. *Ocimum tenuiflorum* (Lamiaceae) leaf hydroalcoholic extract's anti-diabetic and anti-hyperlipidemic properties are assessed, and the biological activities of its phytoconstituents are predicted using in silico analysis and an in vivo anti-diabetic model, respectively.

Mamon et al., (2014) compiled information about medicinal plants used to treat diabetes mellitus through a review. It is an endocrine system metabolic condition that affects around 10% of people worldwide, and the number of affected people is growing daily. The profiles include details about the active chemical agents, the degree of hypoglycemic activity, the plant sections and test model employed, and the scientific and family name. The significance of herbal plants in the treatment of diabetes was amply illustrated by the huge number of plants (108 plant species from 56 families) that were described in this review. These plants have the potential to cure metabolic imbalances and postpone the onset of diabetes consequences.

Singab *et al.*, (2014) Numerous medicinal herbs have been evaluated for their ability to prevent diabetes for centuries, and the results showed that they had strong antidiabetic properties. Additionally, the natural source options have a variety of secondary metabolite forms. This suggests that the human body has multiple locations of action. To develop effective antidiabetic options from natural resources, additional research is needed and focus should be placed on examining the biological activity of hundreds of traditionally used medicinal plants both in vitro and in vivo to evaluate the stated activity.

Manikandan *et al.*, (2013) examined the in vitro anti-diabetic effects of the phytochemical bioactive substances found in the methanolic extract of *Psidium guajava* leaves. The extract shows a dose-dependent increase in inhibitory effect on alpha-glucosidase enzyme (up to 89.4%) and alpha-amylase enzyme (up to 96.3%), which suggests that the presence of bioactive compounds may be responsible for the plant's many medicinal properties, including diabetes. Through in vitro research, the current study demonstrates the methanolic extract of *Psidium guajava* leaves' antidiabetic properties. Alpha-glucosidase and alpha-amylase are intestine digestive enzymes that are essential for the breakdown of carbohydrates. Alpha-glucosidase and alpha-amylase enzyme inhibition is one antidiabetic treatment strategy that lowers blood postprandial glucose levels.

Chandira and Jaykar (2013) developed and assessed the polyherbal combination of the chosen medicinal herbs, including *Albizia odoratissima* (L.F.) bark. Because of their efficacy, lack of adverse effects, and affordability, herbal medications are frequently recommended even when their physiologically active ingredients are unknown. *Eryngium foetidum* Linn's leaves and *Ipomoea digitata* Linn's tuberous root are particularly noteworthy for their anti-diabetic properties. All herbal plants typically have no negative side effects, lower levels of toxicity, superior therapeutic effects, high patient compliance, and are inexpensive, effective medications that benefit society. The aforementioned medicinal plants were chosen for formulation and evaluation of the traditional tablet dosage form for anti-diabetic activity based on a significant number of the aforementioned parameters.

Khan et al., (2012) said to be completely recovered from diabetes. Because they are a rich source of biological elements and many of them are known to be useful against diabetes, numerous medicinal plants have been employed in traditional medical systems around the world to treat diabetes mellitus. Due to their low cost and fewer side effects, medicinal plants with antihyperglycemic properties are becoming more and more popular. It focuses on the several types of plants that have been shown to help people with diabetes. Numerous medicinal plants have been documented for their proven antidiabetic and other health advantages. These include *Phyllanthus amarus*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Gymnema sylvestre*, *Momrodica charantia*, *Ocimum sanctum*, *Panax ginseng*, *Eugenia jambolana*, *Allium sativa*, and *Trigonella foenumgraecum*.

Patel et al., (2012) examined the list of medicinal plants whose antidiabetic properties have been investigated. Based on the information obtained in the literature, it was determined that the secondary metabolite is primarily responsible for the antidiabetic effects of plants. In order to reduce the complications associated with diabetes and related disorders, the information presented in *this* study will assist researchers in developing alternate approaches to treating diabetes mellitus that do not rely on insulin or oral hypoglycemic medications. Despite the fact that the number of diabetes cases is rising daily, no effective treatment has been created up to this point, with the exception of insulin and oral hypoglycemic medications.

Narkhede et al., (2011) revealed that *Caesalpinia digyna* root methanol extract had antidiabetic properties in vitro. The results of our study indicate that *Caesalpinia digyna* methanol extract increases the % inhibitory activity of α -glucosidase enzymes (IC_{50} 402.23 $\pm$ 10.14 μ g/ml) and α -amylase (IC_{50} of 686.94 $\pm$ 3.98 μ g/ml) in a dose-dependent manner. Acarbose was a common medication. A clinical syndrome known as diabetes is typified by hyperglycemia brought on by an absolute or relative lack of insulin. The incidence and prevalence of diabetes mellitus have sharply increased in recent decades. Reducing the synthesis and absorption of glucose in the gastrointestinal tract by inhibiting enzymes that break down carbohydrates, such as α -amylase and α -glucosidase, is one method of treating diabetes.

Sharma (2012) For the qualitative analysis of the different phytochemicals under study, including alkaloids, coumarins, saponins, flavonoids, and steroids, a preliminary phytochemical screening was conducted using the following five plant leaf extracts: *Aegle marmelos* (Bael), *Annona squamosa* (Sitaphal), *Ficus racemosa* (Gular), *Hibiscus rosa sinenses* (Jaswand), and *Psidium guajava* (Guava). The extract from *Aegle marmelos* (leaves) showed positive results in the coumarin filter paper test and aromatic odor test. The extract from *Annona squamosa* leaves showed favorable results in the Shinoda test for flavonoids and Mayer's and Wagner's test for alkaloids. Salkowaski and Liberman-Buchard reactions for steroids and Mayer's and Wagner's tests for alkaloids were both positive in the extract of *Ficus racemosa* (leaves). The Shinoda test for flavonoids revealed positive results from an extract of *Hibiscus rosa sinenses* leaves.

Kumanan et al., (2010) indicated that the Lauraceae plant *Cinnamomum tamala* has strong antidiabetic properties. The plant was chosen because of its ethnopharmacological applications in conventional medicine. The α -amylase inhibition assay was utilized to screen for antidiabetic efficacy in the current investigation, which concentrated on methanol and subsequent water extract of *Cinnamomum tamala* bark. Methanol and a subsequent water extract of *Cinnamomum tamala* bark showed percentage inhibition values of 97.49% and 93.78%, respectively. Similarly, methanol and consecutive water extracts of *Cinnamomum tamala* were reported to have IC₅₀ values of 1.80 and 5.53, respectively. Compared to subsequent water extracts, the methanol extract of *Cinnamomum tamala* exhibited more powerful action.

Chauhan et al., (2010) examined the evidence supporting the use of plants, plant extracts, or plant parts to treat diabetes mellitus. Additionally, it compiles information on plants that have hypoglycemic effects. Experimental research on hypoglycemic plants and their bioactive ingredients is of particular relevance in the current study. The kind of diabetes, associated physiological conditions, and herbal herbs that have potential antidiabetic properties are briefly described. It displays the profiles of hypoglycemic plants that have been documented in the literature.

Malviya et al., (2010) examined the pharmacological test results and the natural medications' mode of action. The advantages of using medicinal plants with hypoglycemic properties to treat diabetes mellitus have been demonstrated by numerous investigations. These plants have the potential to cure metabolic imbalances and postpone the onset of diabetes consequences. According to WHO, preventing diabetes and its complications is not just a significant future challenge, but also necessary if universal health is to be achieved. The identification of plants with antidiabetic properties that might be consumed by humans has thus received a lot of attention in recent years. Additionally, it places a major emphasis on the voluntary and sensible applications of indigenous natural and traditional therapies.

2.1.5 Antimicrobial activity of other plants

Ispiryan et al., (2024) identified the antibacterial properties of the morphological components of *Rubus idaeus L.* and the relationship between them and antioxidant activity and total phenolic content. The writers also sought to assess the plant's potential for producing goods with added value. New features were unveiled for future applications and the creation of innovative, natural products. According to the study's findings, three Gram-positive bacteria might be successfully fought off by raspberry leaves, inflorescences, and fruits. The results showed that extracts from the roots and seeds had the least amount of antibacterial activity among the other plant sections. The antibacterial activity and phenolic content characteristics showed a moderate, weak, or very poor connection, according to the data.

Murugeswari et al., (2023) examined the antimicrobial and antioxidant properties of *Klenia grandiflora* leaf hydroalcoholic extract. By monitoring the Minimum Inhibitory Concentration zone of inhibition against gram-positive and gram-negative bacterial strains, such as *Staphylococcus aureus*, *Bacillus subtilis*, and *E. coli*, a hydroalcoholic extract of *Klenia grandiflora* (leaves) was evaluated for antimicrobial activity. Antibacterial activity was investigated using agar cup plates, while antioxidant activity was evaluated using hydrogen peroxide free radical scavenging. The hydroalcoholic extract of *Klenia grandiflora* showed inhibitory zones against both gram positive and gram negative bacteria. At very low concentrations, the antimicrobial properties of Gentamicin standard demonstrated an inhibitory effect on *E. Coli*, *Staphylococcus aureus*, and *Bacillus subtilis*.

Oloyaet *al.*, (2022) The presence of coumarins, alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, resins, and cardiac glycosides was detected by the phytochemical screening of the extracts, which was evaluated and reported. All of the methanol/DCM extracts from the plant species under study have promising antimycobacterial potential. The selected plant extracts under investigation, with the exception of *A. coriaria*, displayed low levels of acute toxicity and may be safe to use in herbal products.

Sindhu *et al.*, (2020) conducted utilizing two different solvents, acetone and ethyl acetate, to assess the antibacterial activity of *Clerodendrum infortunatum* leaves against gram-negative isolates of *Escherichia coli*. In vitro screening for antibacterial activity was done using well-plate techniques. Flavonoids and terpenoids have the ability to block the enoyl ACP reductase, a crucial bacterial enzyme present in *Escherichia coli*. The enoyl acyl carrier protein reductase of *Escherichia coli* is one of the most desirable targets in diseases associated with the bacteria. Given the previously mentioned results, an effort was made to evaluate the antimicrobial efficacy of the flavonoids and terpenoids present in *Clerodendrum infortunatum* by performing molecular docking studies of these compounds with enoyl ACP reductase (PDB ID: 1C14) using the molinspiration online tool for in silica studies.

Ali *et al.*, (2019) extracts from medicinal plants that have been tested for antibacterial activity and against germs that cause acne. Three solvent extracts of *Camellia sinensis*, *Azadirachta indica*, and *Cassia acutifolia* were tested for their antibacterial properties using the disc diffusion method against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes*. The findings demonstrated that various plant extracts exhibited discernible action against various test organisms. When combination A (C/A) was combined, the acetone extract of that combination demonstrated a 20.33 mm greater mean of antibacterial susceptibility against six bacterial strains with a synergistic impact than other combinations. At a concentration of 10⁻² with a 95% confidence interval, combination A's (C/A) antimicrobial susceptibility was greater than that of the combination group.

Reddy and Jain, (2019) carried out to assess Indian medicinal herbs' antibacterial properties against the causative factors of acne vulgaris. Increased sebum production, hypercornification of the pilosebaceous ducts, aberrant bacterial activity, and inflammation are examples of pathogenic causes. Synthetic medications, which might have serious negative effects, are administered throughout the course of the therapy. Therefore, safer and less harmful drugs are required for the treatment. Natural extracts are employed as medications in herbal or herbal-based treatments, which are safe substitutes. In order to find all of the medicinal plants having anti-acne properties, several databases were searched.

Vora et al., (2018) investigated the antioxidant activity of medicinal plant extracts and their ability to combat acne-causing bacteria. The agar well diffusion method was used to test the antibacterial properties of methanolic extracts of *Rosmarinus officinalis*, *Pelargonium asperum*, *Ocimum tenuiflorum*, *Acacia nilotica*, *Azadirachta indica*, *Matricaria chamomilla*, and *Calendula officinalis* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Kocuria sp.*, *Bacillus subtilis*, and *Propionibacterium acnes*. The findings demonstrated that various plant extracts exhibited discernible action against various test organisms.

Mohamed et al., (2018) Hydromethanolic extracts of different Gram-positive and Gram-negative reference bacterial strains were tested for their antibacterial activity against *Berberis vulgaris*, *Cassia angustifolia*, *Cinnamomum Cassia*, *Cistus monspeliensis*, *Nigella sativa*, *Punica granatum*, *Rhus tripartata*, *Withania frutescens*, and *Zingiber officinale*. Using the disc diffusion method and determining the minimum inhibitory concentration (MIC), the antibacterial activity of several plant extracts was assessed. In terms of MICs, the plant extracts' effectiveness ranged from 0.1 to 12.8 mg/mL, and their zone of inhibition against one or more tested bacteria ranged from 06.0 to 23.0 mm. In terms of MICs and diameters of inhibition, the results of the macerated and refluxed extracts of these plants have been comparatively comparable.

Jhaumeer et al., (2018) examined for their antioxidant and antibacterial qualities as well as their nutritional values in terms of phytochemicals, mineral content, total flavonoid (TFC), total phenolic (TPC), and proximate composition. The mango leaves' respective contents of ash, crude fat, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were 12.61, 3.92, 35.32, 34.98, and 12.86%. While the phosphorus content (0.12%) and crude protein content (13.60%) of common fodders were within the standard

necessary range, the calcium content (2.15%) was over it. The phytochemical analysis revealed that the crude, EtOAc, and MeOH extracts included flavonoids, phenols, alkaloids, saponins, and tannins.

Diso et al., (2017) examined the main antibacterial components (phytochemicals) and antimicrobial properties of *Mangifera indica* (leaves and stem). The outcome demonstrates that certain extracts were effective against the bacteria that were sampled. According to statistical analysis of the results, the extracts showed antibacterial activity against the isolates tested; for the five isolates utilized, the maximum zones of inhibition were 16 mm, 14 mm, 17 mm, 15 mm, and 15.00 mm, respectively. When compared to water extracts, the plant's chloroform extracts displayed larger zones of inhibition.

Disegha et al., (2017) investigated the antifungal effects of several amounts of crude leaf extracts from *Mangifera indica* (mango) on a few chosen fungal species, including *Aspergillus fumigatus*, *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger*. According to the study, extracts from *M. indica* can be utilized as an antifungal drug to treat infectious disorders that are linked to the test organisms. If therapeutic application of aqueous mango extract is to be used in situations implicating the test organisms used in this context, it is advised that higher concentrations of crude mango extracts be used due to the direct proportionality of increasing zones of inhibition with concentration.

Menezes et al., (2015) identified the hydroethanolic extract of *M. paniculata* leaves' (EEMp) polyphenol content, antibacterial properties, and acute toxicological action. HPLC-DAD and the Folin-Ciocalteu and AlCl_3 tests were used to assess the chemical composition. The microdilution method was used to measure the antibacterial and modulatory activity. EEMp was given orally to male and female Swiss mice at doses of 2000 and 5000 mg/kg body weight/day in order to evaluate toxicity. The EEMp samples' flavonoid concentration ranged from 0.3 to 31.1 mg quercetin equivalent/g of extract, while their total phenolic content ranged from 66.5 to 396.8 mg gallic acid equivalent/g of extract. The HPLC-DAD experiment identified ellagic acid as the main component.

Shevale et al., (2015) examined the crude ethanol and acetone extracts of *Asparagus racemosus* roots' in vitro antibacterial properties. Alkaloids, glycosides, phenolic chemicals, tannins, saponins, steroids, flavonoids, and carbohydrates were found in the extracts after phytochemical screening. Ethanolic and acetonetic root extracts of *Asparagus racemosus* shown a range of inhibition on *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *Klebsiella pneumonia*, and *Salmonella typhi*, according to the results of the agar well diffusion method. According to the findings of phytochemical and antimicrobial research, *Asparagus racemosus* is a plant that has promise for the creation of novel medications.

Choi, (2015) Methanolic extracts of *Chamaecyparis obtuse* (CO), *Chrysanthemum boreale* (CB), and *Cryptomeria japonica* (CJ) were evaluated for their antibacterial activity against four bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Yesinia enterocolitica*) using the disc diffusion method. They were evaluated for cytotoxicity and anti-human rhinovirus (HRV) 3 activity using a cytopathic effect reduction technique. The methanolic extracts of CB showed antibacterial activity against the four microorganisms under investigation, whose inhibition zones ranged in diameter from 0.5 to 2.0 mm. *Bacillus cereus* was not significantly inhibited by the other extracts when compared to *Escherichia coli* and *Yesinia enterocolitica*.

Zwetlana, (2014) *E. Coli*, *Pseudomonas*, and *Klebsiella* were tested against the active leaf extracts of six medicinally relevant plants made by aqueous and solvent extraction. Lemon leaf extracts in water were effective at inhibiting *E. coli*. Ethanol preparations of eucalyptus leaves exhibited the strongest antibacterial activity against *Klebsiella*. *Pseudomonas* showed resistance to every solvent, with the exception of the ethanol extracts from Tulsi leaves.

Yang et al., (2013) examined the antimicrobial activity of *Litsea cubeba* essential oil (LC-EO) and the antimicrobial impact (AI) of specific volatile compounds in the LC-EO on the cariogenic bacteria *Streptococcus sanguinis* (*S. sanguinis*), *Streptococcus mutans* (*S. mutans*), and *Streptococcus sobrinus* (*S. sobrinus*). The AI integrates the effects of an antimicrobial substance's amount and minimal microbicidal concentration (MMC). The relative contributions of each of the EO's constituent parts to the total antibacterial activity of the EO can be quantitatively described by the AI. The antibacterial activity of the LC-EO and the majority of the volatile compounds under investigation were greater against *S. sanguinis* than against *S. mutans* or *S. sobrinus*. *S. sanguinis*, *S. mutans*, and *S. sobrinus* without biofilms had MMCs of 375, 750, and 1500 µg/mL, respectively, according to the LC-EO.

Abdelaziz Soukri et al., (2013) was to ascertain the minimum bactericidal and lowest inhibitory concentrations of the antibacterial properties of methanolic extracts of 12 chosen Moroccan plants against the tomato DC3000 isolate of *P. syringae* pv. Although *N. sativa*, a plant used in Morocco as a spice, condiment, and medicinal treatment, yielded the highest values, only the methanolic extracts of *Geranium robertianum*, *Aizoon canariense*, *Rubia perigrina*, and *Nigella sativa* shown clear inhibitory and bactericidal qualities.

Vedhanarayanan, (2013) The disc diffusion method on agar has been used to study *Wrightia tinctoria*'s antibacterial efficacy against human pathogenic bacterial strains, including *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. According to the findings, the extracts might be antibacterial for the species under investigation. The ethanol extract of the leaf showed the largest inhibitory zone among the three solvents that were examined. *Wrightia tinctoria* ethanol extract (24 mm) exhibits the maximum zone of inhibition against *Escherichia coli* (29 mm), *Bacillus subtilis* (24 mm), *Staphylococcus aureus* (30 mm), and *Pseudomonas aeruginosa*. A preliminary phytochemical analysis of *Wrightia tinctoria* revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids, and tannins.

Ofokansi et al., (2013) examined the antibacterial properties of *Phyllanthus muellerianus* (Kuntze) Excell extract and methanol leaf extract against *Salmonella* spp., *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Candida albicans*, *Klebsiella* spp., and *Proteus mirabilis*. The extract's or its fractions' potential interactions with ciprofloxacin were also examined.

Arash Khorasani et al., (2013) The antibacterial efficacy of extracts from *Trifolium pratense* (cultivated plants, including callus) was examined using ethanol and methanol as solvents. Three fungal pathogens and four bacterial pathogens two gramme positive and two gramme negative were examined. The antibacterial activity of methanol extract was found to generate a wider inhibition zone in comparison to ethanol extract. In terms of antibacterial activity, the callus extract performed better than the callus, in vitro, and in vivo growing plants, indicating a new use for *Trifolium pratense* callus.

Kishore *et al.*, (2012) Antibacterial activity was tested using the disc diffusion method and the agar well diffusion method. Antimicrobial activity was tested using the disc diffusion and agar well diffusion techniques. The ethanolic and aqueous extracts had the least amount of antibacterial activity in comparison to methanolic extracts. *Phyllanthus niruri* plant extracts that are said to possess antibacterial properties can be very significant in therapeutic processes.

Khalil, (2012) The antibacterial effectiveness of *Catharanthus roseus* leaf extract from Saudi Arabia was evaluated against a number of human pathogenic pathogens, such as *Staphylococcus aureus* (*Candida albicans*) and pathogenic fungi. The examined extracts demonstrated incredibly strong antibacterial activity against all pathogen types. The disc diffusion method was used to estimate the zone of inhibition in order to evaluate the antibacterial activity. At 100 mg/ml of leaf extract, *Staphylococcus aureus* showed the highest inhibitory activity (15 mm zone), followed by *E. coli* with an 11 mm inhibition zone. Furthermore, the toxic fungus *Candida albicans* is inhibited by ethanol leaf extract (12 mm zone of inhibition).

Gracelin *et al.*, (2011) All of the plant samples exhibited strong antibacterial activity when extracted in methanol, according to the results of an antibacterial screening conducted using the disc diffusion method against the gramme negative bacteria *Aeromonas hydrophila* (an animal pathogen) and *Xanthomonas campestris* (a plant pathogen). The methanol extracts of *S. nigrum*, *S. torvum*, and *S. surattense* showed distinct zones of inhibition against the examined bacteria.

Calvo, (2011) It has been demonstrated that plant extracts and their essential oils have antibacterial properties against bacteria and fungi. To differentiate between the different processes, methods for solid culture medium, broth culture media, micro well techniques, aromagram techniques, and bioautographies will be employed. These techniques also make it possible to ascertain the Minimum Inhibitory Concentration (MIC) of the tested items.

Singh, (2011) induced antimicrobial activity against the selected human pathogens (*Bacillus sp.*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, Non-haemolytic Streptococci, *Streptococcus epidermidis*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*) using natural leaves, roots, and their corresponding calli. The findings demonstrated that callus extracts exhibited the best increased inhibitory effects in

comparison to the natural plant material extracts. The study found that *Premna serratifolia* L. is a promising source for the development of phytomedicines in the future.

Essam Abdel-Sattar (2010) The antibacterial properties of twenty wild plants found in the Kingdom of Saudi Arabia's western regions were investigated. The methanolic extracts from the aerial sections were identified by means of the agar diffusion method. The eight microorganisms employed in this study were *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus subtilis*, *Mycobacterium phlei*, and *Candida albicans*. The results showed that 13 extracts have significant broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. However, the range of action of seven extracts against Gram-positive bacteria was restricted.

Mathur, (2010) The antimicrobial activity and minimum inhibitory concentration (MIC) of several plant extracts from traditionally used medicinal plants, such as *Bidens pilosa* L., *Bixa orellana* L., *Cecropia peltata* L., *Cinchona officinalis* L., *Gliricidia sepium*, *Jacaranda mimosifolia*, and *Justica secunda* Vahl, were tested using ethanol (98%), hexane (99%), and distilled water.

Philip et al., (2009) centered on antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. Consequently, it illustrates the possible effectiveness of many medicinal plants used as antibacterial agents in traditional medicine. None of the plants examined in this study were able to inhibit the growth of *Escherichia coli*. *Curcuma mangga*'s antimicrobial qualities are presently being investigated. The data collected will improve our understanding of the plant's antimicrobial properties.

Rajakaruna et al., (2002) antimicrobial properties against the yeast *Candida albicans*, three Gram-positive and two Gram-negative bacteria, and a non-acid fast bacterium. At least one microbe's methanol extract was effective against 29 species from 12 families. While there was widespread activity against Gram-positive and non-acid fast bacteria, only two taxa a species of *Phyllanthus* L. (Euphorbiaceae) and *Lantana camara* L. (Verbenaceae) were active against the Gram-negative bacterium *Pseudomonas aeruginosa*. None of the species worked well against the other Gram-negative bacterium, *C. albicans*, or *Escherichia coli*. Photoactivity was detected in extracts from ten species from ten families, including the Convolvulaceae, Lamiaceae, and Rhamnaceae, where photoactivity has not yet been reported.

3.1 Plant for the study

3.1.1 *Cassia spectabilis*:

Pharmacies sell a wide variety of antifungal medications. However, these antifungal drugs' ability to penetrate and react chemically with the extracellular polymeric substance known as the biofilm matrix may restrict their effectiveness. Thus, we require a new class of antifungal drugs that are both inexpensive and highly effective. Consequently, we have begun testing compounds based on natural items, like edible medicinal plants. In terms of species, genetic, and habitat variety, Malaysia boasts a wealth of medicinal plant diversity. *Cassia* (C.) *spectabilis* is one of the many medicinally valuable plants found in Malaysia. With over 600 species spread across the globe, the genus *Cassia* is well-known for its vast range of biological and pharmacological effects. In tropical and subtropical regions, *Cassia spectabilis* (sin *Senna spectabilis*) (DC) Irwin Barn (Leguminosae) is commonly grown as an attractive plant. Traditional medicine has always made extensive use of *Cassia spectabilis*. It has been used as a laxative and purgative in traditional Brazilian medicine to cure colds and the flu. Furthermore, a variety of biological activities, including antioxidant, antifungal, and antibacterial properties, have been documented in the literature. Global interest in plant-based therapy has significantly grown in recent years. In developing nations, at least 80% of the world's population gets their primary medical care from plant materials (Meyer B. 2003).



Figure 3.1: *Cassia spectabilis*

Taxonomical classification of *Cassia spectabilis*:

- Kingdom: Plantae
- Phylum: Magnoliophyta
- Class: Magnoliopsida
- Subclass: Rosidae
- Order: Fabales
- Family: Fabaceae Lindl
- Subfamily: Caesalpinioideae
- Tribe: Cassieae
- Subtribe: Cassiinae
- Genus: *Cassia* L.
- Species: *spectabilis*

Synonyms:

- *Cassia trinitatis*
- *Cathartocarpus trinitatis*
- *Cassia humboldtiana*
- *Cassia totonaca*
- *Cathartocarpus speciosus*
- *Cassia speciosa*
- *Cathartocarpus humboldtianus*
- *Cassia edulis*
- *Cassia carnavall.*

Common names:

- Popcorn Bush Cedar
- Archibald's *Cassia*
- Calceolaria *Cassia*
- Golden Shower
- Scented Shower
- Fetid *Cassia*.

Origin:

Cassia is indigenous to the northern parts of South America and Central America. As an ornamental, it has also been brought to Africa, particularly Angola, Burundi, South Africa, and East Africa.

Botanical Description:

With a spreading canopy of 15 to 20 feet (4.5-6 m), this medium-to-large tree can grow to a height of 4.5-8 m. The alternating pinnately complex leaves have fuzzy undersides and 7-15 pairs of up to 3-inch-long leaflets. The tips of leaflets are typically pointy. Bright yellow in hue, the flowers are roughly 1.5 inches across and rise above the foliage in dense racemes up to two feet long. Large, branching, terminal, lateral, leafy panicles that range in length from 15 to 30 cm are known as inflorescences. Numerous aromatic flowers are made up of five ovate, hairy, rounded bracts that are caducous and 4-5 mm long, with velutinous, 2- to 3-mm pedicles. Sepals are 5-7 mm long, oblong to round, orange-yellow, and uneven. The spoon-shaped, yellow, uneven, widely to narrowly obovate petals are 2-3.5 cm long, and the anthers open through a slit and apical pores. There are three tiny, sterile stamens and seven big ones. Hairless, thin, and bent pistil. The ovary is glabrous and recurved, with a subtle style and stigma. Fruits are long, flattened, cylindrical pods that are green at first but turn dark or black as they ripen. Pods terminate in a firm, short, narrow tip that does not divide. The brown, flattened seeds have a diameter of roughly 5 mm (Agro Forestry 2012).

Ethnomedicinal Uses:

In Asia and many other nations, this species of *Cassia* has been used to treat a variety of illnesses. Because of its analgesic, anti-inflammatory, antiulcerogenic, laxative, and antibacterial qualities. It has been utilized as an anti-inflammatory, analgesic, laxative, purgative, antibacterial, and antiulcerogenic in northeastern Brazilian traditional medicine. As a laxative and purgative, it has also been used in traditional Brazilian medicine to cure colds and the flu. These herbs were also utilized to alleviate headaches and fevers in the Ayurvedic medical system. Additionally, the herb was used to treat skin conditions like ringworm in Thai traditional medicine. The leaves of *C. spectabilis* are also used as a traditional remedy to treat constipation, poisoning, and intestinal protozoa infections, as well as to prevent oedema (Mazumder *et al.*, 2008).

Phytochemistry:

Two alkaloids, (+)-spectaline and (-)-iso-6-cassine, were isolated from the leaves of *Cassia spectabilis* in one of the earliest phytochemical investigations of the plant, which began in the middle of the 1970s. For the first time, these piperidin 3-ol alkaloids were documented. These substances were also extracted from the seeds later on, along with two more alkaloids that were identified as (-)-spectalinine and (-) iso-6-camavaline. The latter alkaloid, also known as cassinicine, was isolated for the first time. Δ^5 -sitosterol, stigmasterol, 1,3,8-trihydroxy-2-methylantraquinone, and 1,8-dihydroxy-3-methyl-6-methoxyanthraquinone, also known as physcion, were the four more chemicals that were obtained from this effort. It is rather typical for some researchers to describe specific families of chemicals in phytochemical studies, as shown above. Given the intricacy of the mixture and the presence of the compounds in minute concentrations, it has been noted that the process of isolating and identifying natural products is extremely time-consuming. Furthermore, rather than focusing on the presence of every molecule in the plants, some groups may have been concentrating on the isolation of a particular class of compounds based on activity (Viegas *et al.*, 2007).

Pharmacological Activities of *Cassia spectabilis*:

Antibiofilm Activity:

Scanning electron microscopy (SEM) was used to assess preliminary research on the impact of *Cassia spectabilis* leaf extract on *Candida albicans* biofilm. In comparison to the untreated cells, cells treated with *C. spectabilis* leaf extract for 36 hours showed a significant decrease in adhering cells and biofilm, according to visualization of the ultrastructure overall. This implies that the *C. spectabilis* leaf extract might interfere with the biofilm's metabolism. Since the development and creation of biofilms entail a number of processes, cell reactions, and interactions, any modification to these processes may potentially prevent the formation of biofilms. In addition, the thorough antibiofilm activity of *C. spectabilis* leaf extract was recently described by Torey and Sasidharan. With a MIC value of 6.25 mg/mL, they also discovered that the methanol extract of *C. spectabilis* exhibited good anti-yeast activity against *C. albicans*. The biofilm reduction rates for fluconazole and leaf extract were 95.4% and 96.9%, respectively. Following treatment with *C. spectabilis* leaf extract, the primary alterations seen under scanning electron microscopy were cellular damage and disruption in *C. albicans* biofilms, which were subsequently verified by confocal scanning laser microscopy (Torey *et al.*, 2011).

Antifungal Activity

The disk diffusion experiment was used to assess the antifungal activity of the methanolic extracts of *C. spectabilis* leaves, flowers, stem, and pods against *Aspergillus niger* and *Saccharomyces cerevisiae*. All of the *Cassia spectabilis* extracts were effective against *Aspergillus niger*. Although the inhibition zones of the leaf, flower, stem, and pod extracts were 12 mm, 12 mm, 7 mm, and 11 mm, respectively, they were smaller than the 25 mm inhibition zone of the positive control miconazole nitrate (30µg/mL). *Saccharomyces cerevisiae*, however, showed resistance to every extract that was tested. Miconazole nitrate (30µg/mL) produced a zone of inhibition of 21 mm, but the antifungal activity of *C. spectabilis* leaf extract on *Candida albicans* was examined in another study, yielding a zone of inhibition of 16 mm.

To find the minimum inhibitory concentration (MIC) on *Candida albicans*, the *C. spectabilis* leaf extract was further tested using the broth dilution method in addition to the disk diffusion method. With a MIC of 6.25 mg/mL, the extract demonstrated good antifungal activity. In addition, mice were given a 2.5 g/kg body weight dose of the leaf extract as part of in vivo investigations, and the colony forming units (CFU) were then counted. In comparison to the untreated control cells, the SEM observation after 24 hours of treatment revealed that the cells had a rough appearance and had invaginations. The cells seemed cavitated and fully collapsed after 36 hours of treatment; it was thought that at this

point, the cells had lost all of their metabolic capabilities. The yeast cells ruptured and lysed after 36 hours of treatment, and the cytoplasmic components then began to flow out. These findings showed that the *C. spectabilis* leaf extract might inhibit the proliferation of *Candida albicans* cells by acting on their cell wall or cell membrane (Silva *et al.*, 2006).

Antioxidant Activity:

Cassia spectabilis's antioxidant capacity was assessed through the use of the 2,2-diphenyl-1-picrylhydrazyl (**DPPH**) radical-scavenging assay. At 1.0 mg/mL of extract examined, the extracts of the flower, stem, leaf, and pod showed 54.29%, 53.28%, 45.17%, and 6.18% of radical-scavenging properties, respectively (Sangetha *et al.*, 2008).

Antibacterial Activity:

In the study by Sangetha *et al.*, the antibacterial efficacy of *C. spectabilis* was examined using the disk diffusion test and the minimum inhibitory concentration (MIC) experiment utilizing serial tube dilution technique. *Proteus mirabilis*, *Staphylococcus aureus*, *Bacillus thuringiensis*, *Salmonella typhi*, *Escherichia coli*, *Micrococcus sp.*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Azospirillum lipoferum*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were among the microorganisms that were examined. When compared to the positive control, chloramphenicol, the leaf, flower, stem, and pod extracts demonstrated strong antibacterial action against both Gram-positive and Gram-negative bacteria. The most effective leaf extract was that of *C. spectabilis*, which inhibited the growth of all examined bacterial strains, including *Bacillus subtilis* (30 mm), *Staphylococcus aureus* (30 mm), and *Micrococcus sp.* (35 mm). In reference to the MIC assay, the MIC values ranging from 0.195 to 50.000 mg/mL were obtained against both Gram-positive and Gram-negative bacteria. The MIC results also showed that the leaf extract works against Gram-negative bacteria (50,000 mg/mL for *Pseudomonas aeruginosa*) at a higher dosage than Gram-positive bacteria (0.195 mg/mL for *Bacillus subtilis*). Additionally, Krishnan *et al.* reported that several extracts, including acetone, n-hexane, dichloromethane, ethyl acetate, and methanol leaf extract, had antimicrobial properties against both Gram negative (*Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*) and Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria. They used a microdilution experiment to find the minimum bactericidal concentration (MBC), or MIC. Methyl acetate (4.76%), dichloromethane (8.37%), acetone (6.66%), n-hexane (1.80%), and methanol extract (14.12%) were the next greatest yields in their investigation. The acetone and methanol

extracts had good antibacterial activity, according to their research, with MIC values ranging from 0.625 to 2.5 mg/mL and MBC values from 1.25 to 5 mg/mL.

These extracts' MIC and MBC values were 10-80 times less potent than those of the common antimicrobial medications they employed in their investigation, amoxicillin and miconazole nitrate (Sangetha *et al.*, 2008).

4. AIM AND PLAN OF WORK

4.1 Aim

Investigating the phytochemical makeup and assessing the *in vitro* antioxidant, anti-inflammatory, antibacterial, and antidiabetic capabilities of several solvent extracts of *Cassia spectabilis* stem are the goals of the current study.

4.2 Objectives

1. To obtain and examine *Cassia spectabilis* stems from their native environment.
2. To produce plant material extracts by using petroleum ether, chloroform, ethyl acetate, ethanol, and water in a successive solvent extraction process.
3. To conduct phytochemical screening of the extracts in order to detect the presence of bioactive components such proteins, carbohydrates, alkaloids, glycosides, phenols, tannins, flavonoids, and saponins.
4. To use UV spectrophotometric techniques to quantitatively assess the extracts' total phenolic and flavonoid content.
5. To use High-Performance Liquid Chromatography (HPLC) and Thin-Layer Chromatography (TLC) to detect marker chemicals in the extracts.
6. To assess the extracts' capacity to scavenge free radicals *in vitro* using models of nitric oxide, hydrogen peroxide, and DPPH.
7. To use the albumin denaturation inhibition paradigm to evaluate the anti-inflammatory activity *in vitro*.
8. To assess the extracts' antibacterial activity against certain species of bacteria and fungi.
9. To assess α -amylase and α -glucosidase enzyme inhibition experiments in order to look into the *in vitro* antidiabetic effectiveness.

4.2 Plan of Work

1. Collection and identification of plant

- **Collection and identification:** The plant material of *Cassia spectabilis* will be collected from its natural habitat. Ensure correct identification using botanical references or expert assistance.

2. Extraction of plant material

- **Washing:** Wash the collected material thoroughly in running tap water, then rinse with distilled water.
- **Drying:** Shade dry the plant material in open air.
- **Powdering:** Grind the dried material into a fine powder.

3. Defatting of plant material

- The powdered plant material is subjected to extraction with petroleum ether via maceration method.
- Continue the extraction until the material is defatted.

4. Successive extraction with different solvents

- The defatted plant material is extracted successively with chloroform, ethyl acetate, ethanol and water using maceration.

5. Preliminary phytochemical screening

- The extracts are tested for the presence of the following compounds:
 - Alkaloids
 - Glycosides
 - Phenols
 - Tannins
 - Flavonoids
 - Carbohydrates
 - Protein
 - Saponin

6. Estimation of various phytoconstituents

- **Estimation of total flavonoids content**
 - Aluminium chloride method by UV Spectroscopy.
- **Estimation of total phenolic content**
 - Modified Folin-Ciocalteu method by UV Spectroscopy.

7. Identification of marker compound by TLC

- Thin-Layer Chromatography (TLC) for identifying the marker compound.

8. Identification of marker compound by HPLC

- High-Performance Liquid Chromatography (HPLC) for identifying the marker compound in the extracts.

9. *In vitro* antioxidant and free radical scavenging assays

- DPPH Model
- H₂O₂ Model
- NO Model

10. *In vitro* anti-inflammatory models

- Albumin Denaturation Inhibition Model: To assess anti-inflammatory potential.

11. *In vitro* antimicrobial studies

- Antibacterial Activity: Using bacterial strains.
- Antifungal Activity: Using fungal strains.

12. *In vitro* antidiabetic studies

- α -amylase enzyme inhibition assays
- α -glucosidase enzyme inhibition assays

13. Static analysis of data

- Static analysis of data by using different software.

5. MATERIALS AND METHODS

5.1 Materials and Instruments

Materials used in the investigation are listed in Table 4.1.

Table 5.1: Materials used for study

Sr. No.	Chemicals	Supplier
1.	Bovine serum albumin	Sigma-Aldrich, USA
2.	Iodine	Laba chemie Pvt. Ltd., Mumbai
3.	Potassium Iodide	Laba chemie Pvt. Ltd., Mumbai
4.	Potassium Bismuth Iodide	S. D. Fine Chem. Ltd., Mumbai
5.	Picric acid	Thomas Baker, Mumbai
6.	Sodium nitropruside	Laba chemie Pvt. Ltd., Mumbai
7.	Sodium hydroxide	Laba chemie Pvt. Ltd., Mumbai
8.	Pyridine	S. D. Fine Chem. Ltd., Mumbai
9.	Ferric chloride	Thomas Baker, Mumbai
10.	Gelatin	S. D. Fine Chem. Ltd., Mumbai
11.	Lead acetate	Laba chemie Pvt. Ltd., Mumbai
12.	Nitric acid	S. D. Fine Chem. Ltd., Mumbai
13.	Copper acetate	S. D. Fine Chem. Ltd., Mumbai
14.	Sodium Chloride	S. D. Fine Chem. Ltd., Mumbai
15.	Methanol	Qualigens Fine Chemicals, Mumbai
16.	Ethanol	Qualigens Fine Chemicals, Mumbai
17.	Chloroform	Qualigens Fine Chemicals, Mumbai
18.	Folin-Ciocalteu reagent	Laba chemie Pvt. Ltd., Mumbai
19.	Fehling's solution	Central drug house ltd new Delhi
20.	Gallic acid	HiMedia, Mumbai
21.	Quercetin	HiMedia, Mumbai
22.	Aluminium chloride	Laba chemie Pvt. Ltd., Mumbai
23.	3,5-dinitrosalicylic acid	Laba chemie Pvt. Ltd., Mumbai
24.	Sodium potassium tartrate	Laba chemie Pvt. Ltd., Mumbai
25.	Acarbose	HiMedia, Mumbai

Instruments used in the investigation are listed in Table 4.2.

Table 5.2: Instruments used for study

Sr. No.	Instruments	Supplier
1.	UV -Visible Spectrophotometer	Lab science LS-2704 Mumbai
2.	Micro Centrifuge	REMI laboratory, Mumbai
3.	pH Meter	Accumax India, New Delhi
4.	Electronic Balance	Contech Instruments Ltd. , Mumbai
5.	Hot Air Oven	Oracle Equipments, New Delhi
6.	Vortex Apparatus	Ambros Lab Equipments, Ambala
7.	Rotary Vacuum Evaporator	Microtech Scientific Instruments, New Delhi
8.	Sonicator	Athena Technology, Thane
9.	Water bath	El, Mumbai
10.	HPLC	Waters, Mumbai
11.	UV chamber	Bio Technics India

5.2 Procurement of plant materials

Stem of *Cassia spectabilis* were collected from the Bhopal governing region in February 2023. To prevent the phytochemicals from deteriorating, the plants were cleaned after being collected.

5.2.1 Drying

The main purpose of drying is to remove water after cleaning. This should be done immediately to prevent spoilage or microbial growth of the plant sample. Conditions used for drying the plant material largely depend on the nature of its chemical constituents. Air-drying in room temperature under the sheds, in which the latter prevents direct sun exposure that can wither the plant and lose its heat or light-sensitive bioactives. This method of drying, however, may take a few weeks for complete drying and depends mainly on temperature and humidity.

Stem of *Cassia spectabilis* were shade-dried at room temperature. Using an electric grinder, the dried plant material was ground into a fine powder, sieved, and stored in plastic bags until it was needed.



Figure 5.1: Powdered plant material

5.3 Determination of physio-chemical Parameters

5.3.1 Determination of Ash Values

A. Determination of Total Ash: 2 g of accurately weighed stem powder was incinerated in a tarred platinum or silica dish at a temperature not exceeding 450°C until free from carbon, cooled and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water, the residue was collected on an ashless filter paper, incinerated, along with filter paper, evaporated to dryness and ignited at a temperature not exceeding 450°C. The ash thus obtained was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

B. Determination of Acid Insoluble Ash:

The ash obtained from above procedure was boiled for 5 min. with 25 ml of dilute hydrochloric acid and the insoluble matter was collected in a Gooch crucible, or on an ashless filter paper. The insoluble matter thus obtained was washed with hot water and filter paper was ignited to a constant weight along with filter paper. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

C. Determination of Water Soluble Ash:

The ash was boiled for 5 min. with 25 ml of water, the insoluble matter collected in a Gooch crucible, or on an ashless filter paper, washed with hot water and ignited for 15 min. at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight was the water soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug [83-84].

5.3.2 Determination of Moisture Content (loss on drying):

About 10 g of stem (without preliminary drying and cut in parts of about 3 mm in thickness), after accurately weighing (weight to within 0.01g) was placed in a tarred evaporation dish. It was then dried at 105°C for 5 hours and weighed. Drying was continued and the stem was weighed at 1 h interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was reached when two consecutive weighing after drying for 30 min. and cooling for 30 min. in a desiccator, did not show more than 0.01g difference.

5.4 Extraction by maceration process

An established and popular extraction method for separating bioactive substances from plant sources is maceration. This technique works especially well for removing phytochemicals from plants that include sensitive components that more aggressive extraction techniques that use heat or solvents might break down. The process described below was used to prepare the extract from the powdered and shade dried herbs (Khandelwal, 2005).

5.4.1 Defatting with petroleum ether

After being coarsely ground, 50 grams of stem of *Cassia spectabilis* were shade-dried at room temperature. Using an electric grinder, the dried plant material was ground into a fine powder, sieved, and stored in plastic bags until it was needed. shade dried plant material were macerated and extracted using petroleum ether. The extraction process was carried out until the material had been defatted.



Figure 5.2: Defatting of *Cassia spectabilis* with petroleum ether

5.4.2 Successive extraction with different solvent

Using a 48 hour maceration method, defatted dried powder was extracted using various solvents, including chloroform, ethyl acetate, ethanol and water. By increasing the contact between the plant material and the solvent, the mixture was periodically agitated to improve the extraction efficiency. To separate the liquid extract from the solid plant remains, the mixture is filtered after the soaking period. The dissolved phytochemicals are present in the filtrate. The extract is subsequently concentrated by removing the solvent, typically with a rotary evaporator or by evaporating under lower pressure. To separate particular bioactive components, the concentrated extract can undergo additional processing or analysis.

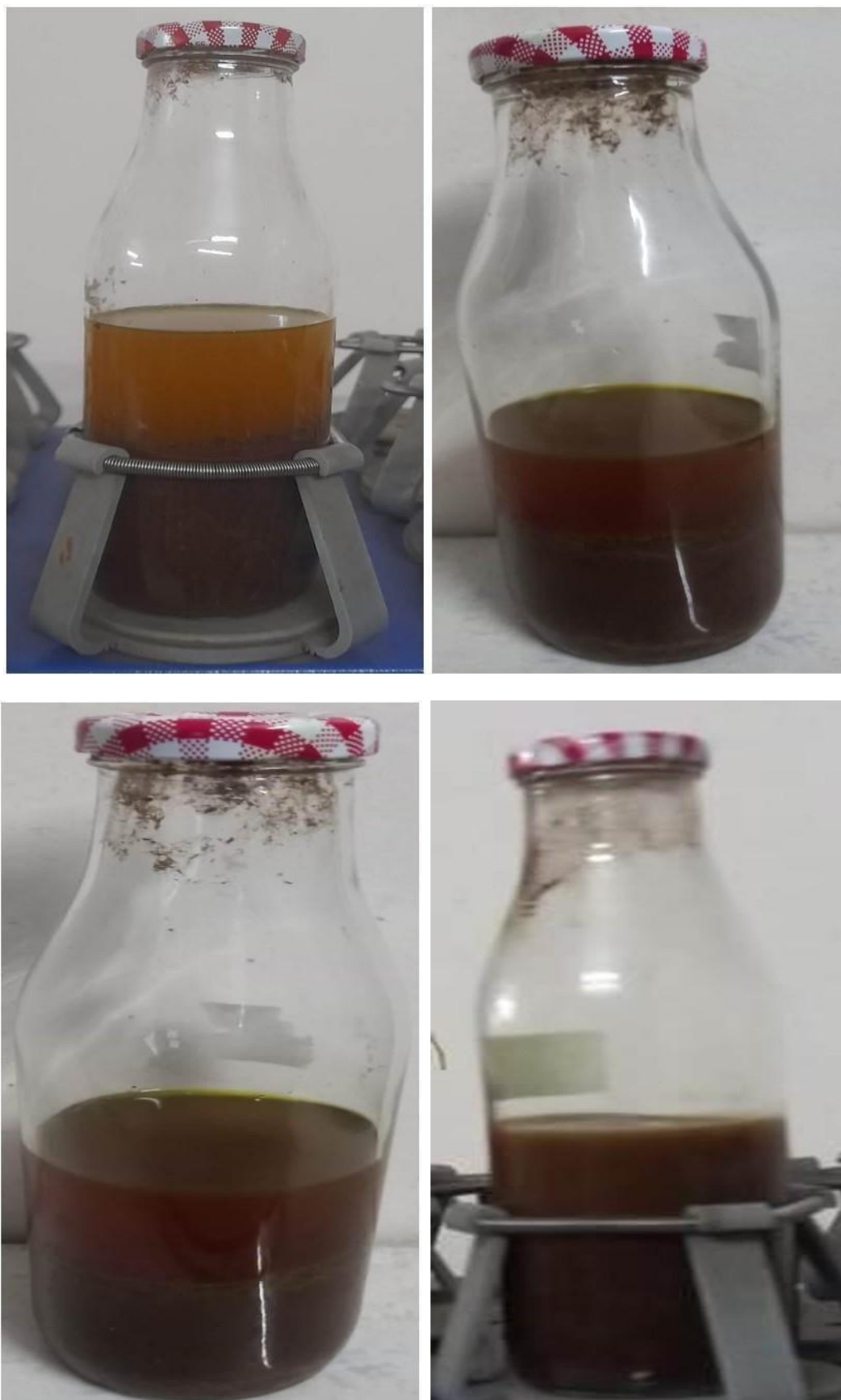


Figure 5.3: Extraction by maceration process

5.4.3 Determination of extractive value (% yield)

In the phytochemical study of plant materials, determining the extractive value also referred to as the yield percentage is an essential step. By measuring the amount of soluble components extracted from a specific amount of plant material, it assesses the extraction process effectiveness. This measure is essential for evaluating the extraction method efficacy and comparing the extraction efficiency of various solvents or circumstances.

5.4.3.1 Importance of extractive value

- **Evaluation of Extraction Efficiency:** The extractive value shows how successfully the target phytochemicals were extracted from the plant material using the selected solvent.
- **Standardization and Quality Control:** Consistent extractive values aid in ensuring the quality and strength of the extracts used in phytochemical research and herbal medicine.
- **Optimization of Extraction Conditions:** Optimizing extraction parameters including solvent type, solvent to plant ratio, extraction time, and temperature can be guided by the extractive value.
- The following formula was used to determine each extracts % yield:

$$\text{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

5.5 Qualitative phytochemical analysis

One essential procedure in the fields of pharmacognosy and natural products chemistry is qualitative phytochemical analysis. It entails identifying the different bioactive substances found in plant materials. Alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds are some examples of these substances, which are referred to as phytochemicals (Kokate, 1994). This analysis aids in the standardization of herbal products and offers useful information about the possible therapeutic qualities of plants. An essential first step in the investigation of therapeutic plants is qualitative phytochemical analysis. Researchers can better comprehend the therapeutic potential of plants, guarantee the quality and consistency of herbal medicines, and open the door for further in depth study and drug development by identifying the different bioactive chemicals present.

1. Detection of alkaloids: Separate extracts were dissolved in diluted hydrochloric acid and then filtered.

a) Hager's Test: Hager's reagent (saturated picric acid solution) was applied to the filtrates. Alkaloids are identified by the appearance of a yellow precipitate.

b) Wagner's test: 1 ml of each extract was mixed with equal parts of Wagner's reagent (2 g of KI and 1.27 g of I₂ were dissolved in 100 ml of distilled water). When a reddish-brown precipitate appears, alkaloids are present.

2. Detection of carbohydrates: Prior to filtering, each extract was individually diluted in 5 ml of distilled water. The filtrates were used to test for the presence of carbohydrates.

a) Fehling's Test: Fehling's A and B solutions were used to heat the filtrates, which were then neutralized with alkali and hydrolyzed with diluted hydrochloric acid. The development of red precipitate indicates the presence of reducing carbohydrates.

b) Benedict's Test: Benedict's reagent was applied to the filtrates, and they were then gently heated. The presence of reducing sugars is indicated by orange red precipitate.

3. Detection of glycosides: After hydrolyzing the extracts with dil. HCl, they were tested for glycosides.

a) Cone. H₂SO₄ Test: A few drops of concentrated sulfuric acid were added to the extract after it had been dissolved in distilled water. The presence of glycoside is shown by the formation of red color.

4. Detection of saponins

a) Froth Test: The extracts were diluted with 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes. The development of a 1 cm layer of foam indicates the presence of saponins.

6. Detection of phenols

a) Ferric Chloride Test: 3-4 drops of ferric chloride solution were added to the extract. The presence of phenols is indicated by the formation of a bluish black color.

b) Folio Ciocalteu Test: 1 ml of extract was mixed with 1 ml of Falin Ciocalteu reagent; the presence of phenols is indicated by the blue green color.

7. Detection of flavonoids

a) Alkaline Reagent Test: A few drops of sodium hydroxide solution were added to the extracts. Flavonoids are indicated by the formation of a bright yellow color that turns colorless when diluted acid is added.

b) Lead acetate Test: A few drops of lead acetate solution were added to the extracts. The presence of flavonoids is shown by the formation of a yellow precipitate.

8. Detection of proteins

a) Xanthoproteic Test: A few drops of concentrated nitric acid were added to the extracts. The presence of proteins is shown by the formation of yellow color.

9. Detection of diterpenes

a) Copper acetate Test: After dissolving the extracts in water, 3-4 drops of copper acetate solution were added. Diterpenes are present when an emerald green color forms.

10. Detection of tannins

a) Gelatin Test: A 1% gelatin solution containing sodium chloride was added to the extract. When tannins are present, a white precipitate will form.

11. Detection of Sterols

a) Salkowski Test: To the extract in chloroform, 3-4 drops of concentrated sulfuric acid were added. The presence of sterols is shown by the formation of a red color at the lower layer.

5.6 Quantitative studies of phytoconstituents

5.6.1 Estimation of total phenol content

The modified folin-ciocalteu method was used to calculate the extracts total phenol concentration (Gaur Mishra *et al.*, 2017). Various aliquots of 5-25 µg/ml were generated by dissolving 10 mg of gallic acid in 10 ml of methanol. After dissolving 10 mg of dried extract in 10 ml of methanol, the mixture was filtered. This extract was used to estimate the amount of phenol in 2 ml (1 mg/ml). 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate were combined with 2 ml of extract and each standard. For color development, the mixture was vortexed for 15 seconds and then left to stand for 10 minutes. Using a spectrophotometer, the absorbance at 765 nm was determined.

5.6.2 Estimation of total flavonoids content

The aluminum chloride technique was used to determine the total flavonoid content (Parkhe and Bharti, 2019). Various aliquots of 5-25 $\mu\text{g/ml}$ were produced by dissolving 10 mg of quercetin in 10 ml of methanol. 10 mg of dried extract were dissolved in 10 ml of methanol, and the resulting mixture was then filtered. The flavonoids in 2 ml (1 mg/ml) were estimated using this extract. 2 ml of 2% AlCh solution were added to 2 ml of extract of each standard, and the combination was then allowed to stand for 15 minutes at room temperature. The absorbance was then measured at 420 nm.



5.7 Separation and Identification of phytoconstituents in ethanolic extract of *Cassia spectabilis* by thin layer chromatography

Each solvent extract was cut with regular household scissors and then sent to thin layer chromatography (TLC) using silica gel 60F254 (7x6 cm; Merck) in accordance with the traditional one-dimensional ascending procedure. Soft pencil was used to make the markings on plates. The sample was spotted for TLC using glass capillaries at a distance of 1 cm at 5 tracks, with a sample volume of 1 μl . For flavonoids, a separate solvent system consisting of toluene, ethyl acetate, and formic acid (5:4:1) was employed in the twin trough chamber (Patel *et al.*, 2017). Following 20 minutes of pre-saturation using the mobile phase, development was employed. The retention factor (Rf), which was computed for many samples, was used to express the mobility of the active component.

Using a TLC cabinet (Electronic India), the produced thin layer chromatographic plates were seen under normal light, short UV light (254 nm), and long UV light (365 nm).

5.7.1 Detection and Calculation of R_f Value

1. Following the development of the chromatogram, the spots R_f Value was determined using the following formula:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

5.8 Identification of Quercetin in ethanolic extract of *Cassia spectabilis* by high-performance liquid chromatography

5.8.1 Reagents and chemicals

Merck Ltd. in New Delhi, India, supplied the HPLC-grade methanol and acetonitrile. The HPLC-grade water used came from Merck Ltd. in New Delhi, India. The Labindia 3000 + UVNIS Spectrophotometer thermospectronic model with 1cm matched quartz cells was used to determine the A_{max} . The N2000 Chromatography Data System, a pump, and a UV visible detector made up the HPLC system (Waters).



To guarantee effective separation and detection of the target compounds, the analysis separation variables were chosen with care. Chromatographic separation was performed using a C18 octadecylsilane bonded phase column that measured 250 mm by 4.60 mm and had a particle size of 5 μm .

Acetonitrile and methanol were mixed 50:50 to create the mobile phase, which offered the best solubility and peak resolution. To ensure consistency in retention durations, the analysis was carried out at room temperature while the flow rate was kept at 1 ml/min. To guarantee the analyte maximal absorbance, a 20 µl sample was added to the device, and detection was done at a wavelength of 256 nm. With a measured retention time of 2.50 ± 0.5 minutes, a quick and effective separation procedure was guaranteed.

5.8.2 Preparation of standard solution

A stock solution of 1000 ppm was created by precisely weighing 10 mg of quercetin, transferring it to a 10 ml volumetric flask, and then adjusting the volume with methanol (Garg, 2021). Standard drug solutions with concentrations of 5, 10, 15, 20, and 25 µg/ml were obtained by taking 1 ml of quercetin stock solutions, diluting them to 10 ml, and then transferring the 0.5, 1.0, 1.5, 2.0, and 2.5 ml solutions to 10 ml volumetric flasks and adding mobile phase to get the volume up to 10 ml.

5.8.3 Analysis of extract

In order to achieve a concentration of 1000 µg/ml, 10 mg of ethanolic extract of *Cassia spectabilis* was transferred to a 10 ml volumetric flask and diluted with methanol to the appropriate level. After passing the resultant solution through Whatmann filter paper, the same solvent was used to volumete it up to the appropriate level. After further filtering with a 0.45µ membrane filter, the resultant solution was sonicated for 10 minutes.

5.9 In-vitro antioxidant activity of ethanolic extract of *Cassia spectabilis*

5.9.1 DPPH method

With a minor modification, the revealed method was applied to assess the extracts overall ability to scavenge free radicals (Parkhe and Jain, 2018). A DPPH solution (6 mg in 100 ml methanol) was made and kept in a dark location. Standard and test concentrations ranging from 10 to 100 µg/ml were generated. 1.5 ml of **DPPH** and 1.5 ml of each standard and test were prepared in a separate test tube; the absorbance of this mixture was immediately measured at 517 nm. 1.5 ml of DPPH and 1.5 ml of methanol were tested as a control absorbance at 517 nm.

Using the following formula, the % inhibition of free radical **DPPH** was determined:

$$\% \text{ inhibition} = \frac{[(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}] \times 100\%}$$

5.9.2 Hydrogen peroxide method

Hydrogen peroxide was used to test the extracts *in-vitro* antioxidant properties (Wasiullah *et al.*, 2023). 2.4 ml of 0.1 M phosphate buffer (pH 7.4), 1.0 ml of ethanolic sample (100-500µg/ml), and 2 ml of hydrogen peroxide (43 mol) were added. After ten minutes of maintaining the resultant solution, the absorbance at 230 nm was measured. The blank was ready without hydrogen peroxide, and the control was made without any sample. It was combined with ascorbic acid as a standard compound. The percentage of free radical hydrogen peroxide scavenging activity has been computed.

$$\% \text{ inhibition} = \frac{[(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}] \times 100\%}$$

5.9.3 Nitric oxide method

The Griess reagent was measured and sodium nitroprusside was converted to nitric oxide. In aqueous solution at physiological pH, sodium nitroprusside spontaneously generates nitric oxide. It then interacts with oxygen to produce nitric ions, which may be measured using the Griess reagent. Reduced nitric oxide production is the result of nitric oxide scavengers competing with oxygen (Bawane *et al.*, 2020). Different extract concentrations were combined with sodium nitroprusside (10 mmol/L) in phosphate buffer saline (PBS), and the mixture was incubated for 150 minutes at 25°C. Specimens were treated with Griess reagent (1% sulfanilamide, 2% H₃PO₄, and 0.1% naphthylethylenediamine dihydrochloride). The absorption of standard ascorbic acid solutions prepared in the same way with Griess reagent as a positive control was measured at 546 nm using the chromophore absorbance produced during the diazotization of sulphanilamide nitrite and subsequent coupling with naphthylethylenediamine. The following formula was used to determine the inhibition proportion:

$$\text{Radical scavenging activity(\%)} = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100$$

Where, A_{test} is the absorption when the extract or standard is present, and A_{control} is the absorption of the control (without extract).

5.10 *In-vitro* anti-inflammatory activity of ethanolic extract of *Cassia spectabilis*

The experiment for protein denaturation was conducted using the (Gambhire *et al.*, 2009) method, with certain adjustments made in accordance with Gunathilake *et al.* (2018). The typical drug was diclofenac sodium, a potent non-steroidal anti-inflammatory medicine.

0.2 mL of 1% bovine albumin, 4.8 mL of phosphate buffered saline (PBS, pH 6.4), and 0.2 mL of extract (10-50 µg/mL) comprised the reaction mixture. After mixing the ingredients, the mixture was incubated for 15 minutes at 37°C and then heated for 5 minutes at 70°C. A UV/VIS spectrometer was used to measure the turbidity at 660 nm after cooling. The control was phosphate buffer solution. The following formula was used to determine the percentage inhibition of protein denaturation:

$$\% \text{ inhibition of denaturation} = (A1 - A2/A1) \times 100$$

Where, A1 denotes the control samples absorption and A2 denotes the test samples.

5.11 *In-vitro* antimicrobial activity of ethanolic extract of *Cassia spectabilis*

5.11.1 Well diffusion method

To cultivate bacteria in the lab, nutrient agar medium (NAM) was made. Petri dishes of the conventional size (100 x 15 mm) are needed for the entire experiment. 13 grams of powder and 1000 milliliters of distilled water were combined to create a homogenous mixture in order to prepare NAM. The NAM mixture was then autoclaved for 25 minutes at 121°C and 15 psi of pressure to sterilize the medium. In order to allow the agar to cool and harden at room temperature, the culture media was then placed into petri dishes at a ratio of 20 ml/dish and left partially covered on the table.

The Agar well-diffusion method was used to evaluate the antibacterial activity of the ethanolic extract of *Cassia spectabilis* (Bauer *et al.*, 1966). Fresh broth cultures of bacteria were used to swab nutrient agar (NA) plates using sterile cotton swabs. Using a sterile cork borer, wells (6 mm in diameter) were created in each of these plates. A solution containing 100 mg/ml, 50 mg/ml, and 25 mg/ml was made using various extracts. After adding roughly 100 µl of various extract concentrations using a sterile micropipette into each well, the wells were left to diffuse for 2 hours at room temperature. Distilled water inoculums were used in control studies.

For 24 hours, the plates were cultured for bacterial pathogens at 37°C. In addition to calculating the activity index, the inhibition zone diameter (mm) was assessed. The experiment was conducted three times, maintaining triplicates. For each replicate, readings were obtained in three fixed directions, and the average values were noted.

5.12 *In vitro* anti-diabetic activity of ethanolic extract of *Cassia spectabilis*

5.12.1 Alpha amylase inhibition assay

The α -amylase inhibition assay was carried out using the 3,5-dinitrosalicylic acid (DNSA) method (Wickramaratne *et al.*, 2016; Miller, 1959). The samples were initially dissolved in a minimal amount of 10% DMSO, and then further diluted in a buffer solution composed of $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ (0.02 M), NaCl (0.006 M) at pH 6.9 to achieve concentrations ranging from 10 to 50 $\mu\text{g}/\text{ml}$. Acarbose was used as a reference standard. To each test tube, 200 μl of α -amylase solution (2 units/ml) was added and mixed with 200 μl of the sample. The mixture was incubated for 10 minutes at 30°C . Following this, 200 μl of a 1% starch solution (w/v) in water was added and the reaction was further incubated for 3 minutes. The reaction was then stopped by adding 200 μl of DNSA reagent, prepared by dissolving 12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of a 96 mM 3,5-dinitrosalicylic acid solution. The mixture was boiled in a water bath at $85\text{--}90^\circ\text{C}$ for 10 minutes, then cooled to room temperature. After cooling, 5 ml of distilled water was added to dilute the mixture, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. A blank with 100% enzyme activity was prepared by replacing the sample with 200 μl of buffer. Another blank was prepared using the sample at each concentration, but in the absence of the enzyme solution. The α -amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below: The % α -amylase inhibition was plotted against the sample concentration and the IC_{50} values were obtained from the graph.

$$\% \text{ inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

5.12.2 Alpha glucosidase inhibition assay

The α -glucosidase inhibitory activity was determined according to the method described by Apostolidis *et al.*, (2007) with some modifications (Molina and Rani, 2020). Stock solutions of extracts were prepared by dissolving up to 10mg of each extract in 10ml of DMSO. A total of 50 μL of extracts (1.25-10mg/mL) and 100 μL of yeast α glucosidase solution in phosphate buffer (pH-6.9) were incubated at 25°C for 10 minutes followed by the addition of 50mL of 5Mmol/L p-nitrophenyl- α -D-glucopyranoside solution in 0.1M phosphate buffer (pH 6.9). The reaction mixture was then incubated at 25°C for 5min the reaction was terminated by adding 3ml of 100Mm sodium carbonate solution into the mixture to stop the reaction and absorbance of liberated p-nitrophenol was read at 405nm

using UV-Visible Spectrophotometer. Acarbose was used a positive control (standard) and the inhibitory activity of alpha-amylase and alpha-glucosidase were calculated by using the following formula:

$$\% \text{ inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

The IC₅₀ values defined as the concentration of the extract that inhibited 50% of the enzyme activity were determined from plots of % inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values.

5.13 Estimation of Quercetin Using HPTLC Method

The quantification of quercetin in the ethanolic leaf extract of *Cassia spectabilis* was carried out using a validated High-Performance Thin Layer Chromatography (HPTLC) method. The analysis was performed using a CAMAG HPTLC system equipped with a Linomat 5 applicator, TLC scanner 3, and Wincats software (version 1.44). Additional accessories included a Hamilton syringe (100 µL), Reprostar 3 documentation system, TLC plate heater, and UV cabinet. The work was conducted at the MFP-PARC HPTLC Facility.

5.13.1 Preparation of Standard Solution

A stock solution of quercetin (1 mg/mL) was prepared in methanol. From this stock, 100 µL was diluted to 1 mL with methanol to obtain a working standard solution of 100 µg/mL. This standard solution was used for preparing calibration spots on the TLC plate in varying volumes.

5.13.2 Preparation of Extract Sample

The ethanolic leaf extract of *Cassia spectabilis* was accurately weighed and dissolved in methanol to prepare a solution of 1 mg/mL concentration. From this, 100 µL was diluted with 950 µL of methanol to obtain a working concentration of 100 µg/mL. The solution was filtered and used for spotting on the TLC plate.

Table 5.3 Chromatographic Conditions

Parameter	Specification
Instrument	CAMAG Automatic TLC Sampler with Wincats software
Stationary Phase	Precoated Silica gel 60 _{F254} TLC plates (20 x 10 cm, 0.2 mm thickness, E. MERCK KGaA)
Mobile Phase	Toluene : Ethyl acetate : Formic acid (5 : 4 : 1 v/v/v)
Development Chamber	Twin trough glass chamber (20 x 10 cm)
Development Mode	Ascending
Distance Run	75mm
Detection Wavelength	254nm
Band Length	8mm
Number of Tracks	8
Standard Injection Volumes (μL)	2, 4, 6, 8, 10, 12
Sample Injection Volumes (μL)	2,4
Development Time	15 minutes
Drying Method	Room temperature air drying

5.13.3 Preparation and Development of TLC Plates

The TLC plates were pre-washed with methanol and activated at 110 °C for 5 minutes prior to use. Using a LINOMAT IV applicator, the standard and sample solutions were applied as narrow bands (8 mm width) on the silica gel plate. The chamber was saturated with the mobile phase for 20 minutes before plate development.

After development, plates were air-dried and scanned densitometrically at 254 nm using a CAMAG TLC Scanner 3. The peak areas corresponding to quercetin were recorded, and a calibration curve was constructed by plotting concentration (μg/spot) versus mean peak area.

5.13.4 Estimation of Quercetin in Ethanolic Extract of *Cassia spectabilis*

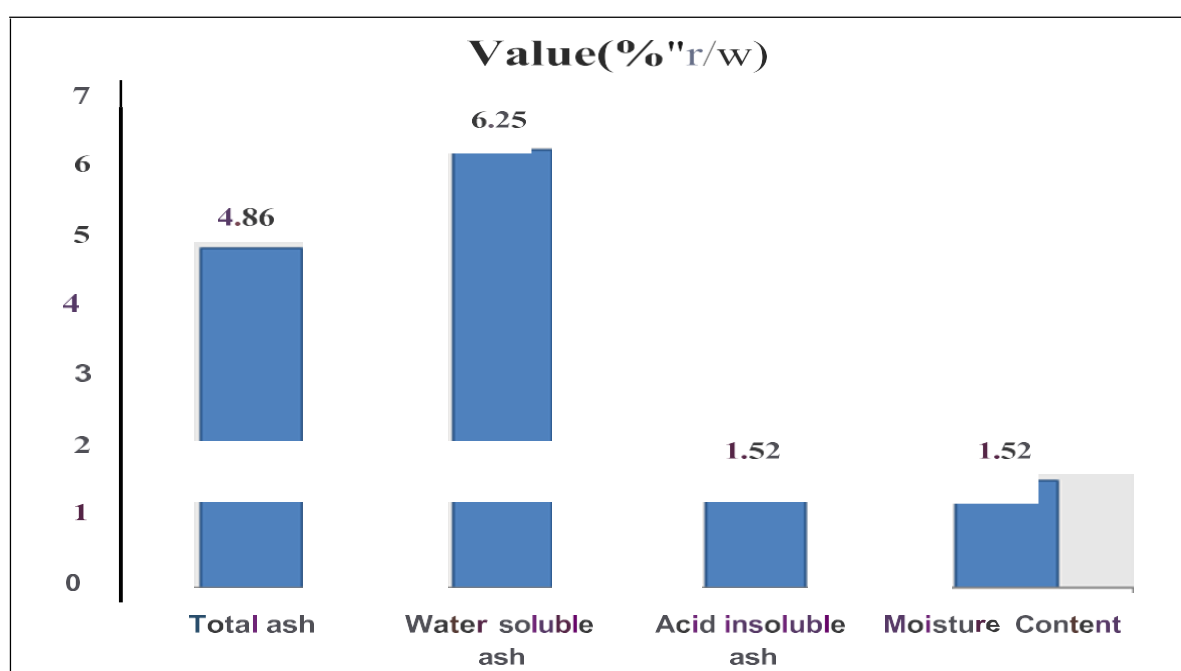
The ethanolic extract sample (2 μL and 4 μL application) showed a well-defined peak at $R_f = 0.33 \pm 0.01$, corresponding to that of standard quercetin. The concentration of quercetin in the extract was calculated using the regression equation obtained from the calibration plot.

6. RESULTS AND DISCUSSION

6.1 Results of physicochemical test of plant powder

Table 6.1: Physicochemical test of *Cassia spectabilis*

S. No.	Parameters	Value(% w/w)
1	Total ash	4.86
2	Water soluble ash	6.25
3	Acid insoluble ash	1.52
4	Moisture Content	1.52

Figure 6.1: Physicochemical test of *Cassia spectabilis*

The physicochemical analysis of *Cassia spectabilis* was carried out to determine parameters such as total ash, water-soluble ash, acid-insoluble ash, and moisture content. The results, as presented in Table 5.1, revealed that the total ash value was 4.86% w/w, indicating the presence of both physiological and non-physiological ash derived from the plant tissue and external matter such as soil or inorganic salts. The water-soluble ash value of 6.25% w/w suggests the presence of water-soluble inorganic components, which may include potassium, sodium, and calcium salts. The acid-insoluble ash value of 1.52% w/w reflects the minimal presence of silica or other earthy impurities, indicating good purity of the plant material. The moisture content was found to be 1.52% w/w, which is within acceptable

limits, demonstrating low moisture level and, therefore, less susceptibility to microbial contamination or degradation. These values confirm that the plant material used in the study meets standard physicochemical quality parameters, ensuring its suitability for further phytochemical and biological investigations.

6.2 Results of extractive values

Table 6.2: Extractive values of *Cassia spectabilis*

S. No.	Extracts	% Yield (w/w)
1.	Chloroform	1.25
2.	Ethyl acetate	2.62
3.	Ethanolic	7.23
4.	Aqueous	11.47

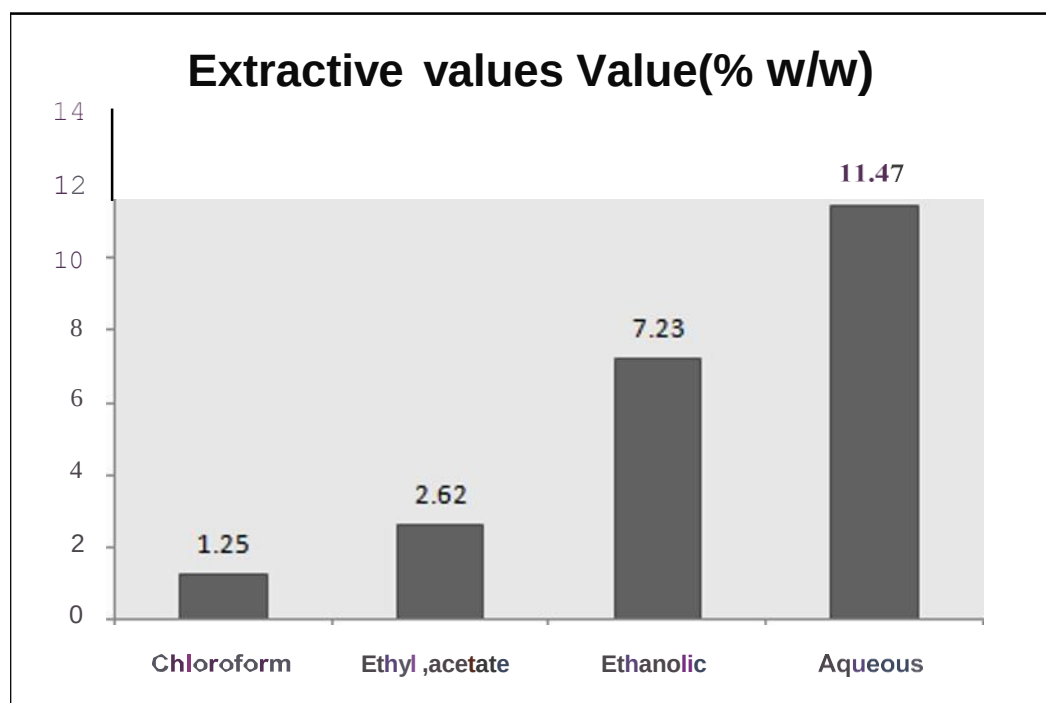


Figure 6.2: Extractive values of *Cassia spectabilis*

The extractive values of *Cassia spectabilis* obtained using different solvents are presented in Table 5.2. The results revealed that the aqueous extract showed the highest yield of 11.47% w/w, followed by the ethanolic extract with 7.23% w/w, the ethyl acetate extract with 2.62% w/w, and the chloroform extract with 1.25% w/w. The variation in extractive values among the solvents can be attributed to differences in their polarity, which influence the solubility of various phytoconstituents. The higher yield in the aqueous and ethanolic extracts indicates the presence of a greater number of polar bioactive compounds such as

phenolics, flavonoids, glycosides, and tannins, which are more soluble in polar solvents. Conversely, the lower yields obtained with chloroform and ethyl acetate suggest that non-polar or moderately polar constituents are present in smaller amounts. These findings suggest that polar solvents are more effective for extracting a broad spectrum of phytochemicals from *Cassia spectabilis*, making the ethanolic and aqueous extracts suitable for further pharmacological evaluation.

6.3 Results of phytochemical screening of extract of *Cassia spectabilis*

Table 6.3: Result of phytochemical screening of chloroform extract of *Cassia spectabilis*

S. No.	Constituents	Chloroform extract
1.	Alkaloids Wagner's Test: Hager's Test:	-ve -ve
2.	Glycosides Cone. H ₂ SO ₄ Test:	-ve
3.	Flavonoids Lead acetate Test: Alkaline test:	-ve -ve
4.	Diterpenes Copper acetate Test:	-ve
5.	Phenol Ferric Chloride Test: Falin Ciocalteu Test:	-ve -ve
6.	Proteins Xanthoproteic Test:	+ve
7.	Carbohydrate Fehling's Test: Benedict's Test	-ve -ve
8.	Saponins Froth Test:	-ve
9.	Tannins	

	Gelatin test:	-ve
10.	Sterols Salkowski Test:	-ve

+ve= present, -ve=negative

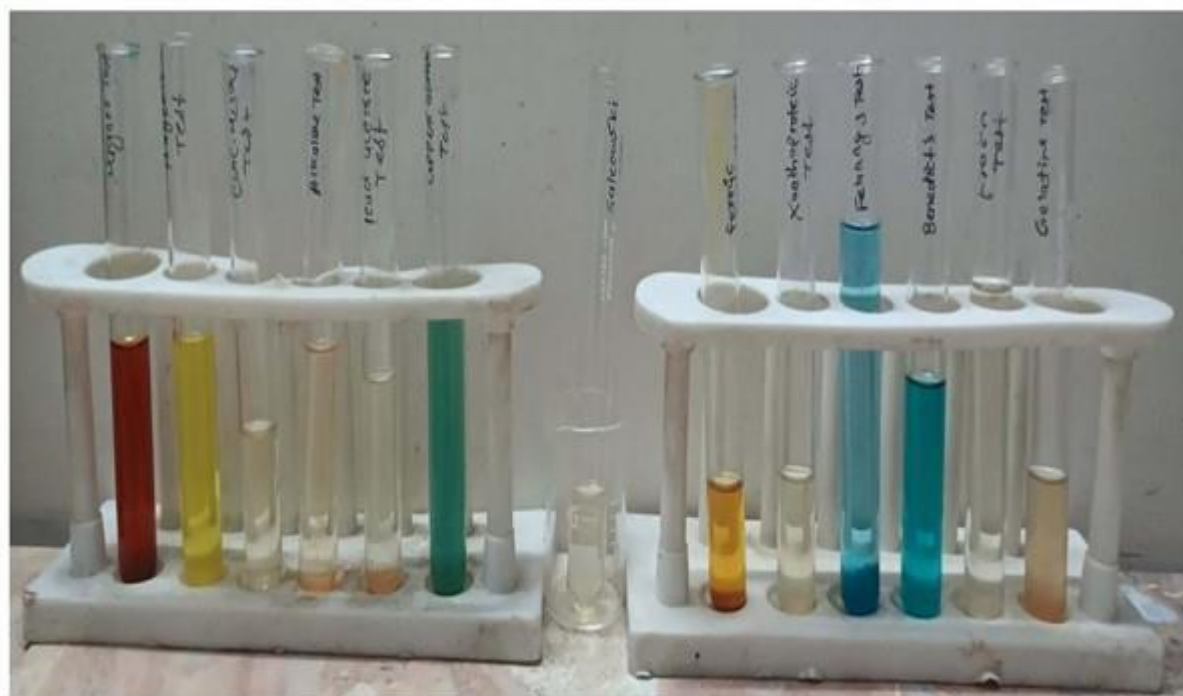


Figure 6.3: Phytochemical screening of chloroform extract of *Cassia spectabilis*

The preliminary phytochemical screening of the chloroform extract of *Cassia spectabilis*, as shown in Table 5.3, revealed the presence of proteins, while all other tested phytoconstituents such as alkaloids, glycosides, flavonoids, diterpenes, phenols, carbohydrates, saponins, tannins, and sterols were found to be absent. The positive result for proteins in the chloroform extract indicates that certain non-polar or moderately polar proteinaceous compounds may be extractable in chloroform under the given conditions. The absence of most other phytoconstituents suggests that chloroform, being a less polar solvent, is less efficient in extracting polar bioactive compounds such as flavonoids, glycosides, and phenols, which are more soluble in polar solvents like ethanol and water. Hence, the chloroform extract is expected to contain mainly non-polar constituents in negligible amounts, and the results confirm that *Cassia spectabilis* is rich in polar compounds, making polar solvents more suitable for further phytochemical and pharmacological studies.

Table 6.4: Result of phytochemical screening of ethyl acetate extract of *Cassia spectabilis*

S. No.	Constituents	Ethyl acetate extract
1.	Alkaloids Wagner's Test: Hager's Test:	-ve -ve
2 .	Glycosides Cone. H ₂ SO ₄ Test:	+ve
3 .	Flavonoids Lead acetate Test: Alkaline test:	+ve -ve
4.	Diterpenes Copper acetate Test:	-ve
5 .	Phenol Ferric Chloride Test: Polin Ciocalteu Test:	-ve -ve
6.	Proteins Xanthoproteic Test:	+ve
7.	Carbohydrate Fehling's Test: Benedict's Test	-ve -ve
8.	Saponins Froth Test:	-ve
9.	Tannins Gelatin test:	-ve
10.	Sterols Salkowski Test:	-ve

+ve= present, -ve=negative

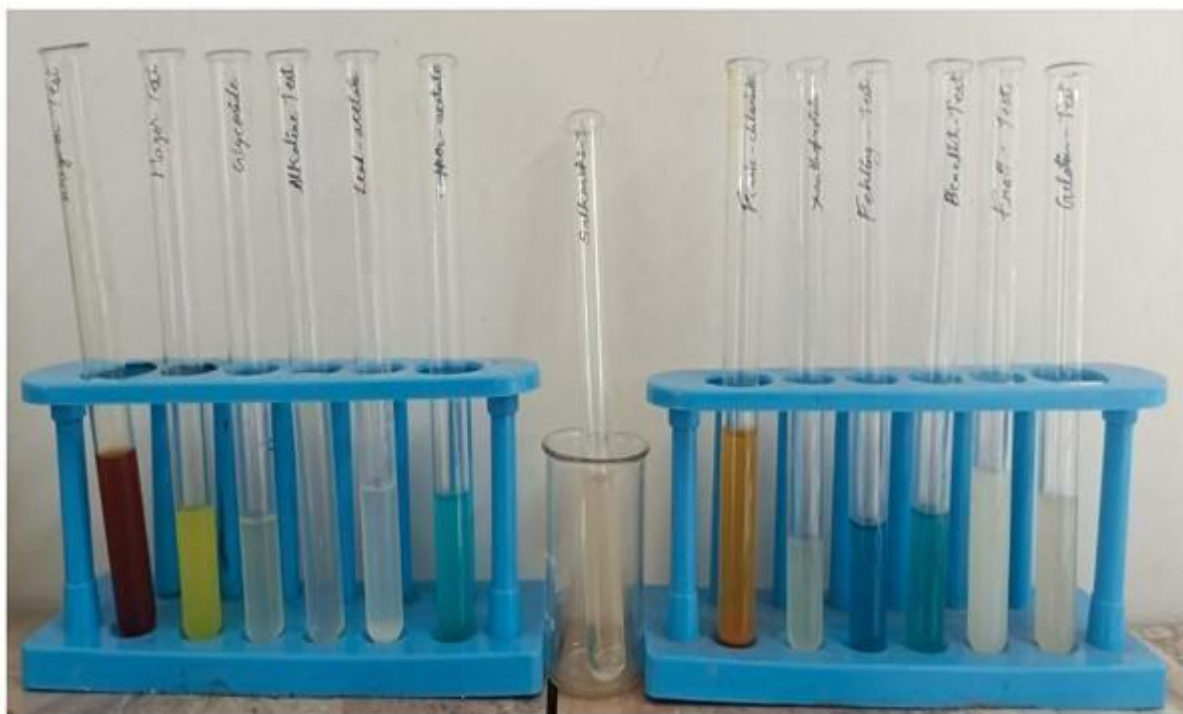


Figure 6.4: Phytochemical screening of ethyl acetate extract of *Cassia spectabilis*

The phytochemical screening of the ethyl acetate extract of *Cassia spectabilis* (Table 5.4) revealed the presence of glycosides, flavonoids, and proteins, while other constituents such as alkaloids, diterpenes, phenols, carbohydrates, saponins, tannins, and sterols were found to be absent. The detection of glycosides and flavonoids indicates that ethyl acetate, being a moderately polar solvent, is capable of extracting certain semi-polar bioactive compounds from the plant material. The presence of these compounds suggests potential biological activities, as flavonoids and glycosides are known for their antioxidant, anti-inflammatory, and antimicrobial properties. The positive test for proteins also supports the presence of nitrogenous compounds in the extract. The absence of other constituents such as tannins, phenols, and saponins may be due to their higher solubility in more polar solvents like ethanol or water. The results indicate that the ethyl acetate extract of *Cassia spectabilis* contains selective bioactive components, particularly glycosides and flavonoids, which may contribute to its pharmacological potential.

Table 6.5: Result of phytochemical screening of ethanolic extract of *Cassia spectabilis*

S. No.	Constituents	Ethanolic extract
1.	Alkaloids Wagner's Test: Hager's Test:	-ve -ve
2.	Glycosides Cone. H ₂ SO ₄ Test:	-ve
3.	Flavonoids Lead acetate Test: Alkaline test:	+ve +ve
4.	Diterpenes Copper acetate Test:	-ve
5.	Phenol Ferric Chloride Test: Folin Ciocalteu Test:	-ve +ve
6.	Proteins Xanthoproteic Test:	+ve
7.	Carbohydrate Fehling's Test: Benedict's Test	+ve -ve
8.	Saponins Froth Test:	+ve
9.	Tannins Gelatin test:	-ve
10.	Sterols Salkowski Test:	-ve

+ve= present, -ve=negative



Figure 6.5: Phytochemical screening of ethanolic extract of *Cassia spectabilis*

The phytochemical screening of the ethanolic extract of *Cassia spectabilis*, as presented in Table 5.5, revealed the presence of flavonoids, phenols, proteins, carbohydrates, and saponins, while alkaloids, glycosides, diterpenes, tannins, and sterols were found to be absent. The positive results for flavonoids and phenols indicate that ethanol, being a polar solvent, efficiently extracts polyphenolic compounds known for their potent antioxidant and anti-inflammatory activities. The presence of saponins suggests potential membrane-stabilizing and antimicrobial effects, while the detection of carbohydrates and proteins reflects the nutritional or structural constituents of the plant. The absence of alkaloids and tannins may be due to their low concentration or poor solubility in ethanol under the extraction conditions used. The ethanolic extract showed a rich composition of polar bioactive compounds, particularly flavonoids and phenols, which could significantly contribute to the pharmacological activities of *Cassia spectabilis* and justify the use of ethanol as an effective extraction solvent for further biological evaluations.

Table 6.6: Result of phytochemical screening of aqueous extract of *Cassia spectabilis*

S. No.	Constituents	Aqueous extract
1.	Alkaloids Wagner's Test: Hager's Test:	-ve -ve
2.	Glycosides Cone. H ₂ SO ₄ Test:	-ve
3.	Flavonoids Lead acetate Test: Alkaline test:	+ve +ve
4.	Diterpenes Copper acetate Test:	+ve
5.	Phenol Ferric Chloride Test Falin Ciocalteu Test	+ve +ve
6.	Proteins Xanthoproteic Test:	+ve
7.	Carbohydrate Fehling's Test: Benedict's Test	+ve +ve
8.	Saponins Froth Test:	+ve
9.	Tannins Gelatin test:	+ve
10.	Sterols Salkowski Test:	-ve

+ve= present, -ve=negative

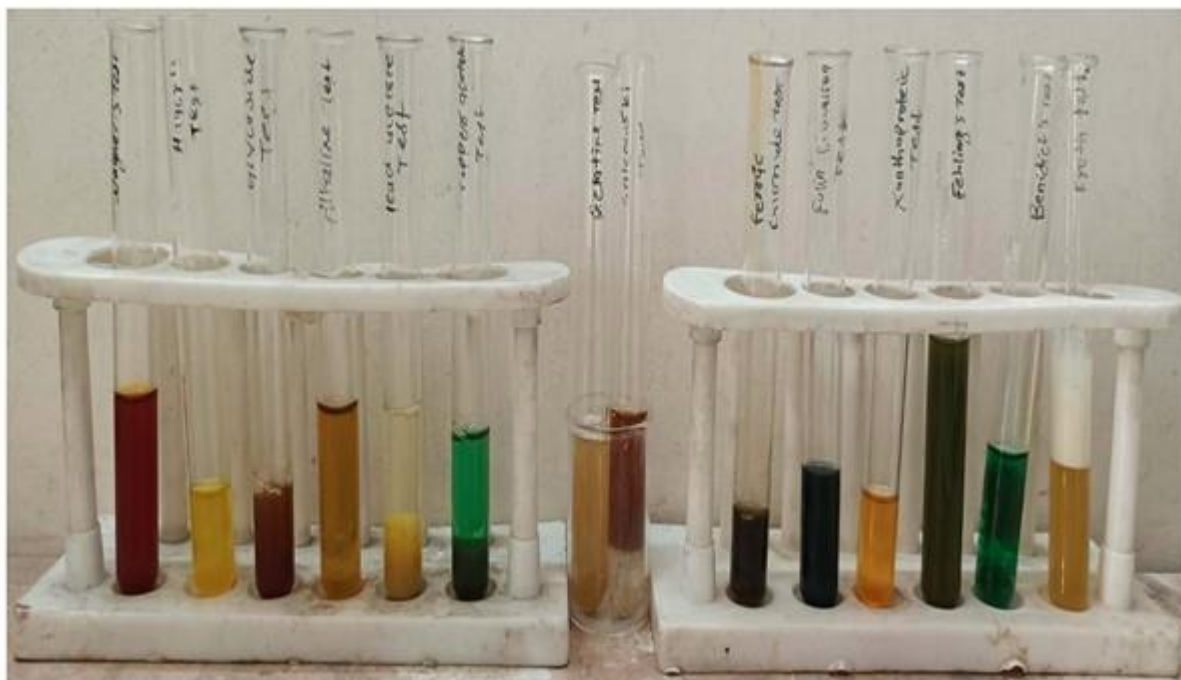


Figure 6.6: Phytochemical screening of aqueous extract of *Cassia spectabilis*

The phytochemical screening of the aqueous extract of *Cassia spectabilis*, as shown in Table 5.6, revealed the presence of a wide range of bioactive constituents, including flavonoids, diterpenes, phenols, proteins, carbohydrates, saponins, and tannins, while alkaloids, glycosides, and sterols were found to be absent. The strong presence of flavonoids and phenols indicates that the aqueous extract is rich in polyphenolic compounds, which are well known for their antioxidant, anti-inflammatory, and antidiabetic properties. The detection of saponins and tannins suggests potential antimicrobial and membrane-stabilizing activities, while the presence of carbohydrates and proteins reflects the nutritive and structural components of the plant. The positive test for diterpenes further supports the occurrence of secondary metabolites that may contribute to anti-inflammatory and cytoprotective effects. The absence of alkaloids and glycosides could be attributed to their poor solubility in water. The aqueous extract exhibited the highest diversity of phytochemicals among all tested extracts, suggesting that water is an effective solvent for extracting a broad range of polar compounds responsible for the medicinal properties of *Cassia spectabilis*.

6.4 Result of estimation of total phenol and flavonoids content of *Cassia spectabilis* extract

6.4.1 Estimation of total phenolic content (TPC)

The folin-ciocalteu (FC) reagent method is frequently used to measure the total phenolic content (TPC) of plant extracts. This technique relies on phenolic chemicals reducing phosphomolybdic-phosphotungstic acid complexes to produce a blue complex that can be measured using spectrophotometry.

Calibration Curve of Gallic acid

Table 6.7: Preparation of Calibration curve of Gallic acid

S. No.	Concentration ($\mu\text{g/ml}$)	Mean Absorbance
1	5	0.164
2	10	0.339
3	15	0.526
4	20	0.673
5	25	0.851

(n=3, Mean $\pm$ SD)

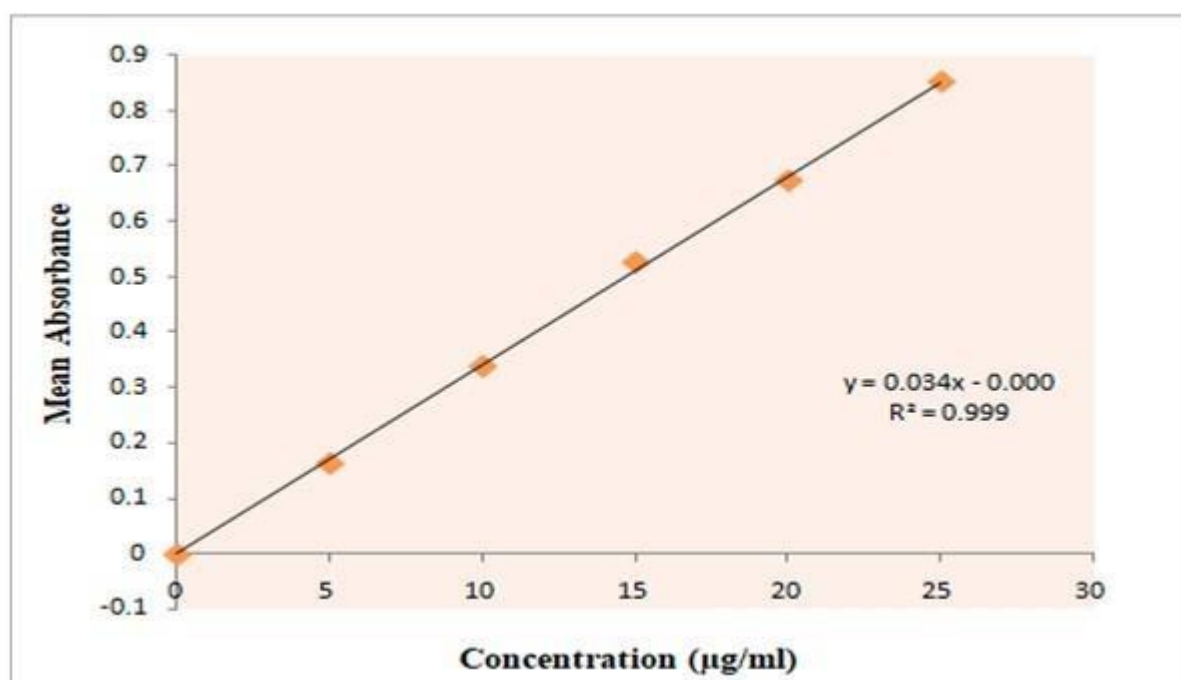


Figure 6.7: Graph of calibration curve of Gallic acid

Table 6.8: Statistical parameters related to standard curve of Gallic acid

Parameters	Values
Beer's Law Range	5-25 µg/ml
Regression Coefficient	$R^2 = 0.999$
Regressed line equation ($y = mx + c$)	$y = 0.034x - 0.000$

6.4.2 Estimation of total flavonoids content (TFC)

The aluminum chloride (AlCh) Colorimetric Method is commonly used to assess the total flavonoid content (TFC) of plant extracts. The foundation of this technique is the spectrophotometric quantification of the yellow complex that forms between flavonoids and aluminum chloride.

Calibration curve of Quercetin**Table 6.9:**

S. No.	Concentration (µg/ml)	Mean Absorbance
1	5	0.257
2	10	0.468
3	15	0.701
4	20	0.894
5	25	1.102

(n=3, Mean±SD)

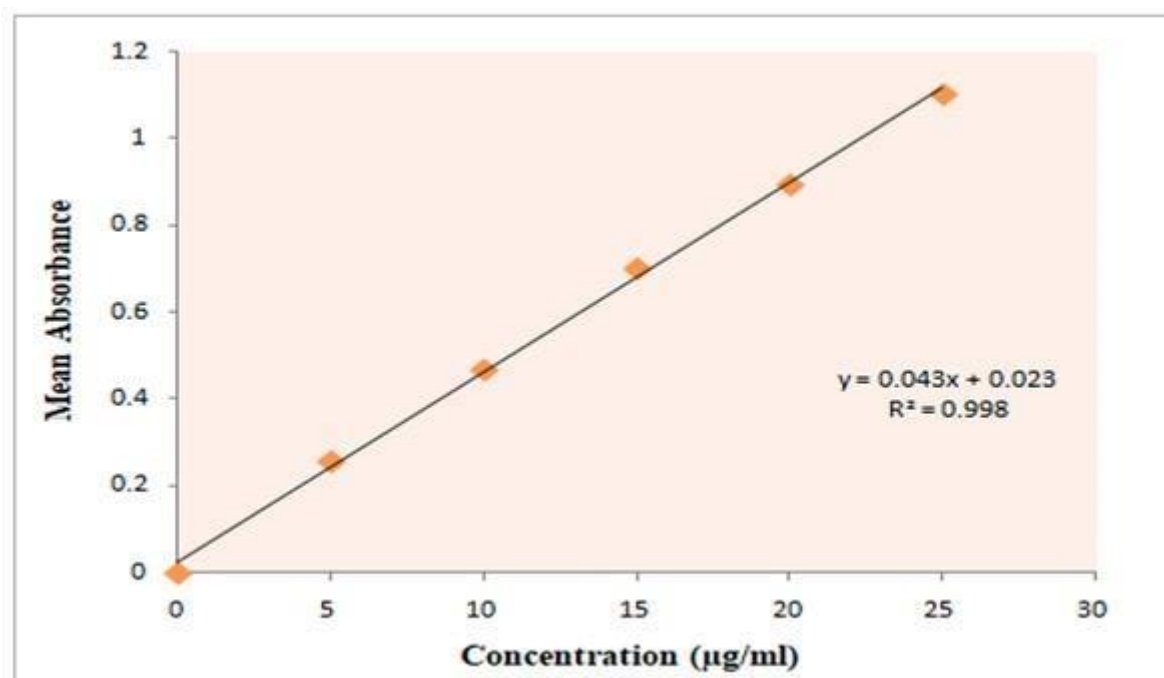


Figure 6.8: Graph of calibration curve of Quercetin

Table 6.10: Statistical parameters related to standard curve of Quercetin

Parameters	Values
Beer's Law Range	5-25 µg/ml
Regression Coefficient	$R^2 = 0.998$
Regressed line equation ($y = mx + c$)	$y = 0.043x + 0.023$

Table 6.11: Estimation of total phenol and flavonoids content of *Cassia spectabilis*

S. No.	Extracts	Total phenol content	Total flavonoids content
		Gallic acid equivalent (GAE) mg/ 100 mg	Quercetin equivalent (QE) mg/ 100 mg
1.	Ethyl acetate	-	0.21
2.	Ethanollic	0.75	
3.	Aqueous	0.62	0.83

The quantitative estimation of total phenolic and flavonoid content of *Cassia spectabilis* extracts, as presented in Table 5.11, revealed that the ethanolic extract contained the highest amounts of both phenols (0.75 mg GAE/100 mg) and flavonoids (0.96 mg QE/100 mg), followed by the aqueous extract with 0.62 mg GAE/100 mg of phenols and 0.83 mg QE/100 mg of flavonoids. In contrast, the ethyl acetate extract showed the lowest flavonoid content (0.21 mg QE/100 mg) and no detectable phenolic content. The higher concentration of

phenolic and flavonoid compounds in the ethanolic and aqueous extracts suggests that these polar solvents are more efficient in extracting polyphenolic constituents compared to the moderately polar ethyl acetate solvent. Since phenols and flavonoids are known for their strong antioxidant, anti-inflammatory, and antidiabetic activities, the richness of these compounds in the ethanolic extract indicates its greater potential for biological efficacy. Thus, these findings highlight the ethanol extract of *Cassia spectabilis* as a promising source of natural antioxidants and bioactive phytochemicals.

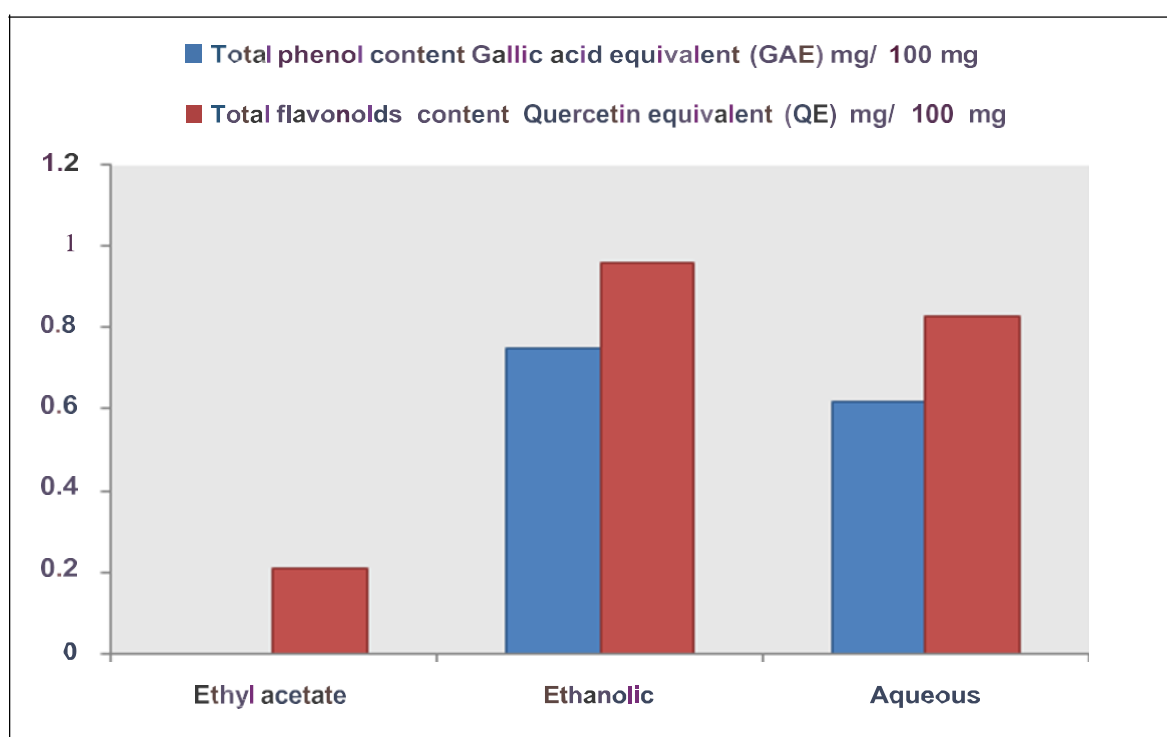


Figure 6.9: Graph of total phenol and flavonoids content of *Cassia spectabilis*

6.5 Results of TLC of ethanolic extract of *Cassia spectabilis*Table 6.12: TLC of ethanolic extract of *Cassia spectabilis*

S. No.	Mobile phase Toluene: Ethyl acetate Formic acid (5:4:1)	R _f value
1.	(Quercetin) Dis. travel by mobile phase= 5.0 cm No. of spot at normal light = 1 No. of spot at short UV = 1 No. of spot at long UV = 1	Normal- 0.52 Short- 0.52 Long- 0.52
2.	(Ethyl acetate extract) No. of spot at normal light = 1 No. of spot at short UV= 3 No. of spot at long UV = 5	Normal- 0.92 Short- 0.64, 0.72, 0.92 Long- 0.52, 0.58, 0.64, 0.72, 0.92
3.	(Ethanolic extract) No. of spot at normal light = 1 No. of spot at short UV= 3 No. of spot at long UV = 7	Normal- 0.92 Short- 0.64, 0.72, 0.92 Long- 0.021, 0.52, 0.58, 0.60, 0.64, 0.72, 0.92
4.	(Aqueous extract) No. of spot at normal light = 0 No. of spot at short UV= 1 No. of spot at long UV = 5	Normal- 0 Short- 0.70 Long- 0.021, 0.52, 0.60, 0.64, 0.92

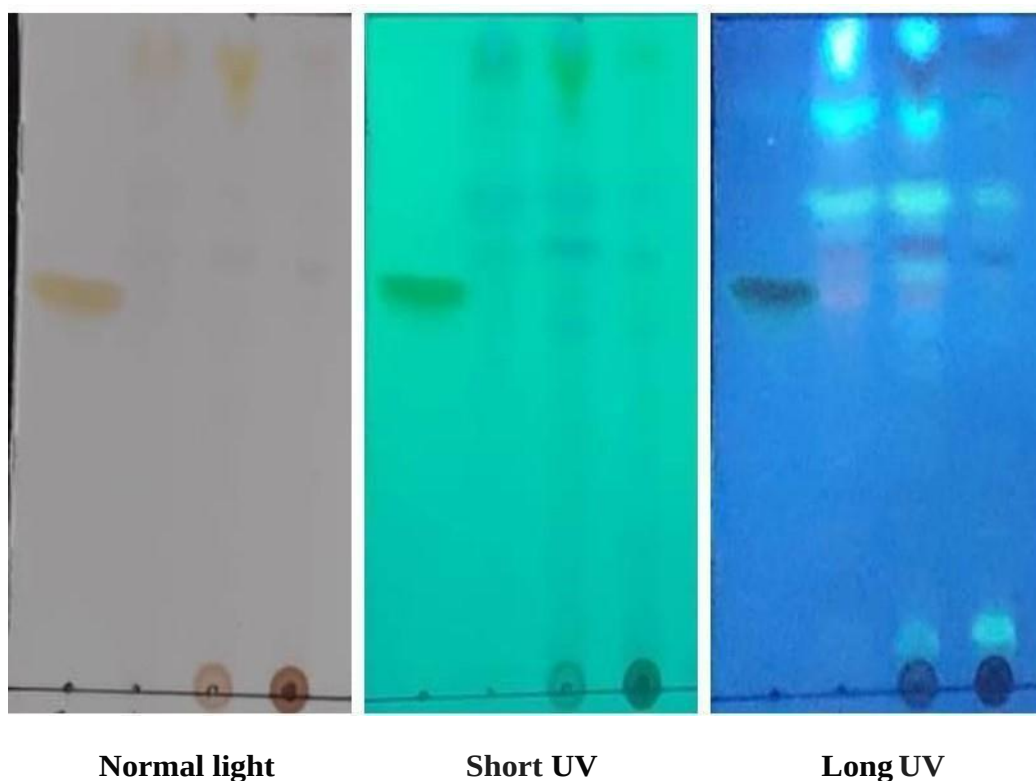


Figure 6.10: TLC of Flavonoids

1st spot= Standard Quercetin, 2nd spot= Ethyl acetate extract, 3rd spot= Ethanolic extract, 4th spot= Aqueous extract

Thin Layer Chromatography (TLC) analysis was performed on the ethanolic, ethyl acetate, and aqueous extracts of *Cassia spectabilis*, using quercetin as the standard reference compound. The solvent system selected for development was Toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v). The mobile phase travelled a distance of 5.0 cm, and the developed plates were examined under visible light, short-wave UV (254 nm), and long-wave UV (366 nm) to visualize and compare the separated phytochemical components.

The standard quercetin produced a single, distinct spot with an R_f value of 0.52 across all detection modes, confirming its purity and stability in the chosen solvent system. This R_f value served as a comparative reference for identifying quercetin or similar flavonoid compounds in the extracts of *Cassia spectabilis*.

The ethyl acetate extract exhibited one visible spot under normal light (R_f 0.92), three spots under short UV (R_f 0.64, 0.72, and 0.92), and five fluorescent spots under long UV (R_f 0.52, 0.58, 0.64, 0.72, and 0.92). The multiple bands observed under UV light suggest the presence of several phytochemical constituents, including flavonoids, phenolic acids, and terpenoids. The band at R_f 0.52, which corresponds to that of quercetin, indicates that the extract contains quercetin or compounds of a similar chemical nature.

In the case of the ethanolic extract, one spot was visible under normal light (R_f 0.92), three under short UV (R_f 0.64, 0.72, and 0.92), and seven under long UV (R_f 0.021, 0.52, 0.58, 0.60, 0.64, 0.72, and 0.92). The greater number of fluorescent spots indicates that ethanol effectively extracted a wide range of bioactive compounds, particularly flavonoids and phenolic constituents. The appearance of a spot at R_f 0.52 confirms the presence of quercetin in the ethanolic extract. This aligns with previous findings that *Cassia* species are rich in flavonoids such as quercetin, which contribute to their antioxidant and therapeutic properties.

The aqueous extract showed no visible spots under normal light, one spot under short UV (R_f 0.70), and five under long UV (R_f 0.021, 0.52, 0.60, 0.64, and 0.92). The relatively fewer spots suggest that water extracted only a limited number of UV-active compounds, likely due to its polarity restricting the extraction of less polar constituents. Nonetheless, the consistent presence of a band at R_f 0.52 indicates that quercetin or its derivatives are present, even in minor amounts, in the aqueous extract.

The TLC analysis revealed that ethanol was the most efficient solvent for extracting a broad spectrum of phytoconstituents from *Cassia spectabilis*. The detection of a spot matching the R_f of quercetin in all extracts confirms its presence in the plant. These observations highlight the phytochemical richness of *Cassia spectabilis* and support its potential

pharmacological importance, particularly in antioxidant and therapeutic applications associated with flavonoids like quercetin.

6.6 Results of HPLC of ethanolic extract of *Cassia spectabilis*

Table 6.13: Preparation of calibration curve of Quercetin

S. No.	Concentration ($\mu\text{g/ml}$)	MeanAUC	SD	%RSD
1	0	0	0	0
2	5	112852	165.65	0.1468
3	10	236989	220.25	0.0929
4	15	341187	255.69	0.0749
5	20	449058	274.95	0.0612
6	25	559154	245.22	0.0439
7	Sample	5898	14.65	0.2483

*Average of three determination, Mean $\pm$ SD

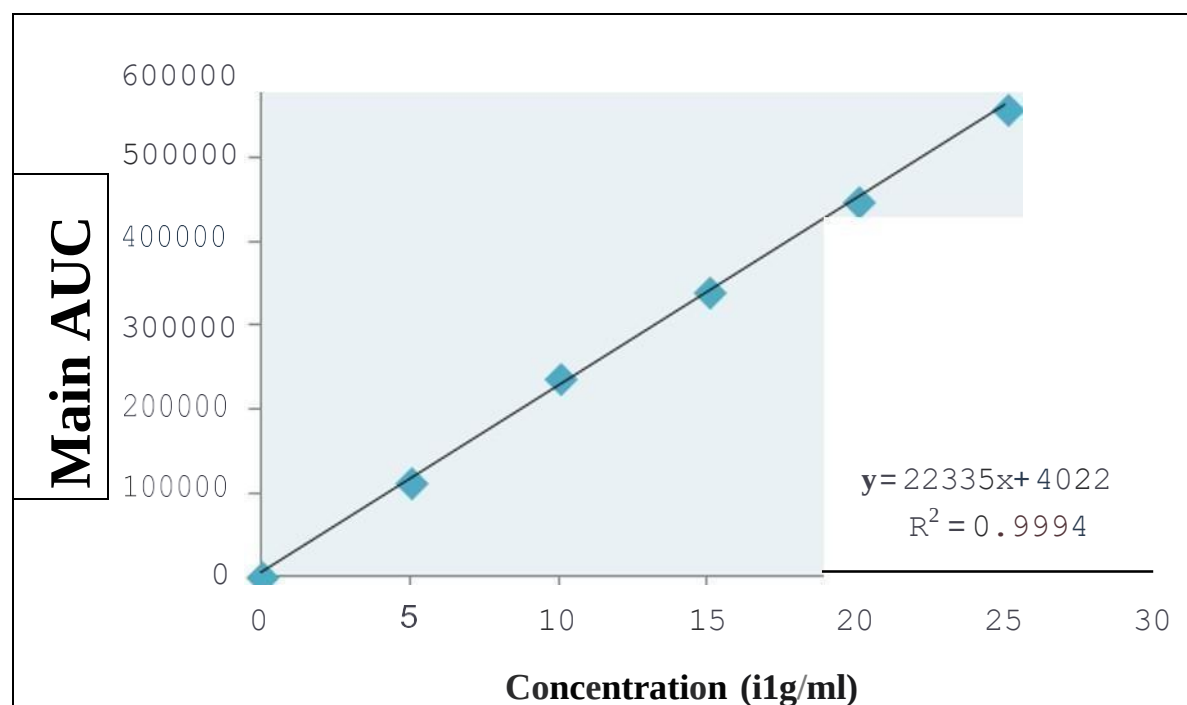


Figure 6.11: Calibration curve of Quercetin

The calibration curve for quercetin was prepared using standard solutions in the concentration range of 0-25 µg/ml, and their corresponding mean peak areas (AUC) were recorded by HPLC analysis. Each concentration was analyzed in triplicate to ensure precision and reliability of the data. The mean AUC values increased linearly with the increase in concentration, indicating a direct proportional relationship between concentration and detector response.

The regression analysis of the calibration curve yielded the equation $Y = 22335X + 4022$, where Y represents the peak area and X represents the concentration of quercetin (µg/ml). The correlation coefficient ($r^2 = 0.9994$) demonstrates excellent linearity across the selected concentration range, confirming the suitability of the developed method for quantitative estimation of quercetin.

The standard deviation (SD) and percentage relative standard deviation (%RSD) values for all concentration levels were found to be very low (ranging from 0.0439% to 0.1468%), which indicates high precision and reproducibility of the analytical method. The minimal variation in replicate readings confirms the robustness and accuracy of the technique.

The sample extract showed a mean AUC of 5898 with an SD of 14.65 and %RSD of 0.2483, reflecting consistent analytical performance even at low concentrations. These results verify that the developed HPTLC method is precise, accurate, and linear for the estimation of quercetin in herbal extracts.

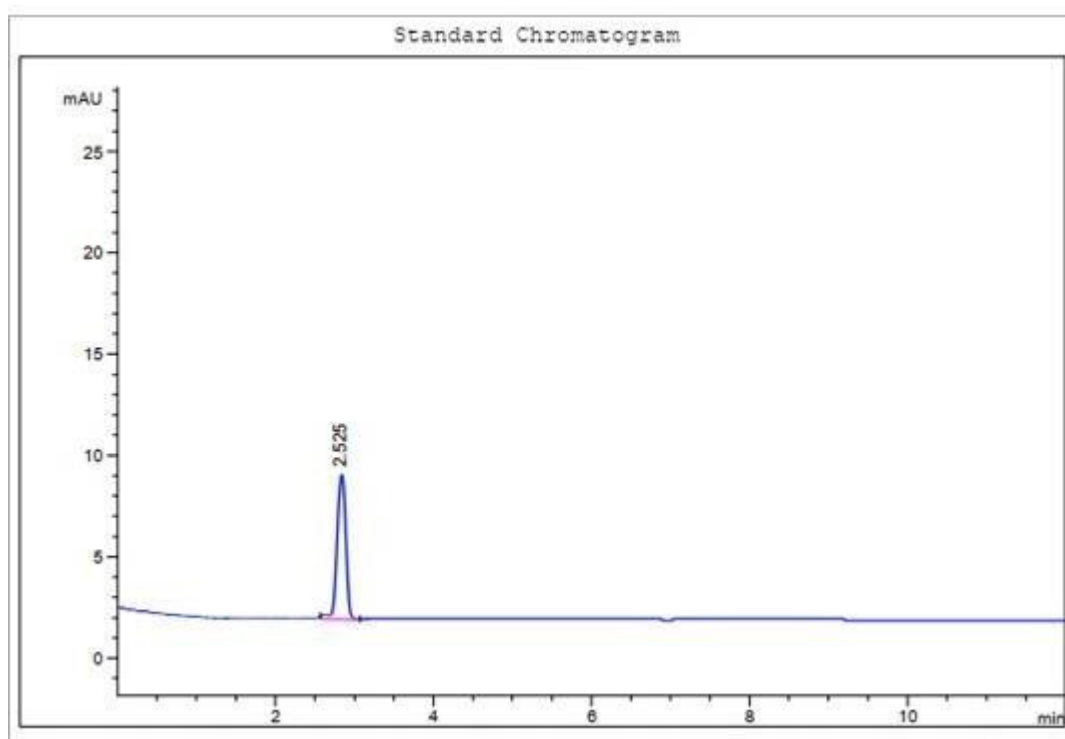


Figure 6.12: Chromatogram of standard Quercetin

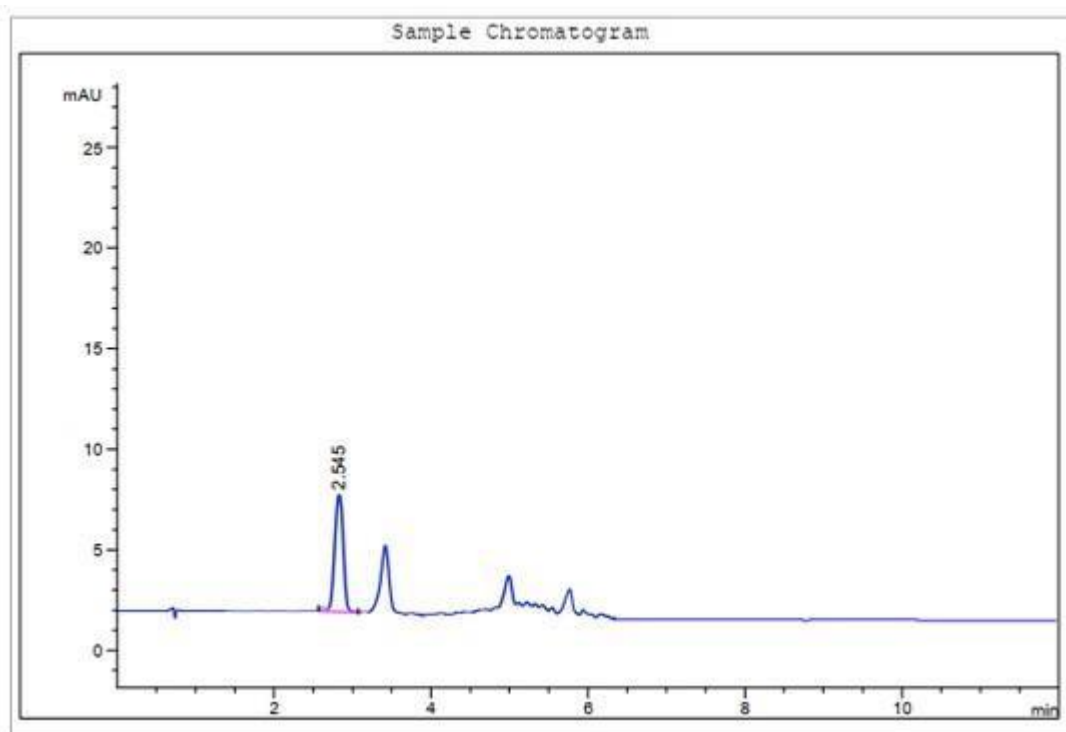


Figure 6.13: Chromatogram of ethanolic extract of *Cassia spectabilis*

Table 6.14: Quantitative HPLC estimation of Quercetin in *Cassia spectabilis* extract

S. No.	Ethanolic extract	RT	Area	% Quercetin content
1.	<i>Cassia spectabilis</i>	2.525	5898	0.248

The quantitative HPLC analysis of the ethanolic extract of *Cassia spectabilis* revealed a retention time (RT) of 2.525 minutes, corresponding closely to that of the standard quercetin, confirming its presence in the extract. The chromatogram showed a distinct, well-resolved peak with an area of 5898, indicating good separation and detection of the compound. The percentage of quercetin content was found to be 0.248%, suggesting that *Cassia spectabilis* contains a measurable amount of this bioactive flavonoid. This result supports the phytochemical potential of the plant, aligning with previous reports highlighting its richness in flavonoids responsible for antioxidant and therapeutic activities.

6.7 Results of antioxidant activity of ethanolic extract of *Cassia spectabilis*

6.7.1 Results of antioxidant activity using DPPH model

Table 6.15: % Inhibition of ascorbic acid and ethanolic extract of using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	46.25	29.57
2	20	51.69	32.46
3	40	62.78	40.29
4	60	67.05	43.97
5	80	75.91	54.14
6	100	89.34	61.25
IC ₅₀ value		15.91	71.51

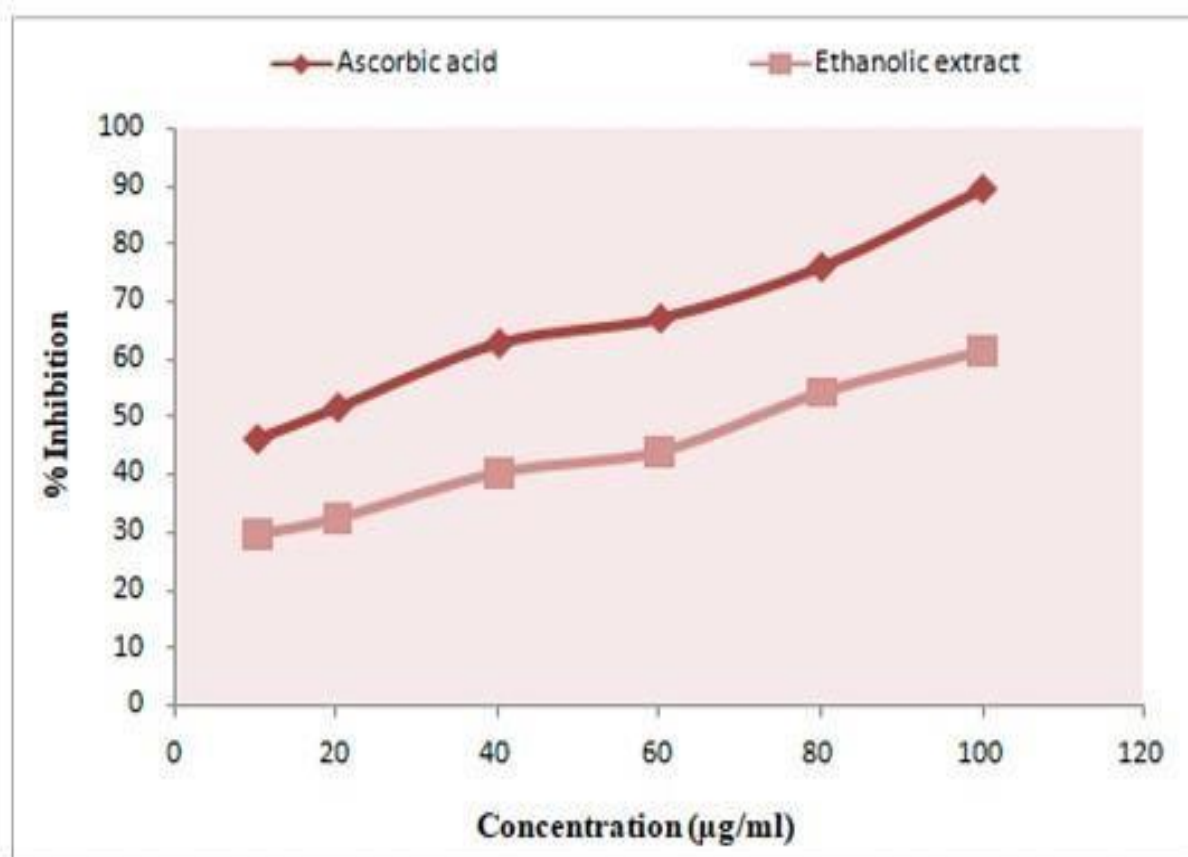
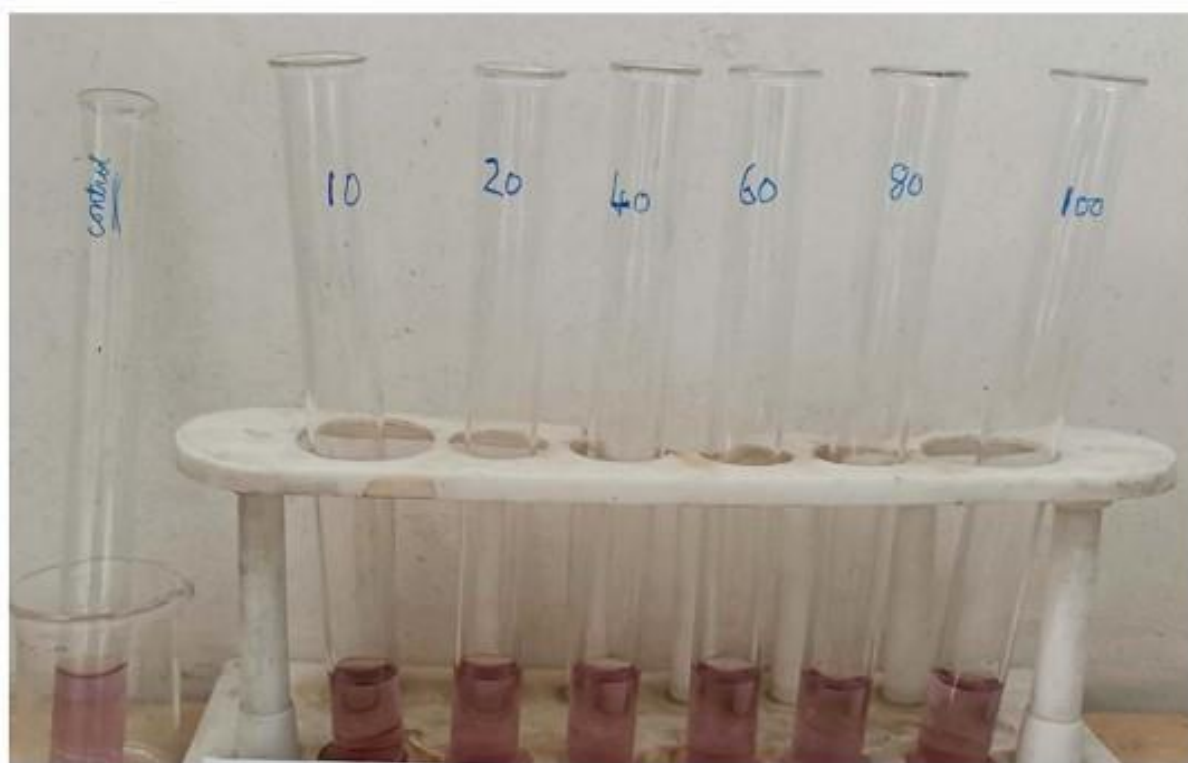


Figure 6.14: % Inhibition of ascorbic acid and ethanolic extract of *Cassia spectabilis* by DPPH method

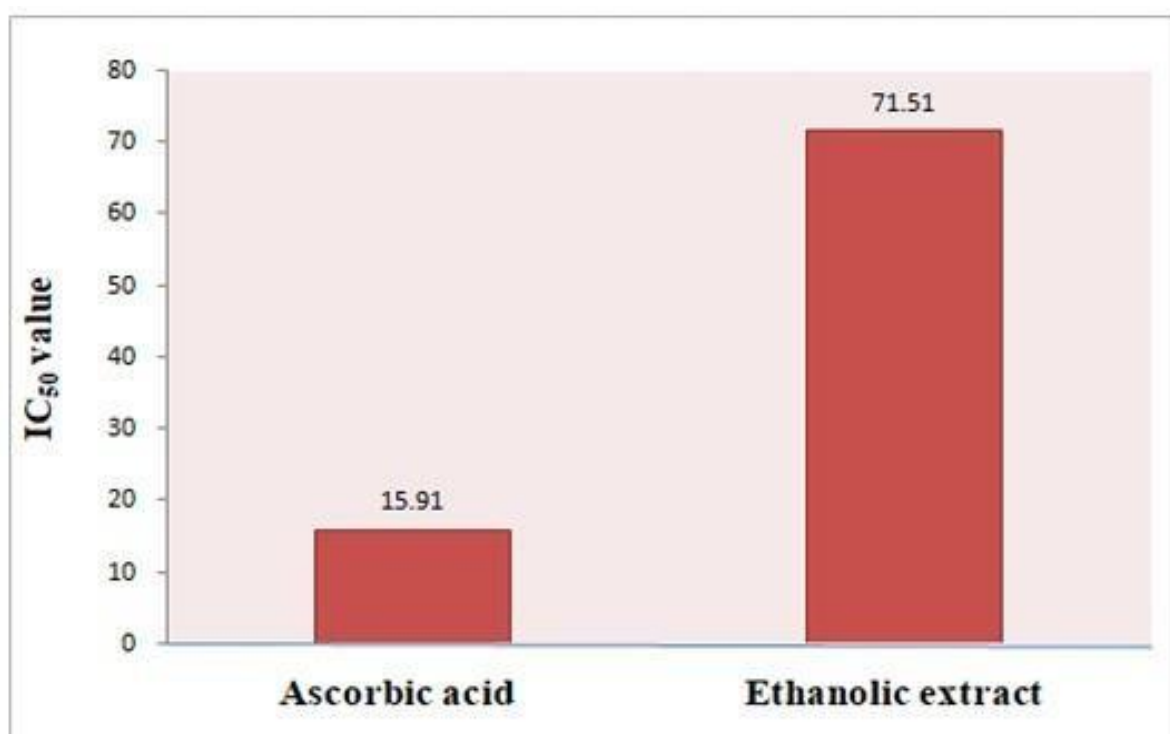


Figure 6.15: Graph of IC₅₀ value of ascorbic acid and ethanolic extract of *Cassia spectabilis* by DPPH method

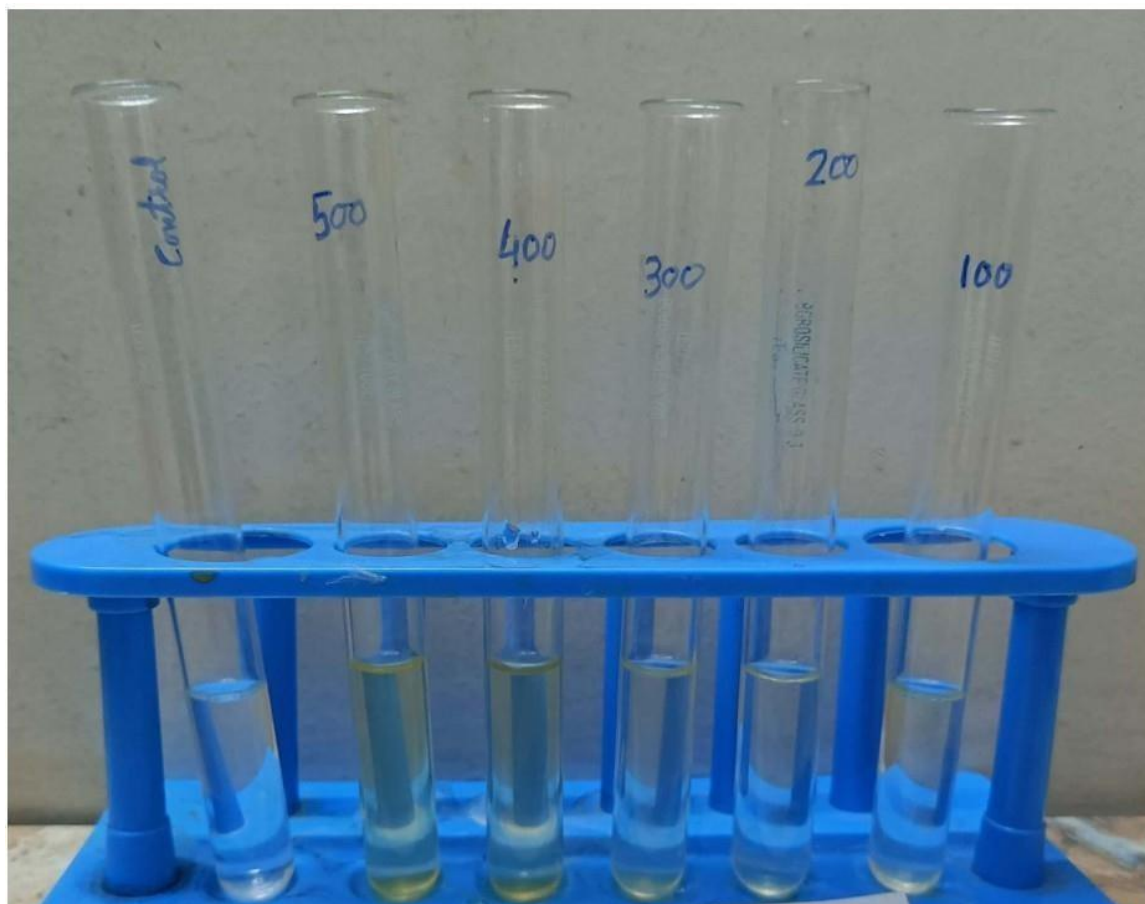
The antioxidant activity of the ethanolic extract of *Cassia spectabilis* was evaluated using the DPPH radical scavenging assay and compared with the standard antioxidant, ascorbic acid. The results showed that the percentage inhibition of DPPH radicals increased in a concentration-dependent manner for both ascorbic acid and the ethanolic extract. However, the extract exhibited comparatively lower scavenging activity than ascorbic acid at all tested concentrations.

The IC₅₀ value, which represents the concentration required to inhibit 50% of DPPH radicals, was found to be 15.91 µg/ml for ascorbic acid and 71.51 µg/ml for the ethanolic extract, indicating that the standard possesses stronger antioxidant potential. Although the extract's IC₅₀ value was higher, it still demonstrated notable free radical scavenging ability, suggesting the presence of bioactive phytoconstituents such as flavonoids and phenolic compounds contributing to its antioxidant activity.

6.7.2 Results of antioxidant activity using hydrogen peroxide model

Table 6.16: % Inhibition of ascorbic acid and ethanolic extract of using hydrogen peroxide method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	100	49.79	31.54
2	200	64.87	40.98
3	300	75.03	52.76
4	400	85.41	62.54
5	500	93.56	73.81
IC ₅₀ value		80.55	278.39



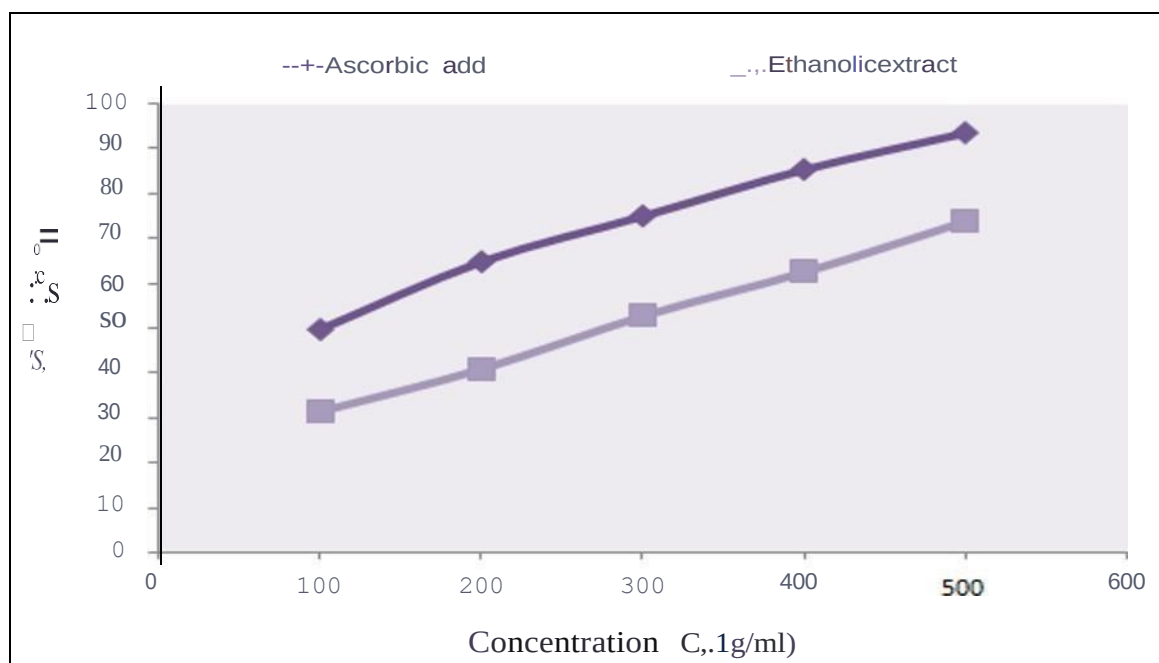


Figure 6.16: % Inhibition of ascorbic acid and ethanolic extract of *Cassia spectabilis* by H₂O₂ method

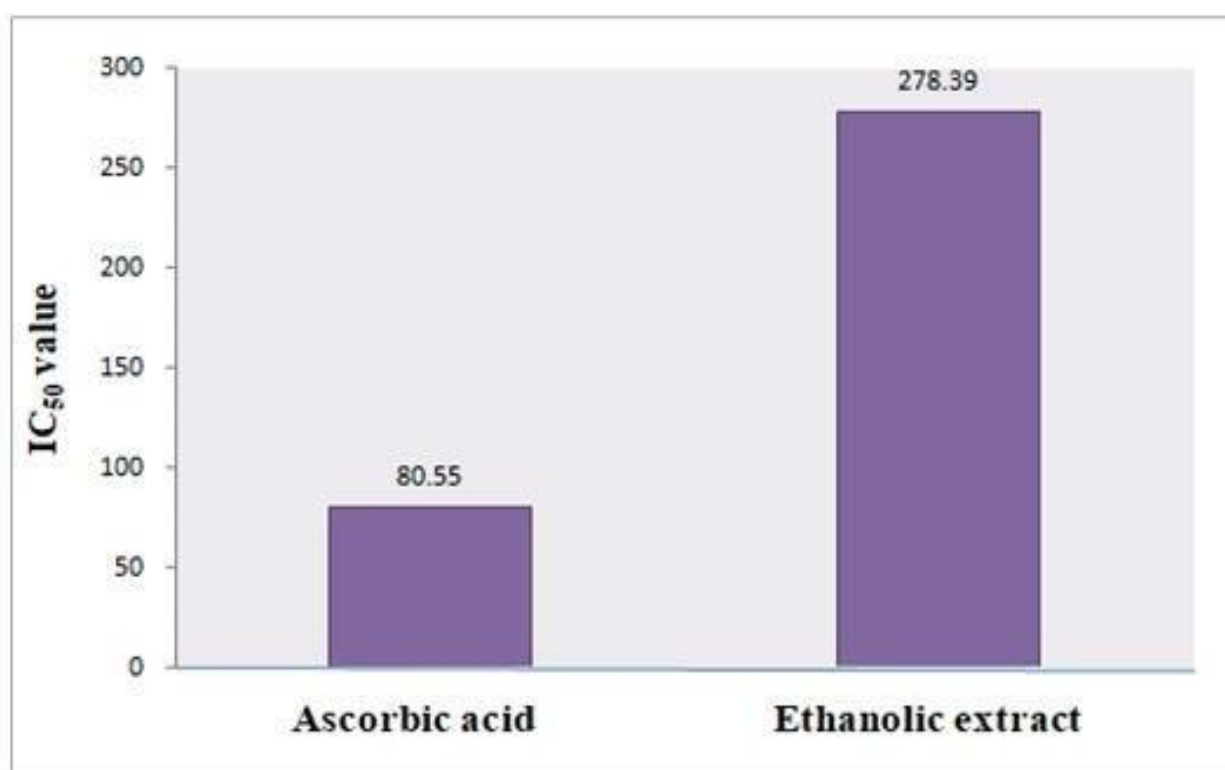


Figure 6.17: Graph of IC₅₀ value of ascorbic acid and ethanolic extract of *Cassia spectabilis* by H₂O₂ method

The hydrogen peroxide scavenging assay demonstrated that both ascorbic acid and the ethanolic extract of *Cassia spectabilis* exhibited concentration-dependent antioxidant activity. The percentage inhibition of hydrogen peroxide radicals increased with increasing concentration for both samples. However, the ethanolic extract showed comparatively lower scavenging efficiency than the standard ascorbic acid at all tested concentrations.

The IC₅₀ value of ascorbic acid was found to be 80.55µg/ml, whereas the ethanolic extract showed an IC₅₀ value of 278.39µg/ml. This higher IC₅₀ value indicates that a greater concentration of the extract is required to achieve the same level of radical inhibition as the standard, reflecting a moderate antioxidant potential. The observed activity of the extract could be attributed to the presence of phenolic and flavonoid compounds that act as hydrogen donors and neutralize reactive oxygen species.

6.7.3 Results of antioxidant activity using Nitric oxide model

Table 6.17: % Inhibition of ascorbic acid and ethanolic extract of using Nitric oxide method

S.No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	20	40.25	27.35
2	40	65.78	37.91
3	60	70.75	49.58
4	80	79.24	56.74
5	100	86.46	60.89
IC ₅₀ value		25.08	68.25

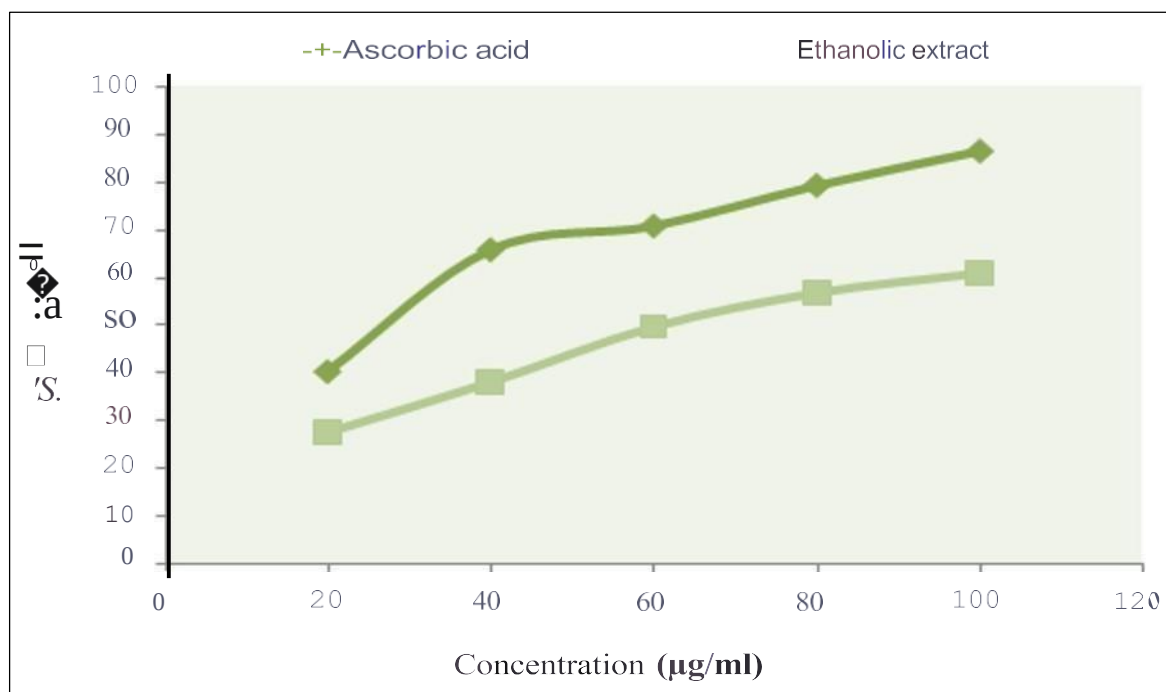


Figure 6.18: % Inhibition of ascorbic acid and ethanolic extract of *Cassia spectabilis* by NO method

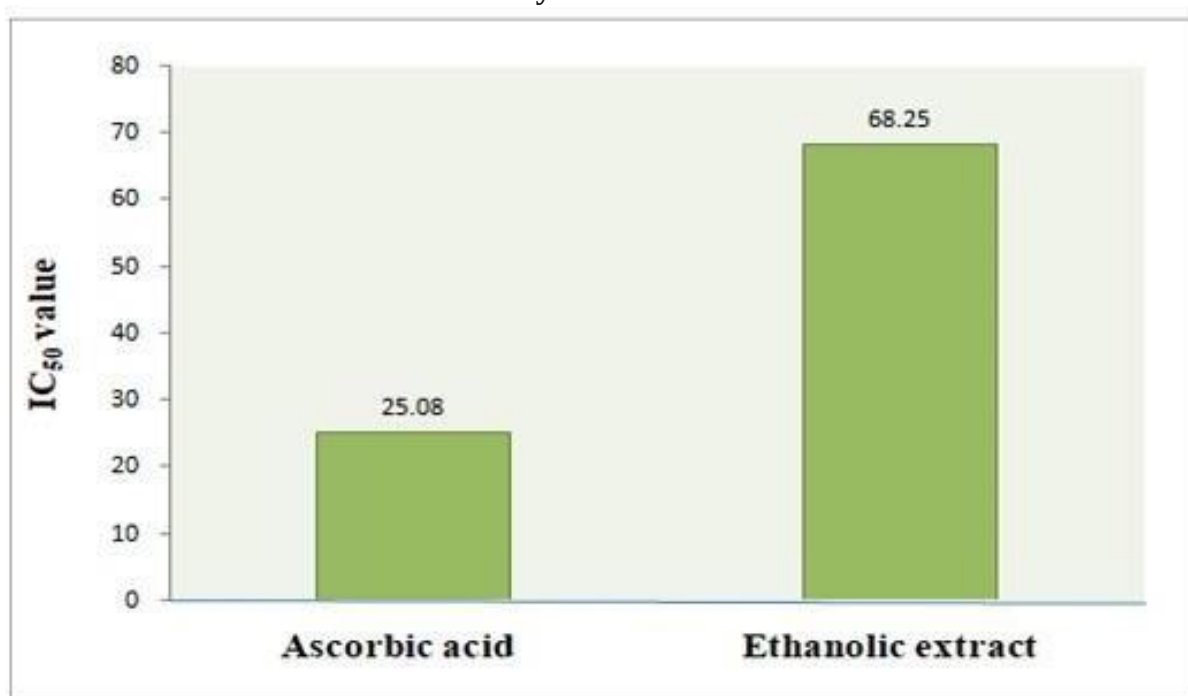


Figure 6.19: Graph of IC₅₀ value of ascorbic acid and ethanolic extract of *Cassia spectabilis* by NO method

The nitric oxide scavenging assay revealed that both ascorbic acid and the ethanolic extract of *Cassia spectabilis* exhibited a dose-dependent increase in percentage inhibition. As the concentration increased from 20 to 100 µg/ml, the scavenging activity of both samples significantly improved. However, ascorbic acid demonstrated a much higher inhibition compared to the ethanolic extract across all concentrations.

The IC₅₀ value of ascorbic acid was recorded as 25.08 µg/ml, whereas the ethanolic extract showed an IC₅₀ value of 68.25 µg/ml, indicating that the standard antioxidant was more potent. Nonetheless, the ethanolic extract exhibited appreciable nitric oxide scavenging activity, suggesting the presence of bioactive compounds such as flavonoids and phenolics capable of reducing oxidative stress by neutralizing reactive nitrogen species.

6.8 Results of *in-vitro* anti-inflammatory activity of ethanolic extract of *Cassia spectabilis*

Table 6.18: % Inhibition of Diclofenac sodium and ethanolic extract of *Cassia spectabilis*

S. No.	Concentration (µg/ml)	% Inhibition	
		Diclofenac sodium	Ethanolic extract
1	100	49.58	30.25
2	200	67.25	42.63
3	300	77.34	56.41
4	400	85.96	61.32
5	500	92.65	68.96
IC ₅₀ value		66.34	280.41

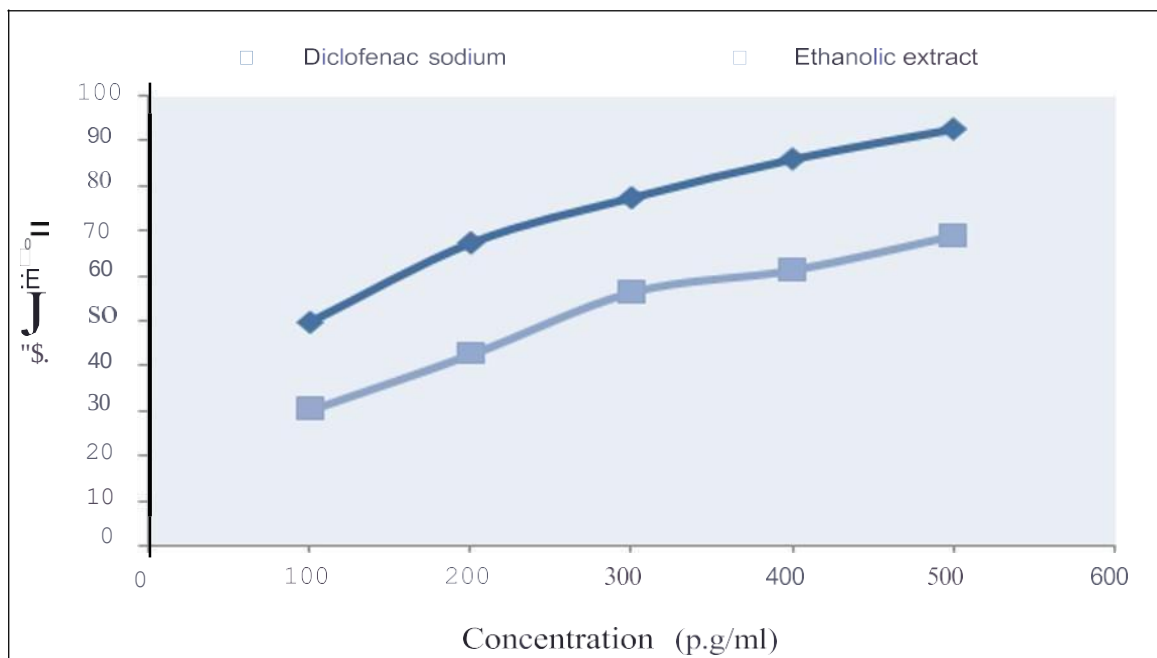


Figure 6.20: Graph of % Inhibition of Diclofenac sodium and ethanolic extract of *Cassia spectabilis*

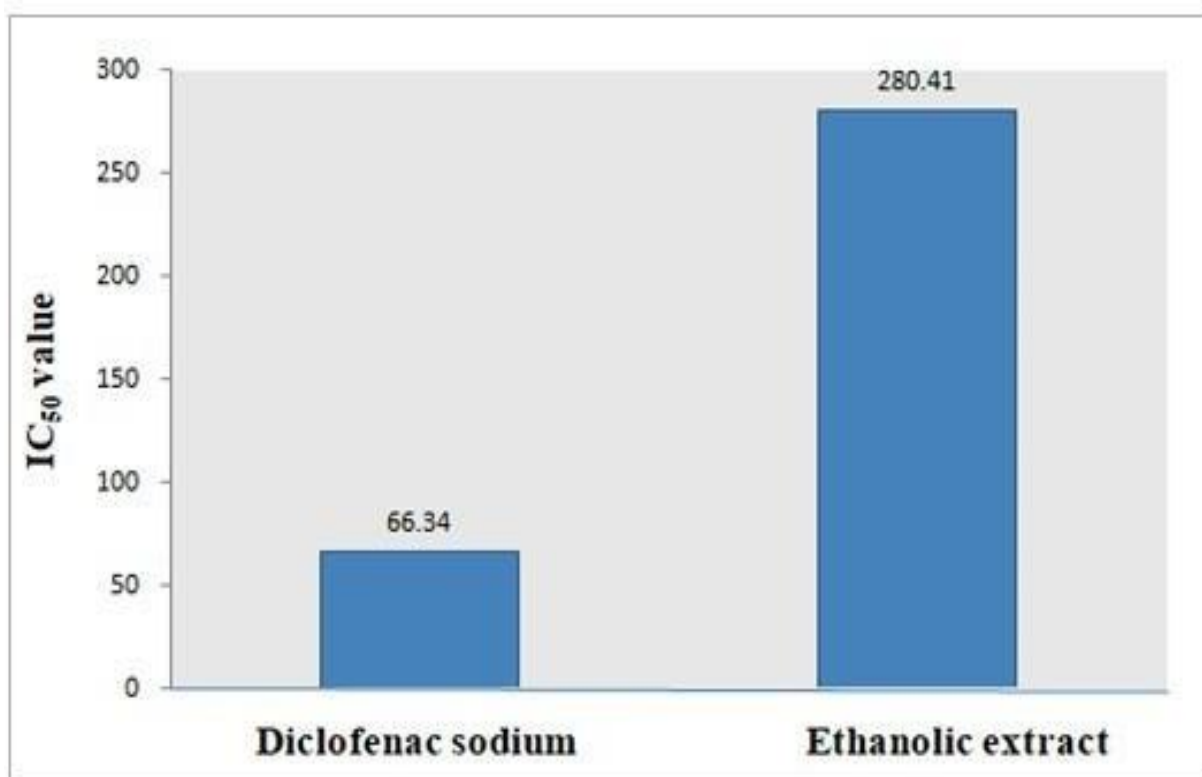


Figure 6.22: Graph of IC₅₀ value of Diclofenac sodium and ethanolic extract of *Cassia spectabilis*

The anti-inflammatory activity, assessed using the standard Diclofenac sodium and the ethanolic extract of *Cassia spectabilis*, showed a clear dose-dependent increase in percentage inhibition. As the concentration increased from 100 to 500 µg/ml, the inhibition of the inflammatory response progressively improved for both the standard and the extract.

Diclofenac sodium exhibited higher activity with an IC₅₀ value of 66.34 µg/ml, whereas the ethanolic extract displayed moderate activity with an IC₅₀ value of 280.41 µg/ml. These results indicate that while the extract is less potent than the standard anti-inflammatory drug, it still possesses significant inhibitory effects, likely due to bioactive phytoconstituents such as flavonoids and phenolic compounds that contribute to its anti-inflammatory potential.

6.9 Results of *in-vitro* antimicrobial activity of ethanolic extract of *Cassia spectabilis*

Table 6.19: Antimicrobial activity of standard drug against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Ofloxacin	<i>Streptococcus mutans</i>	17±0	20±0	22±0
2.	Ciprofloxacin	<i>Bacillus subtilis</i>	15±0	24±0	29±0
		<i>Klebsiella pneumoniae</i>	30±0.3	35±0	40±0.5
		<i>Escherichia coli</i>	22±0.3	27±0	30±0
3.	Fluconazole	<i>Candida albicans</i>	26±0	30±0	32±0

The antimicrobial activity of the selected standard drugs was evaluated against various microbial strains, and the results were expressed as the diameter of the zone of inhibition in millimeters at different concentrations (10, 20, and 30 µg/ml).

Ofloxacin showed effective antibacterial activity against *Streptococcus mutans*, with zones of inhibition increasing from 17 mm at 10 µg/ml to 22 mm at 30 µg/ml, indicating a dose-dependent response. Ciprofloxacin demonstrated broad-spectrum antibacterial activity, with notable inhibition against *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Escherichia coli*. The zones of inhibition increased consistently with higher concentrations, reaching a maximum of 29 mm, 40 mm, and 30 mm, respectively, reflecting the drug's strong bactericidal potential.

Fluconazole, tested against *Candida albicans*, also exhibited significant antifungal activity, with inhibition zones expanding from 26 mm at 10 µg/ml to 32 mm at 30 µg/ml. This demonstrates that the standard drugs were effective against their respective microbial strains

in a concentration-dependent manner. The results provide a benchmark for comparing the antimicrobial efficacy of plant extracts or novel formulations in subsequent studies.

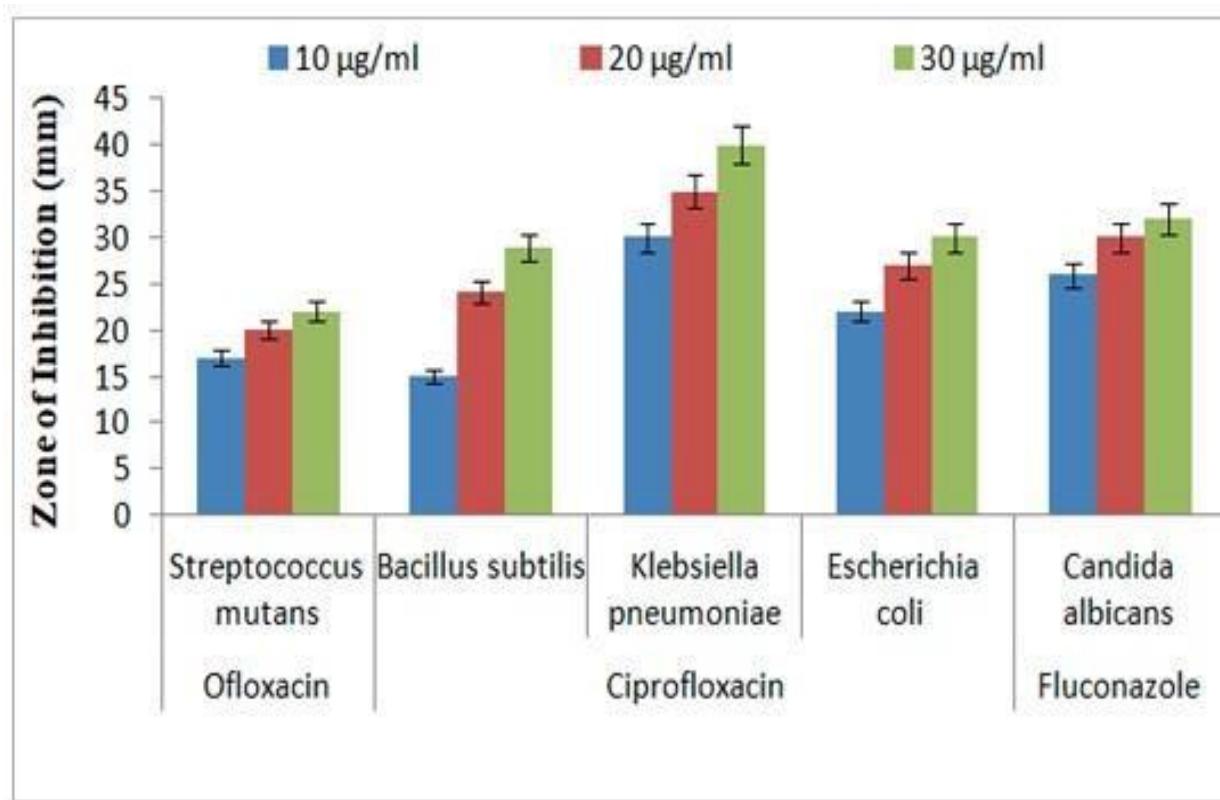


Figure 6.22: Graph of Antimicrobial activity of standard drug against selected microbes



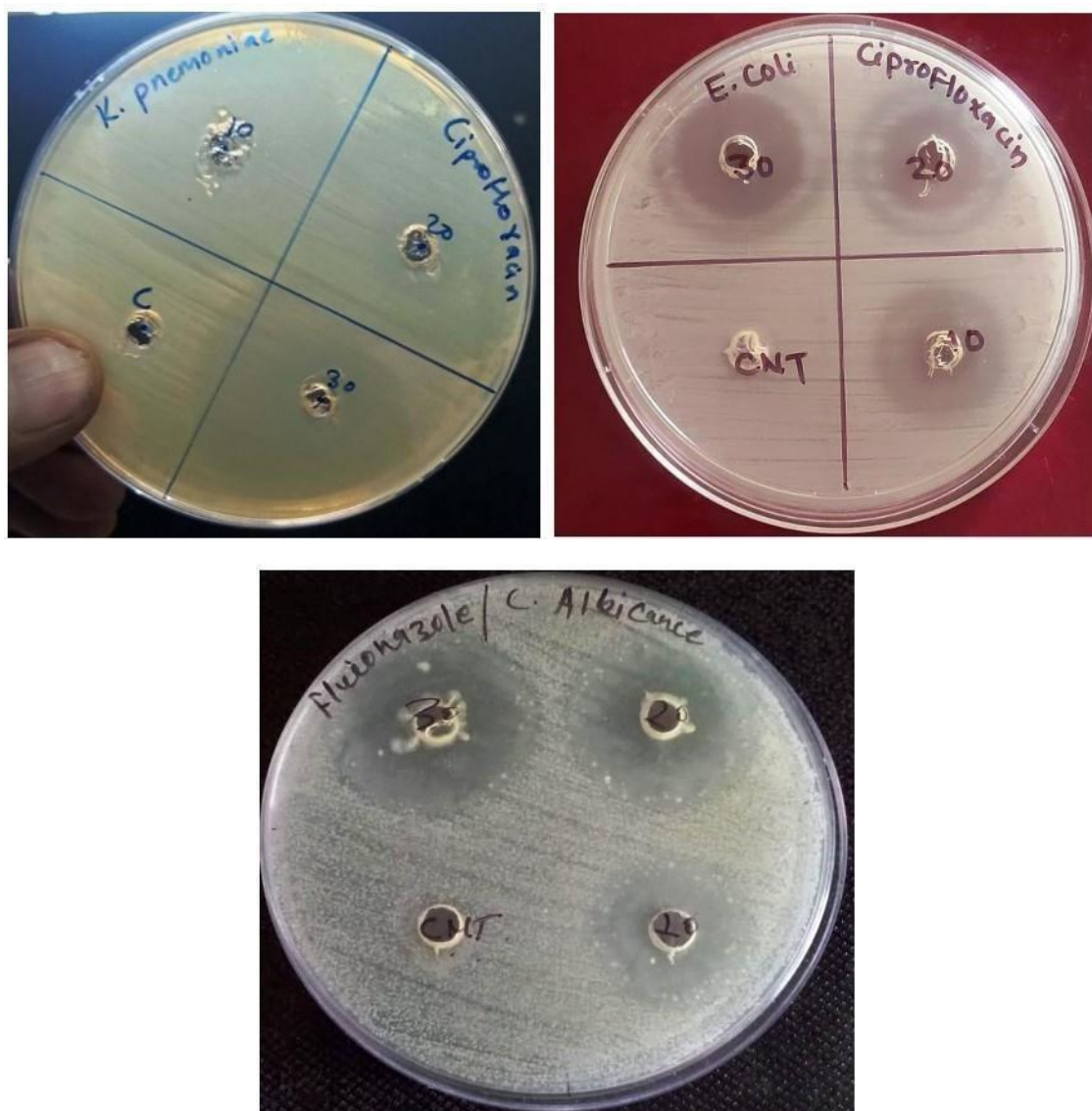


Figure 6.23: Photoplate of antimicrobial activity of standard drug against selected microbes

Table 6.20: Antimicrobial activity of ethanolic extract of *Cassia spectabilis*

S. No.	Name of microbes	Zone of inhibition (mm)		
		25mg/ml	50 mg/ml	100mg/ml
1.	<i>Streptococcus mutans</i>	11±0.6	14±0.5	16±0.3
2.	<i>Bacillus subtilis</i>	12±0.3	14±0.6	17±0.74
3.	<i>Klebsiella pneumoniae</i>	9±0.47	10±0	12±0.47
4.	<i>Escherichia coli</i>	9±0.94	12±0.5	15±0.86
5.	<i>Candida albicans</i>	10±0	12±0	14±0

The antimicrobial activity of the ethanolic extract of *Cassia spectabilis* was evaluated against a panel of bacterial and fungal strains, and the results were expressed as the diameter of the zone of inhibition in millimeters at different concentrations (25, 50, and 100 mg/ml).

The extract exhibited moderate antibacterial activity against *Streptococcus mutans*, with the zone of inhibition increasing from 11 mm at 25 mg/ml to 16 mm at 100 mg/ml, indicating a concentration-dependent effect. Similarly, *Bacillus subtilis* showed enhanced sensitivity to the extract, with inhibition zones ranging from 12 mm to 17 mm as the concentration increased. *Klebsiella pneumoniae* and *Escherichia coli* also demonstrated susceptibility, though the zones of inhibition were comparatively smaller, ranging from 9-12 mm and 9-15 mm, respectively, highlighting that Gram-negative bacteria were less sensitive than Gram-positive strains.

The ethanolic extract showed antifungal activity against *Candida albicans*, with inhibition zones increasing from 10 mm at 25 mg/ml to 14 mm at 100 mg/ml, confirming the presence of bioactive compounds capable of inhibiting fungal growth. Overall, the results indicate that the ethanolic extract of *Cassia spectabilis* possesses broad-spectrum antimicrobial properties in a dose-dependent manner, although the activity is moderate compared to standard antimicrobial drugs. These findings support its potential application in developing plant-based antimicrobial formulations.

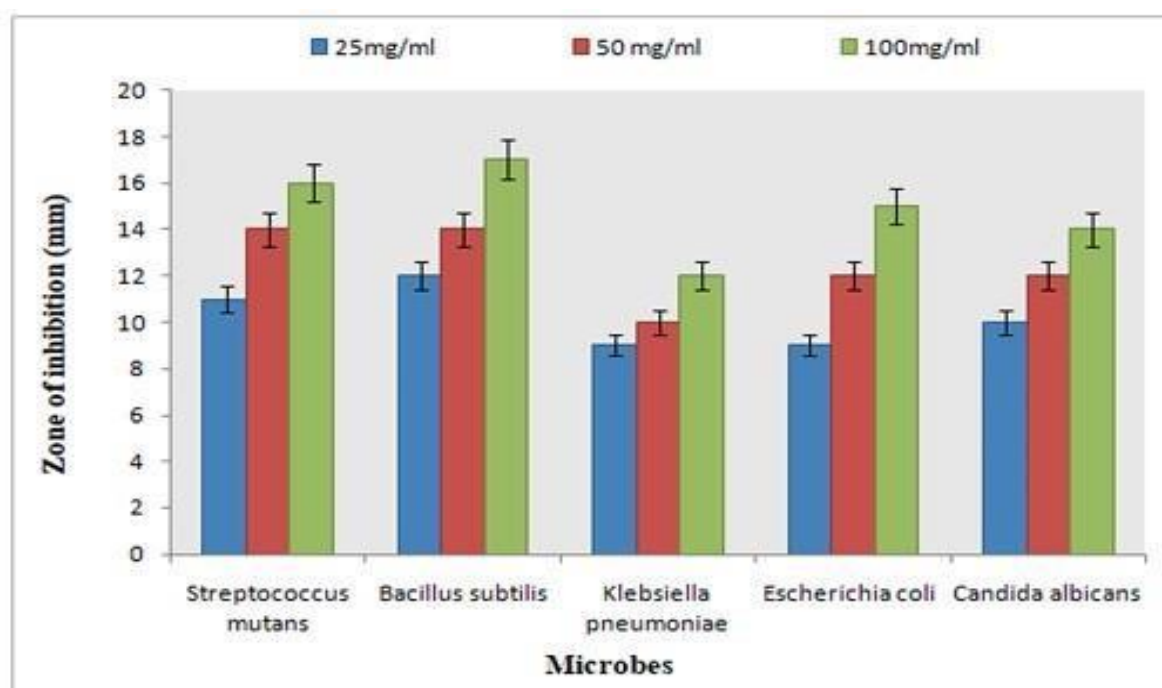


Figure 6.24: Graph of Antimicrobial activity of ethanolic extract of *Cassia spectabilis*

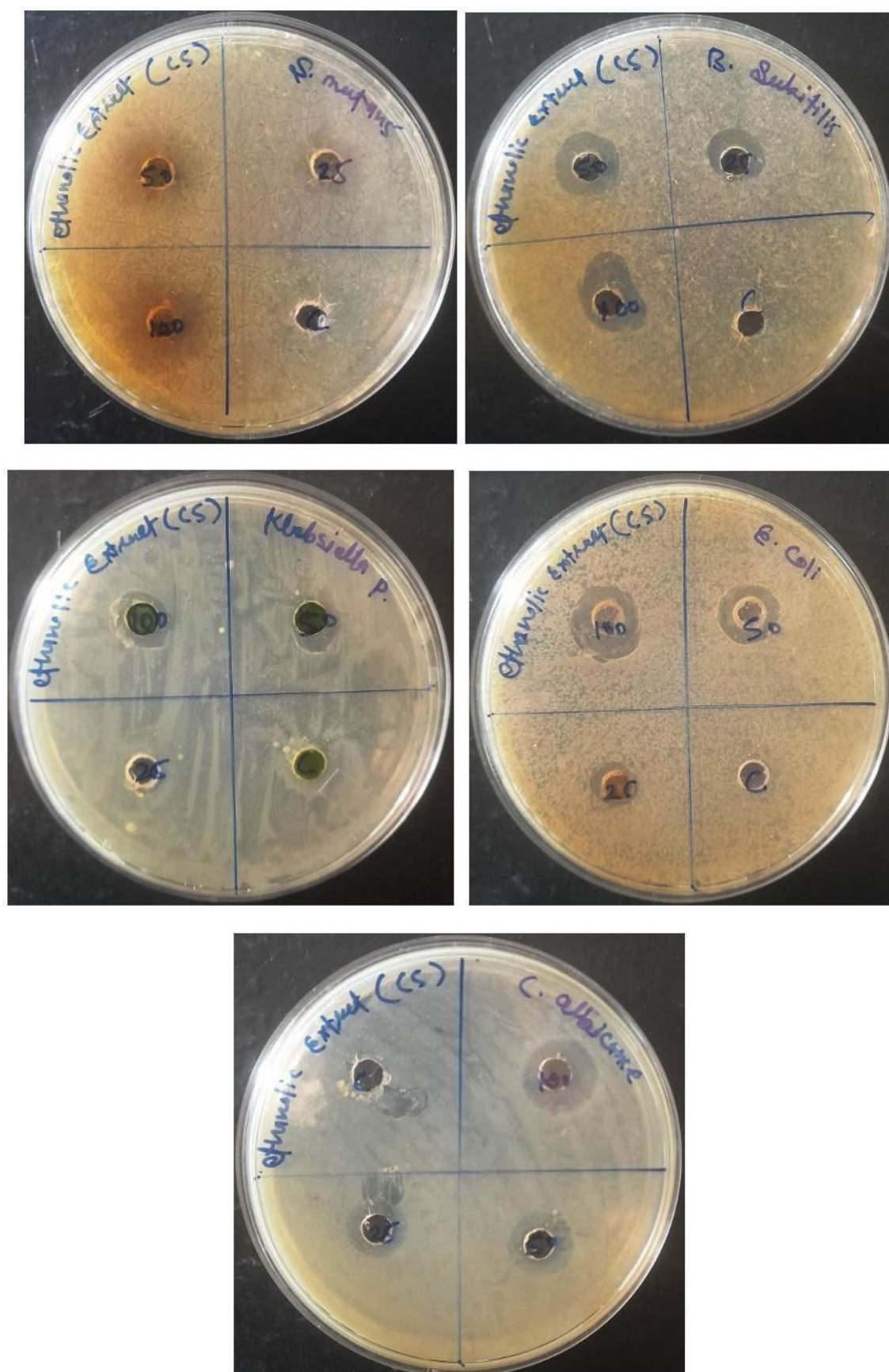


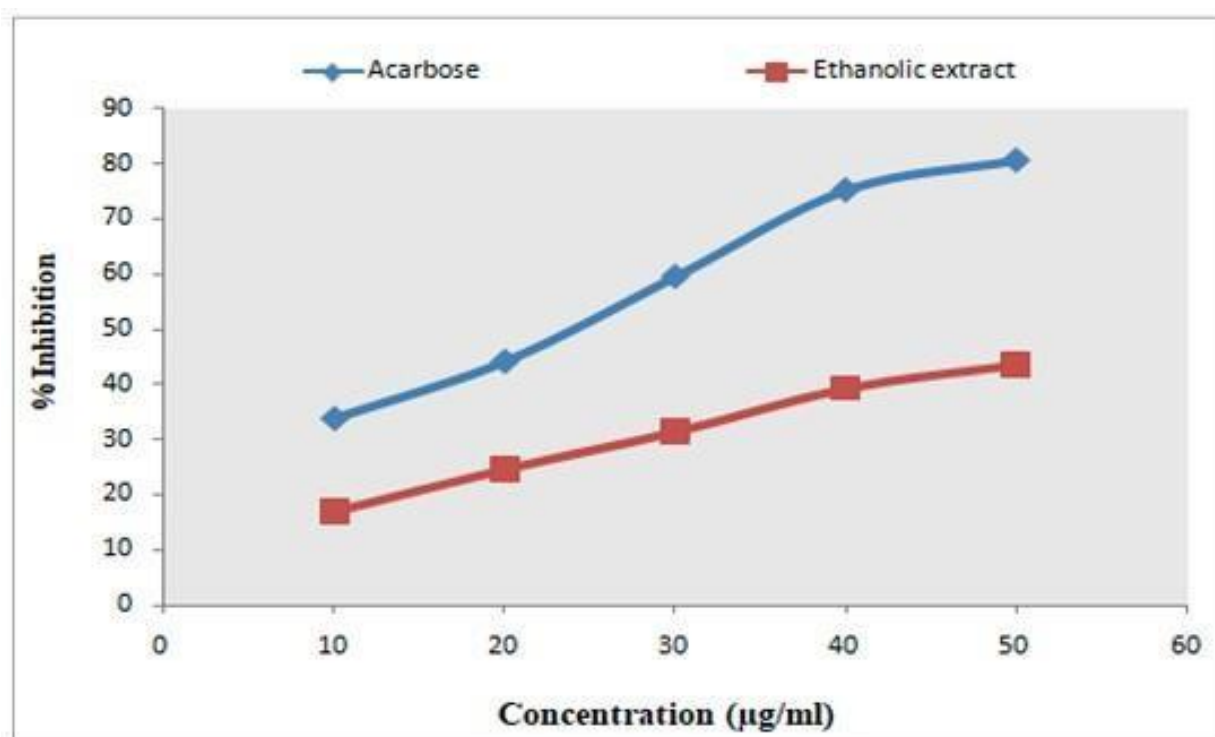
Figure 6.25: Photoplate of Antimicrobial activity of ethanolic extract of *Cassia spectabilis*

6.10 Results of *in vitro* antidiabetic studies of ethanolic extract of *Cassia spectabilis*

6.10.1 Results of Alpha amylase inhibition assay

Table 6.21: Results of % Inhibition of Acarbose and ethanolic extract of *Cassia spectabilis*

S. No.	Concentration ($\mu\text{g/ml}$)	% Inhibition	
		Acarbose	Ethanolic extract
1.	10	33.94	17.04
2.	20	44.21	24.68
3.	30	59.57	31.45
4.	40	75.16	39.26
5.	50	80.52	43.51
IC ₅₀ value		27.31	57.88

**Figure 6.26: Graph of % Inhibition of Acarbose and ethanolic extract by alpha amylase**

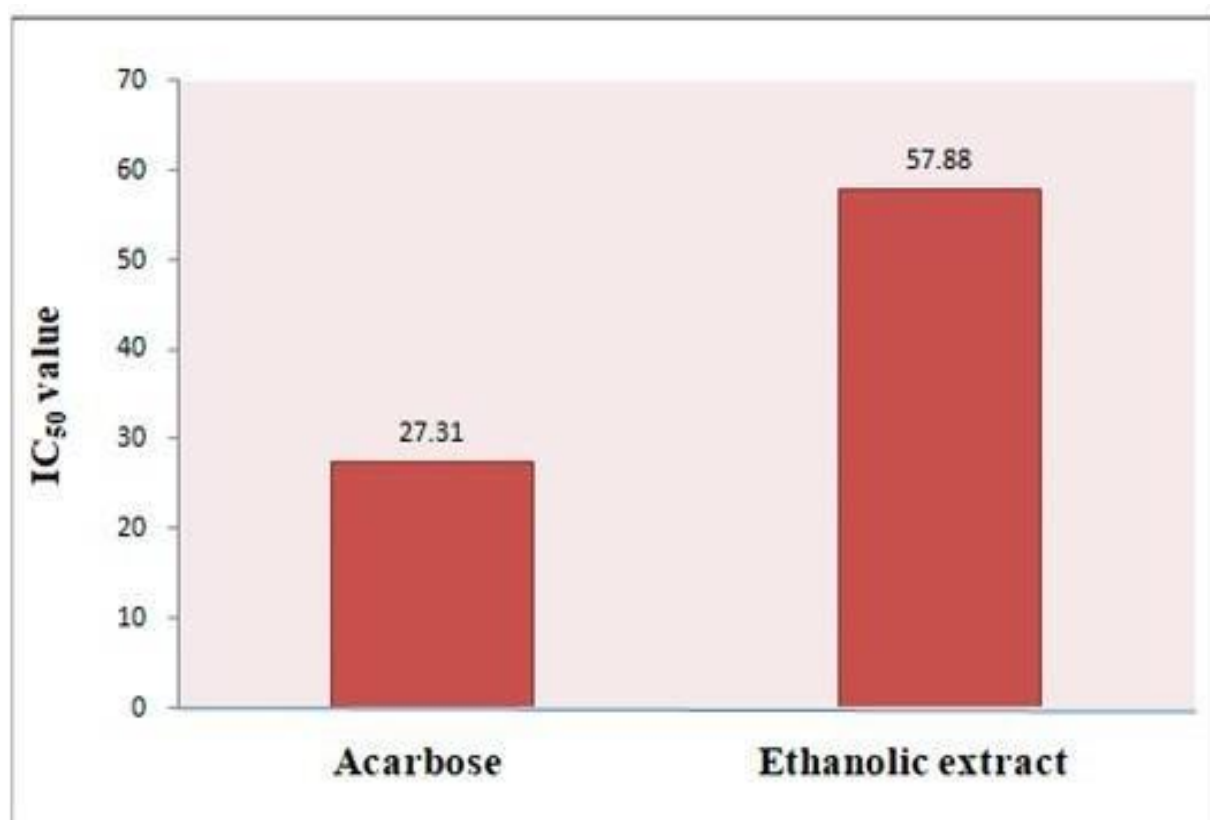


Figure 6.27: Graph of IC₅₀ value of Acarbose and ethanolic extract by alpha amylase

The in vitro antidiabetic activity of the ethanolic extract of *Cassia spectabilis* was evaluated using the α -glucosidase inhibitory assay, with Acarbose serving as the standard reference drug. The percentage inhibition of α -glucosidase by the extract increased in a concentration-dependent manner, ranging from 17.04% at 10 μ g/ml to 43.51% at 50 μ g/ml. The standard drug Acarbose exhibited higher inhibitory activity, with values ranging from 33.94% to 80.52% over the same concentration range.

The IC₅₀ value, representing the concentration required to inhibit 50% of enzyme activity, was calculated as 57.88 μ g/ml for the ethanolic extract, compared to 27.31 μ g/ml for Acarbose, indicating that the extract possesses moderate α -glucosidase inhibitory potential. The gradual increase in inhibition with concentration suggests the presence of bioactive compounds, such as flavonoids and phenolics, in the extract that may interfere with carbohydrate-hydrolyzing enzymes, thereby contributing to its antidiabetic effect.

6.10.2 Results of alpha glucosidase inhibition assay

Table 6.22: Results of % Inhibition of Acarbose and ethanolic extract of *Cassia spectabilis*

S. No.	Concentration (µg/ml)	% Inhibition	
		Acarbose	Ethanolic extract
1.	12.5	40.16	20.85
2.	25	51.45	29.41
3.	50	62.07	32.65
4.	100	74.96	44.08
5.	200	80.14	51.69
IC ₅₀ value		16.66	172.31

The in vitro antidiabetic activity of the ethanolic extract of *Cassia spectabilis* was evaluated using the α-glucosidase inhibitory assay, with Acarbose as the standard reference. The percentage inhibition of α-glucosidase by the extract increased with concentration, ranging from 20.85% at 12.5 µg/ml to 51.69% at 200 µg/ml. In comparison, Acarbose demonstrated higher inhibition at all tested concentrations, ranging from 40.16% to 80.14%.

The IC₅₀ value of the ethanolic extract was calculated as 172.31 µg/ml, while that of Acarbose was 16.66 µg/ml. This indicates that the extract has moderate α-glucosidase inhibitory activity but is less potent than the standard drug. The concentration-dependent increase in inhibition suggests the presence of bioactive phytoconstituents, such as flavonoids, phenolics, and other secondary metabolites, which may contribute to the enzyme inhibitory effect.

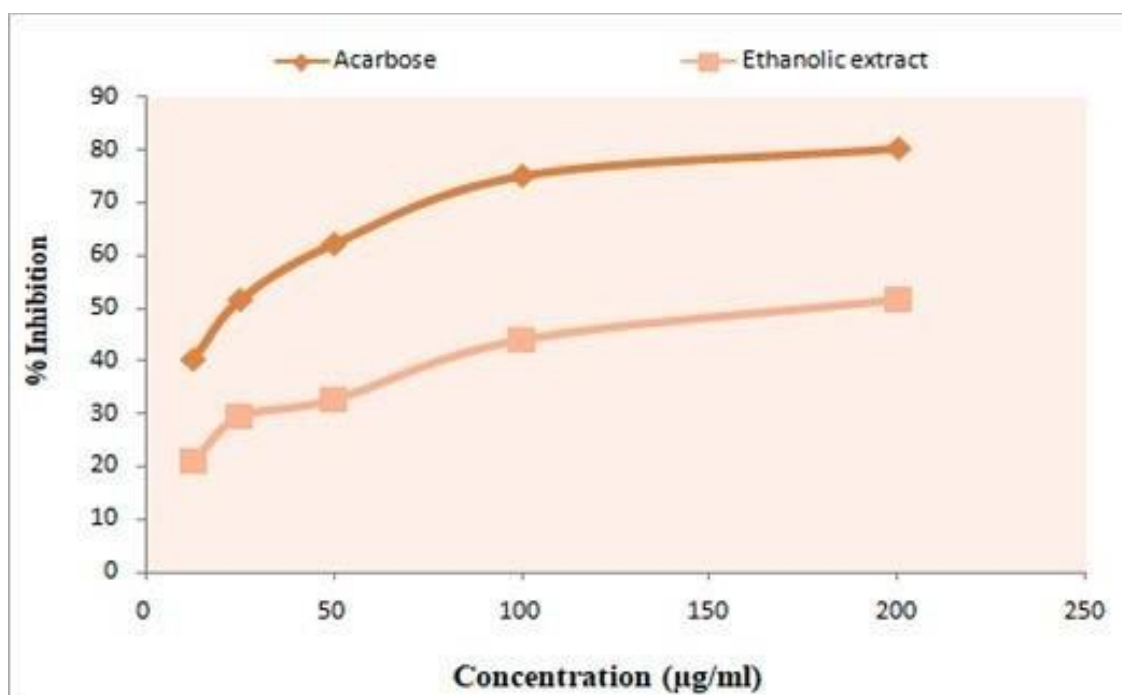


Figure 6.28: Graph of % Inhibition of Acarbose and ethanolic extract by alpha glucosidase

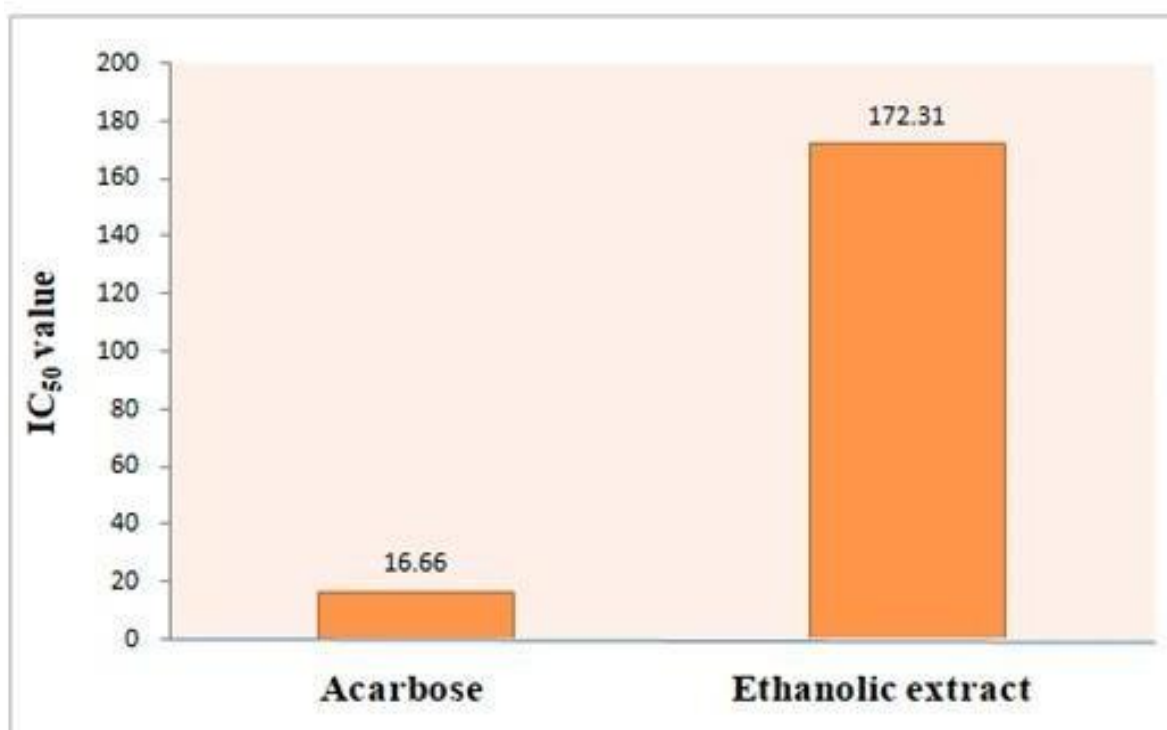


Figure 6.29: Graph of IC₅₀ value of Acarbose and ethanolic extract by alpha glucosidase

6.11 Results of HPTLC analysis

6.11.1 Preparation of calibration Curve of quercetin by HPTLC

Table 6.23: Preparation of calibration Curve of quercetin by HPTLC

S. No.	Concentration ($\mu\text{g/mL}$)	Mean Area $\pm$ SD
1	0	0.00 $\pm$ 0.00
2	2	8245.2 $\pm$ 25.6
3	4	16524.5 $\pm$ 30.4
4	6	24589.7 $\pm$ 35.2
5	8	32657.1 $\pm$ 42.8
6	10	40326.6 $\pm$ 48.3
7	12	48410.4 $\pm$ 56.7

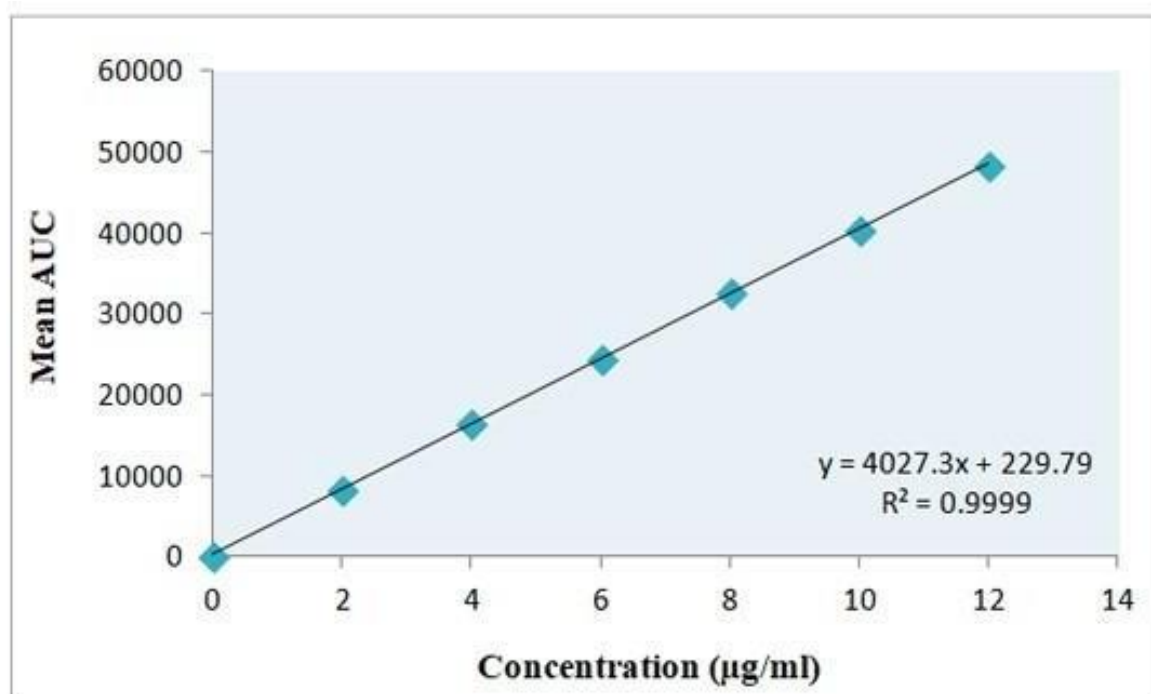


Figure 6.30: Calibration Curve of quercetin by HPTLC

Regression analysis

Concentration	2-12 $\mu\text{g/ml}$
Slope	4027.3
Intercept	229.79
Regression Coefficient	0.9999

The HPTLC analysis was performed to quantify quercetin in the ethanolic extract using a validated calibration curve. Standard solutions of quercetin were prepared in the concentration range of 2-12 $\mu\text{g/mL}$, and their corresponding mean peak areas were measured. The data exhibited a consistent increase in peak area with increasing concentration, indicating a strong linear relationship between concentration and response.

Regression analysis of the calibration curve yielded a slope of 4027.3, an intercept of 229.79, and a correlation coefficient (R^2) of 0.9999. The high R^2 value demonstrates excellent linearity and confirms the reliability of the method for the quantitative estimation of quercetin within the selected range.

These results indicate that the developed HPTLC method is precise, reproducible, and suitable for the accurate quantification of quercetin in plant extracts. The linear response over the tested concentration range also supports its applicability for routine quality control and standardization of *Cassia spectabilis* extracts.

6.11.2 Thin layer chromatographic image of standard and extract samples



Figure 6.31: Thin layer chromatography in Normal light

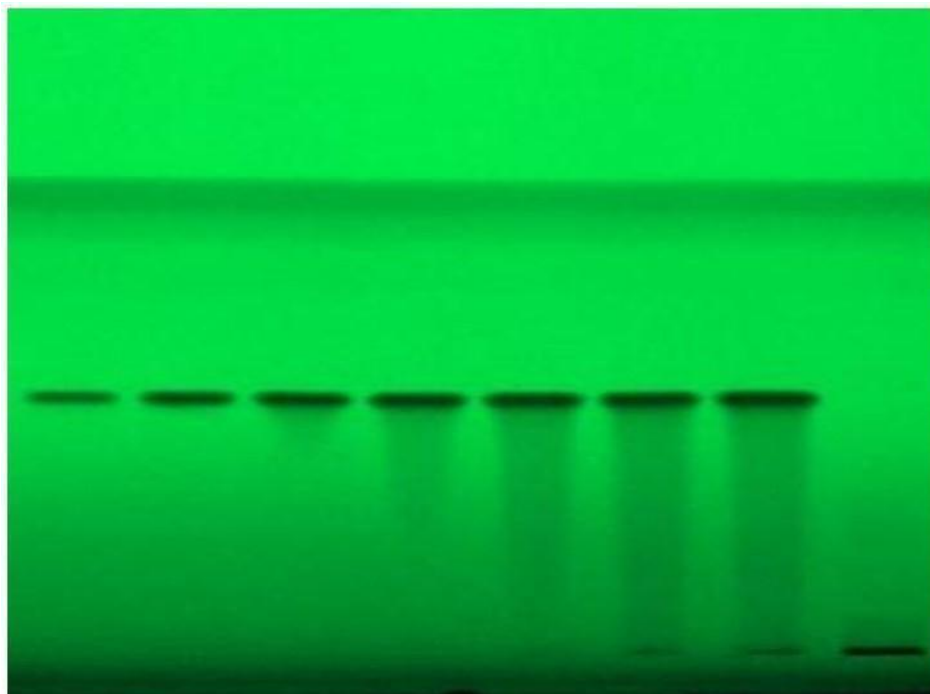


Figure 6.32: Thin layer chromatography in Short U.V

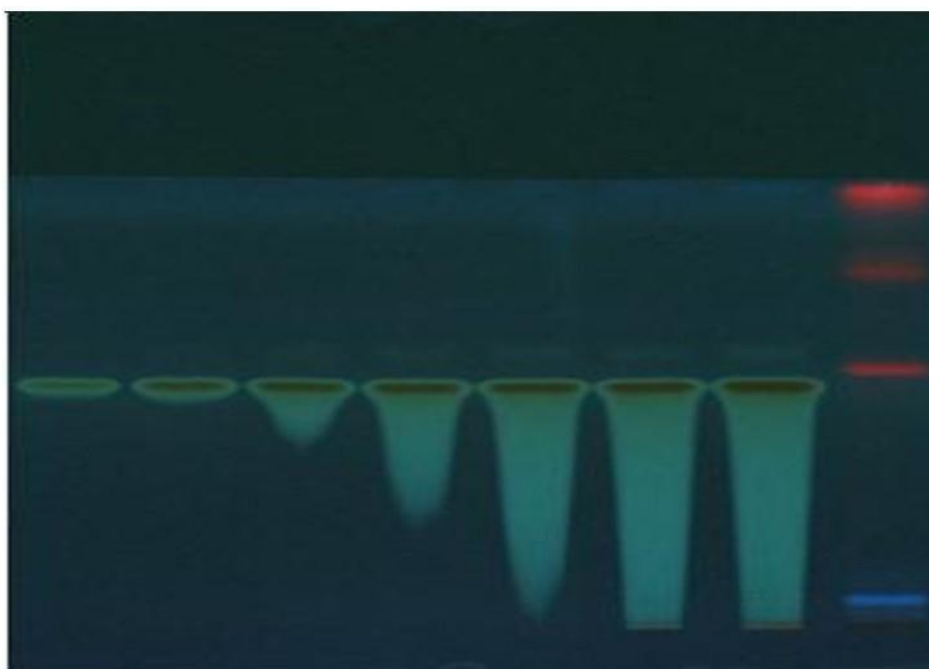


Figure 6.33: Thin layer chromatography in long U.V

6.11.3 Chromatogram of standard quercetin

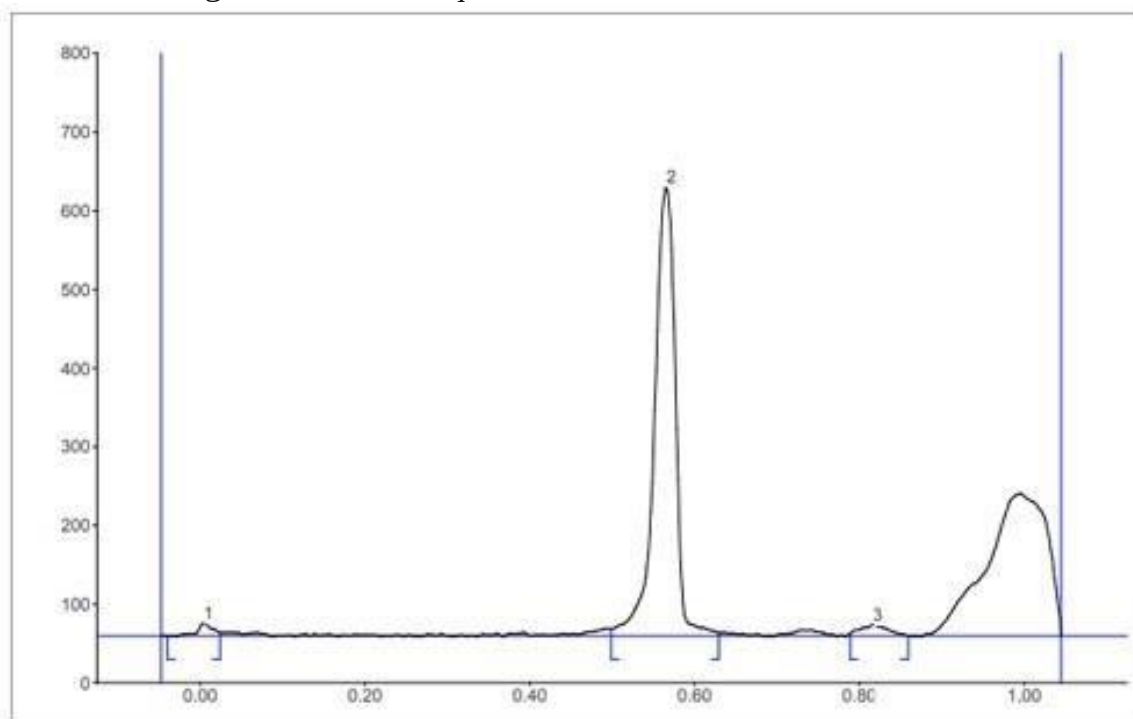


Figure 6.34: Chromatogram of standard quercetin 2µg/ml

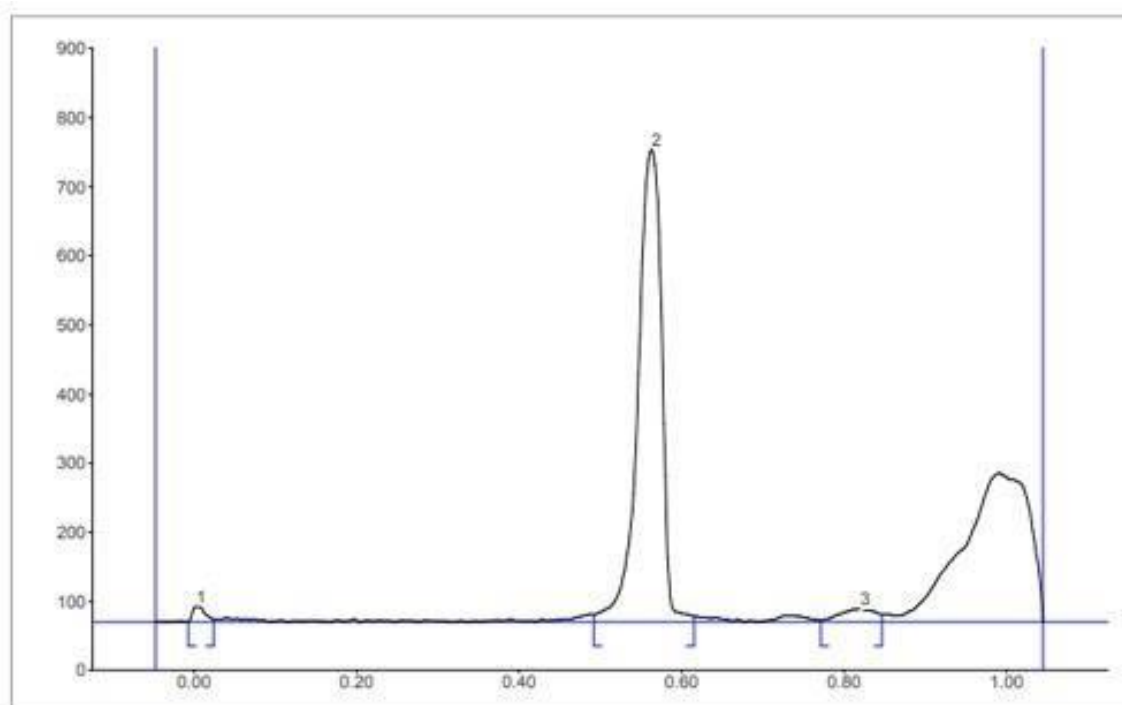


Figure 6.35: Chromatogram of standard quercetin 4µg/ml

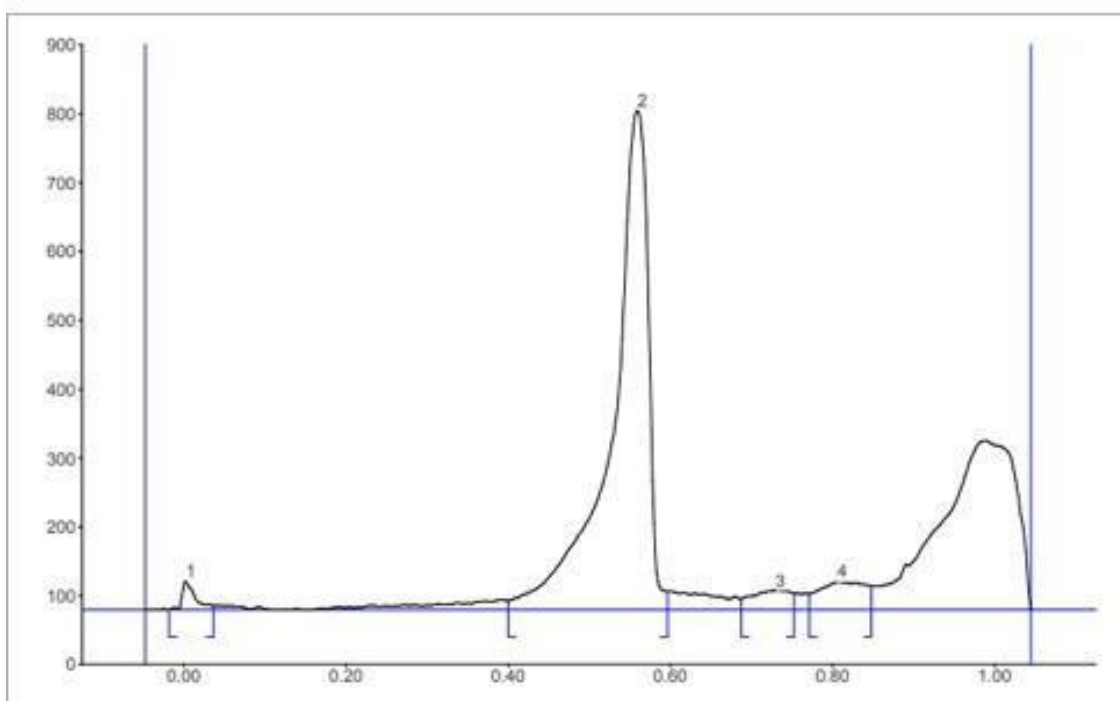


Figure 6.36: Chromatogram of standard quercetin 6µg/ml

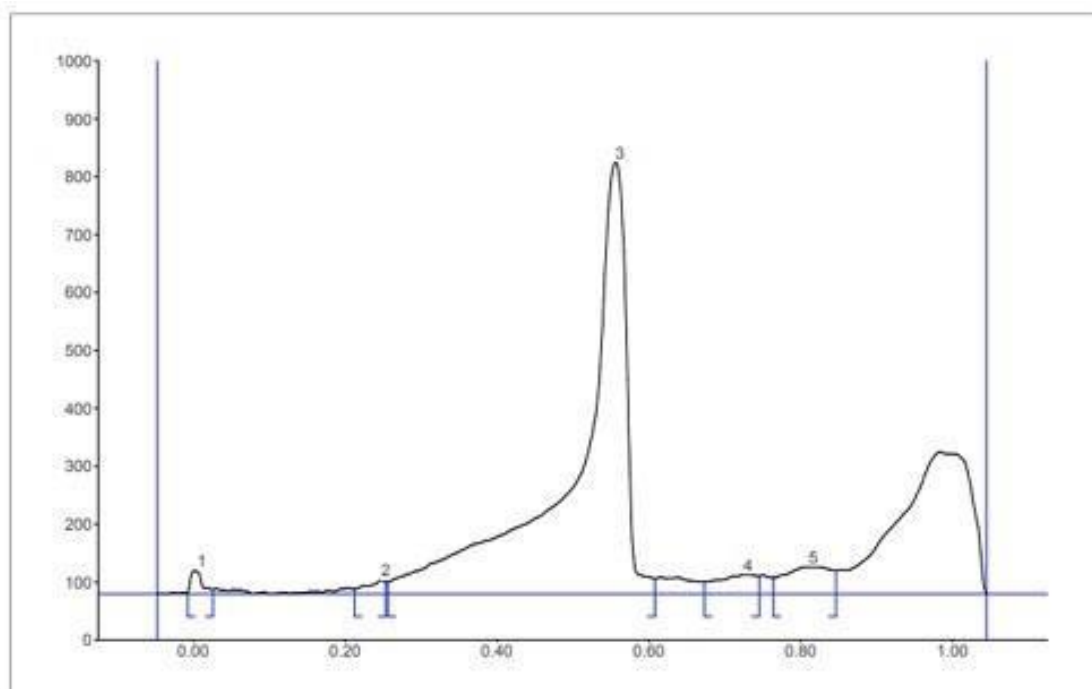


Figure 6.37: Chromatogram of standard quercetin 8µg/ml

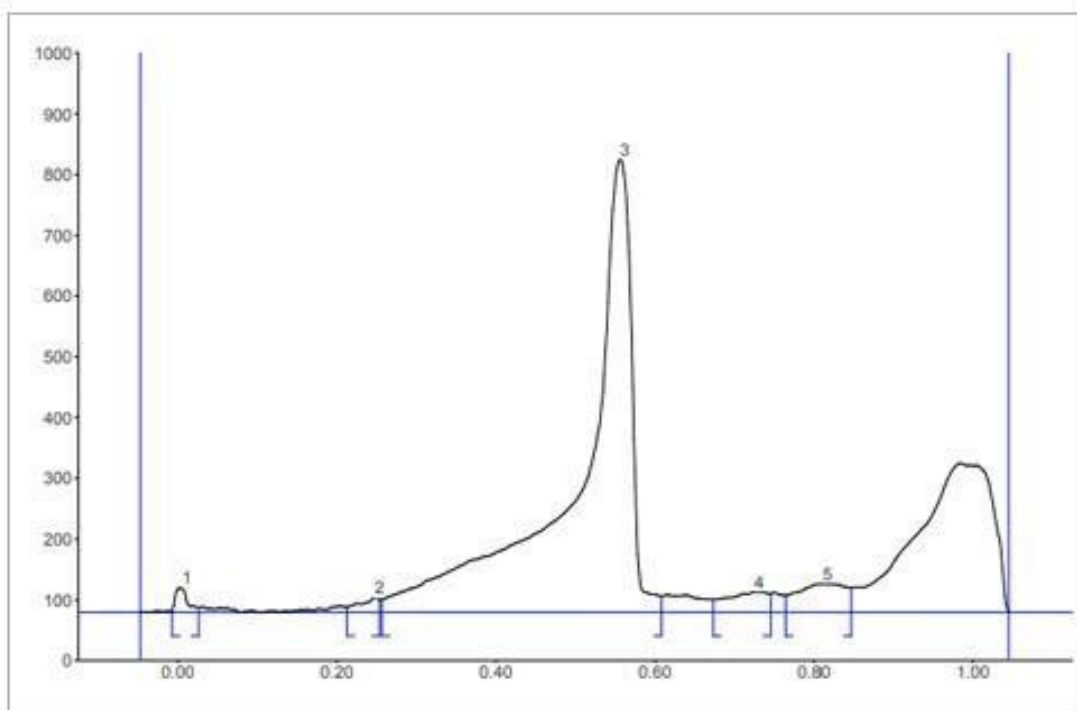


Figure 6.38: Chromatogram of standard quercetin 10µg/ml

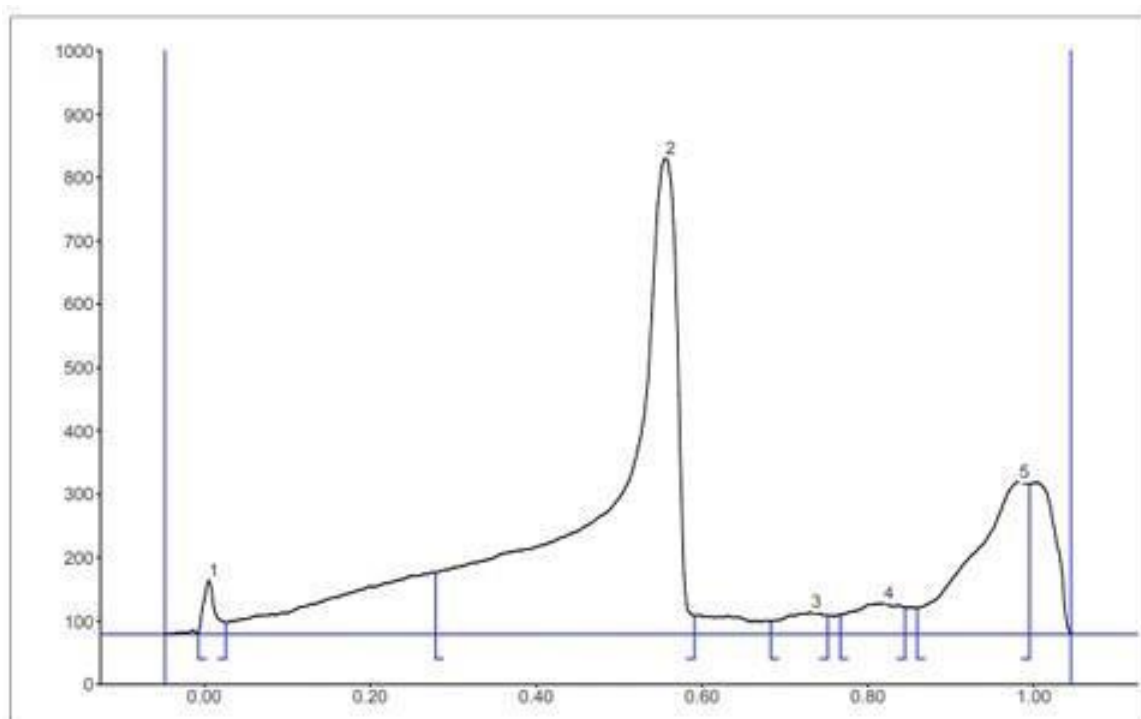


Figure 6.39: Chromatogram of standard quercetin 12µg/ml

6.11.4 Chromatograms of sample

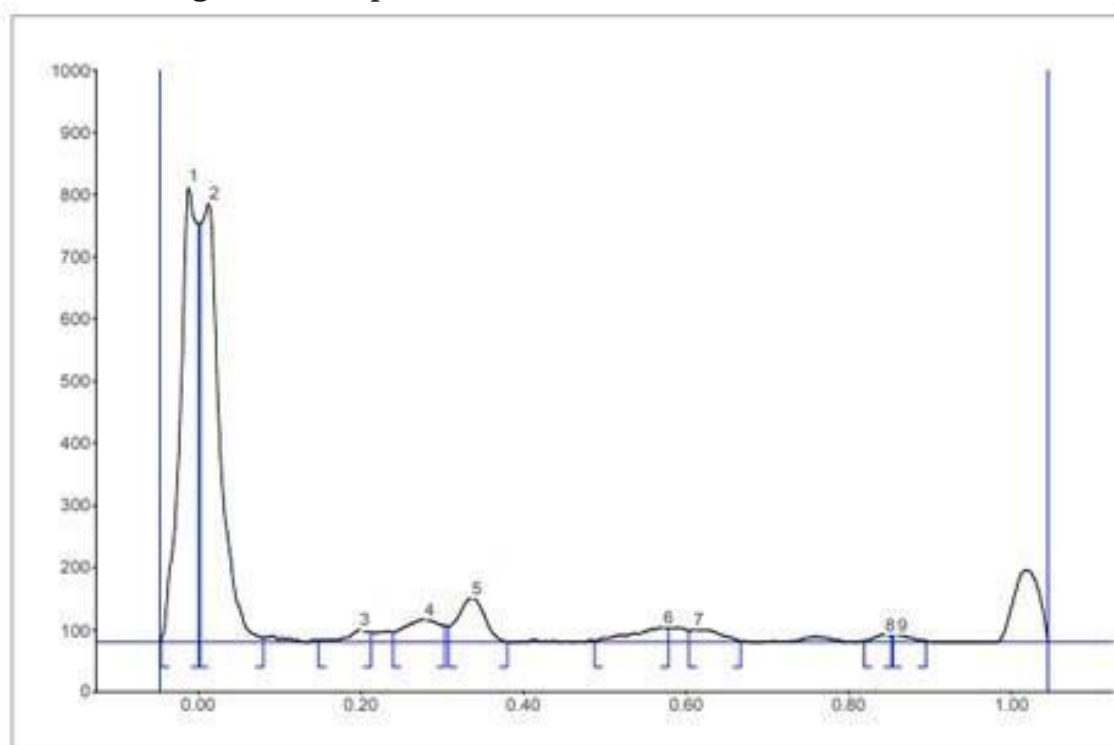


Figure 6.40: HPTLC chromatogram of ethanolic extract of *Cassia spectabilis*

6.11.5 Results of HPTLC estimation

Table 6.24: Results of HPTLC estimation

Estimation	Ethanolic extract
Percentage Found	0.265

The HPTLC estimation of quercetin in the ethanolic extract of *Cassia spectabilis* revealed a content of 0.265%. This indicates that quercetin is present in measurable quantities in the extract, albeit at a relatively low concentration.

The result confirms the presence of quercetin as one of the major flavonoid constituents, which may contribute to the antioxidant, antimicrobial, and antidiabetic activities observed in the extract. The HPTLC method provided a reliable, precise, and reproducible means of quantifying quercetin, supporting its use for standardization and quality control of the plant extract for further pharmacological or nutraceutical applications.

7. SUMMARY AND CONCLUSION

7.1 Summary

The physicochemical analysis of *Cassia spectabilis* leaves provided crucial details about the plant material's quality and purity. The overall mineral composition of the leaves was indicated by the total ash content, which was found to be 4.86%. While acid-insoluble ash, which represents siliceous or other insoluble materials, was 1.52%, water-soluble ash, which represents the amount of inorganic elements soluble in water, was tested at 6.25%. The leaves' low moisture content of 1.52% makes them ideal for long-term storage and guards against microbial contamination and phytoconstituent breakdown.

The solubility profile of phytochemicals in various solvents was revealed by the extractive values. With the highest yield (11.47% w/w), the aqueous extract was followed by the ethanolic (7.23% w/w), ethyl acetate (2.62% w/w), and chloroform (1.25% w/w) extracts. This suggests that whereas nonpolar chemicals are few in the chloroform extract *Cassia spectabilis* is abundant in polar compounds that are better extracted using aqueous and ethanolic solvents.

A wide variety of bioactive components were identified by phytochemical analysis of the various extracts. Only proteins were present in the chloroform extract; no alkaloids, glycosides, flavonoids, phenols, carbohydrates, saponins, tannins, or sterols were found. This implies that the plant contains very few nonpolar components. Glycosides, flavonoids, and proteins were detected in the ethyl acetate extract, suggesting that ethyl acetate is an effective solvent for extracting moderately polar substances.

A wealth of bioactive phytochemicals, including flavonoids, phenolic compounds, and glycosides, which have anti-inflammatory, anti-bacterial, antioxidant, and antidiabetic qualities, are found in *Cassia spectabilis* leaves, according to the results. The significance of choosing suitable extraction techniques based on the intended pharmacological activity is shown by the variance in phytochemical profiles among various solvent extracts.

The findings show that the leaves of *Cassia spectabilis* are a rich source of bioactive phytochemicals, including flavonoids, phenolic compounds, and glycosides, which have anti-inflammatory, anti-bacterial, antioxidant, and antidiabetic qualities. Depending on the

intended pharmacological activity, the phytochemical profiles of various solvent extracts vary, which highlights the need of choosing the right extraction techniques.

Standard spectrophotometric techniques were used to assess the total phenolic content (TPC) and total flavonoid content (TFC) of *Cassia spectabilis* extracts. TPC, which quantifies the capacity of phenolic compounds to degrade phosphomolybdic-phosphotungstic acid complexes to create a blue chromogen observable at a particular wavelength, was estimated using the Folin-Ciocalteu reagent method. With a regression equation of $y = 0.034x - 0.000$ and a correlation coefficient (R^2) of 0.999, gallic acid, which served as the calibration curve's standard, showed a linear response in the concentration range of 5-25 $\mu\text{g/ml}$, demonstrating exceptional linearity and precision.

The aluminium chloride (AlCl₃) colorimetric approach, which is based on the production of a yellow complex between flavonoids and aluminium ions, was used to determine TFC. The standard, quercetin, demonstrated a linear response in the 5-25 $\mu\text{g/ml}$ range, with a correlation coefficient (R^2) of 0.998 and a regression equation of $y = 0.043x + 0.023$, demonstrating the method's dependability.

With 0.75 mg GAE/100 mg and 0.96 mg QE/100 mg, respectively, the ethanolic extract of *Cassia spectabilis* had the highest phenolic and flavonoid contents, according to quantitative analysis, demonstrating its abundance in bioactive antioxidant chemicals. The aqueous extract has somewhat lower concentrations of flavonoids (0.83 mg QE/100 mg) and phenolics (0.62 mg GAE/100 mg). Interestingly, the ethyl acetate extract had a minimal phenolic content and a relatively low flavonoid content (0.21 mg QE/100 mg), indicating that most polar phenolic and flavonoid chemicals are preferentially removed in polar solvents like water and ethanol.

These findings suggest that the leaves of *Cassia spectabilis* are a promising source of flavonoid and phenolic chemicals, especially in the ethanolic and aqueous extracts, which may help explain their pharmacological, anti-inflammatory, and antioxidant properties. Ethanol and water extracts were chosen for additional bioactivity research and formulation development because to their greater TPC and TFC.

The mobile phase Toluene:Ethyl acetate:Formic acid (5:4:1 v/v/v) was used to separate and identify phytoconstituents in *Cassia spectabilis* extracts using Thin Layer Chromatography

(TLC), with quercetin serving as the reference standard. Under normal light, short UV, and long UV, the standard quercetin displayed a single spot with an R_f value of 0.52, indicating its purity.

Multiple UV-active phytochemicals, including quercetin (R_f 0.52), were present in the ethyl acetate extract, as evidenced by the presence of one spot under normal light, three spots under short UV, and five spots under long UV. With quercetin's presence confirmed at R_f 0.52, the ethanolic extract displayed one spot under normal light, three spots under short UV, and seven spots under long UV, indicating a greater diversity of bioactive chemicals. While quercetin was still identifiable, the aqueous extract displayed no spots under normal light, one spot under short UV, and five spots under long UV, suggesting less UV-active ingredients. The TLC results confirm the presence of quercetin in all extracts and reveal that the ethanolic extract contains the richest profile of phytoconstituents, supporting its use in further bioactivity and phytochemical studies.

Using a proven HPLC approach, the amount of quercetin in the ethanolic extract of *Cassia spectabilis* was quantitatively estimated. Peak areas were recorded for each standard solution in a calibration curve for quercetin that covered the concentration range of 5-25 $\mu\text{g/mL}$. With a regression coefficient (R^2) near 1, the calibration curve showed excellent linearity and the mean peak area grew proportionately with concentration, demonstrating the correctness and dependability of the procedure.

Analysed using the same HPLC parameters, the ethanolic extract of *Cassia spectabilis* revealed a retention time (RT) of 2.525 minutes. Based on the calibration curve, the quercetin content was determined to be 0.248% w/w, and the sample's matching peak area was 5898. These findings show that HPLC offers an accurate and repeatable technique for measuring this significant flavonoid in plant extracts and validate the presence of quercetin in the ethanolic extract, albeit in a comparatively low concentration.

Three distinct in vitro tests **DPPH** radical scavenging, hydrogen peroxide scavenging, and nitric oxide scavenging were used to assess the antioxidant capacity of the ethanolic extract of *Cassia spectabilis*, with ascorbic acid serving as the standard reference.

A concentration-dependent increase in radical scavenging activity was demonstrated by the ethanolic extract in the DPPH experiment; the percentage inhibition ranged from 29.57% at

10µg/mL to 61.25% at 100µg/mL. The extract's IC₅₀ value, which indicates the concentration needed to block 50% of the DPPH radicals, was determined to be 71.51 µg/mL, while ascorbic acid's was 15.91 µg/mL. These findings show that although the extract has significant free radical scavenging activity, it is not as effective as the conventional antioxidant.

The extract's ability to scavenge hydrogen peroxide also showed a dose-dependent effect. **With** an IC₅₀ value of 278.39µg/mL, the percentage inhibition rose from 31.54% at 100 µg/mL to 73.81% at 500 µg/mL, while ascorbic acid showed an IC₅₀ of 80.55 µg/mL. This implies that, even at higher concentrations than the norm, the ethanolic extract can neutralise reactive oxygen species.

The extract also successfully suppressed nitric oxide radicals in a concentration-dependent manner, according to the nitric oxide scavenging assay, with percent inhibition ranging from 27.35% at 20µg/mL to 60.89% at 100µg/mL. The extract's IC₅₀ value was 68.25 µg/mL, while ascorbic acid's was 25.08 µg/mL. These results demonstrate that *Cassia spectabilis*'s ethanolic extract exhibits strong antioxidant activity against a variety of radical systems, which could support some of its possible medicinal benefits.

Diclofenac sodium was used as the reference medication in the in vitro protein denaturation procedure to assess the anti-inflammatory properties of the ethanolic extract of *Cassia spectabilis*. The percentage of protein denaturation inhibition increased from 30.25% at 100µg/mL to 68.96% at 500µg/mL, demonstrating a concentration-dependent effect of the extract. Diclofenac sodium, on the other hand, showed more inhibitory action, varying between 49.58% and 92.65% at the same doses. The ethanolic extract's IC₅₀ value, which indicates the concentration needed to prevent 50% of protein denaturation, was determined to be 280.41µg/mL, while Diclofenac sodium's IC₅₀ was 66.34µg/mL. These findings imply that the ethanolic extract may have some potential as a natural anti-inflammatory agent, but its effectiveness is less than that of the common medication, diclofenac sodium.

In comparison to common medications like Ofloxacin, Ciprofloxacin, and Fluconazole, the antibacterial activity of the ethanolic extract of *Cassia spectabilis* was assessed against a panel of bacterial and fungal species. With zone of inhibition (ZOI) values varying from 15 mm to 40 mm based on the pathogen and concentration, the conventional medications demonstrated potent antibacterial action. Ciprofloxacin had broad-spectrum efficacy against

Bacillus subtilis, *Klebsiella pneumoniae*, and *Escherichia coli* (15-40 mm), Ofloxacin effectively inhibited *Streptococcus mutans* (17-22 mm), and Fluconazole exhibited strong antifungal activity against *Candida albicans* (26-32 mm).

Cassia spectabilis ethanolic extract exhibited concentration-dependent, mild antibacterial activity. Maximum inhibition was seen at 100 mg/mL for *Streptococcus mutans* (16 mm), *Bacillus subtilis* (17 mm), *Klebsiella pneumoniae* (12 mm), *Escherichia coli* (15 mm), and *Candida albicans* (14 mm). The ZOI rose as the extract concentration increased. These findings imply that the extract may be used as a natural antibacterial agent since, despite being less effective than conventional antimicrobial medications, it exhibits strong inhibitory activity against strains of both Gram-positive and Gram-negative bacteria as well as fungi.

Using α -amylase inhibition experiments, the ethanolic extract of *Cassia spectabilis* was tested for its in vitro antidiabetic potential, with Acarbose acting as the standard reference medication. Acarbose and the ethanolic extract both caused a concentration-dependent rise in the % inhibition of α -amylase. With an JC_{50} value of 27.31 μ g/mL, Acarbose demonstrated substantial inhibitory efficacy for the first set of doses (10-50 μ g/mL), attaining up to 80.52% inhibition. With an JC_{50} of 57.88 μ g/mL and a significant inhibition of 43.51% at 50 μ g/mL, the ethanolic extract showed considerable antidiabetic action while being less strong than the standard.

Similar patterns were seen in the second assay, which used a larger concentration range (12.5-200 μ g/mL). Acarbose significant α -amylase inhibitory capacity was confirmed by its maximum inhibition of 80.14% at 200 μ g/mL and JC_{50} of 16.66 μ g/mL. With an JC_{50} of 172.31 μ g/mL, the ethanolic extract showed increased inhibition with concentration, reaching 51.69% at 200 μ g/mL. These findings imply that, although its effectiveness is less than that of the common medication Acarbose, the ethanolic extract of *Cassia spectabilis* has considerable α -amylase inhibitory activity, which may help explain its possible antidiabetic impact. This demonstrates how well the extract works as a natural remedy for postprandial hyperglycemia.

Using a conventional calibration method, the flavonoid content of the ethanolic extract of *Cassia spectabilis* was measured by HPTLC analysis of quercetin. Over a concentration range of 2-12 μ g/mL, a quercetin calibration curve was created. The mean peak areas

showed excellent linearity, increasing linearly with concentration from $8,245.2 \pm 25.6$ at $2\mu\text{g/mL}$ to $48,410.4 \pm 56.7$ at $12\mu\text{g/mL}$. With a slope of 4,027.3, an intercept of 229.79, and a regression coefficient (R^2) of 0.9999, the calibration curve's regression analysis demonstrated a very accurate and dependable measurement of quercetin.

The presence of this bioactive flavonoid was confirmed by using this calibration to estimate the quercetin level in the ethanolic extract of *Cassia spectabilis*, which came out to be 0.265%. These findings show that quercetin is present in substantial amounts in the ethanolic extract, which may be responsible for some of its pharmacological properties, such as its anti-inflammatory and antioxidant properties.

7.2 Conclusion

The thorough analysis of *Cassia spectabilis* leaves shows that this plant is a great source of phytochemicals that are bioactive and have a lot of pharmacological potential. Acceptable moisture and ash concentrations were found by physicochemical examination, guaranteeing the plant material's stability and purity. According to extractive values, bioactive substances are effectively extracted by polar solvents, especially ethanol and water, while nonpolar elements are scarce. Flavonoids, phenols, glycosides, proteins, carbohydrates, saponins, and tannins were all validated by phytochemical screening; the ethanolic extract showed the widest range of ingredients.

The ethanolic extract had the highest levels of total phenolic and flavonoid contents, according to quantitative analysis (0.75 mg GAE/100 mg and 0.96 mg QE/100 mg, respectively), suggesting that it has a great deal of potential as an antioxidant source. All extracts included quercetin, according to TLC and HPTLC tests; the ethanolic extract showed a varied phytochemical profile and a detectable quercetin level of 0.265%.

Significant in vitro biological activity was demonstrated by the ethanolic extract. In **DPPH**, hydrogen peroxide, and nitric oxide scavenging tests, it demonstrated strong antioxidant capacity, but marginally less than that of conventional antioxidants. Protein denaturation inhibition revealed moderate anti-inflammatory activity, and the extract demonstrated concentration-dependent antibacterial activity against *Candida albicans* and both Gram-positive and Gram-negative bacteria. Additionally, via inhibiting α -amylase, the ethanolic extract showed moderate in vitro antidiabetic action, indicating that it may be used to treat

postprandial hyperglycemia. The study highlights *Cassia spectabilis* as a promising medicinal plant with rich phenolic and flavonoid content, antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties. The ethanolic extract, in particular, can serve as a potential source of bioactive compounds for further pharmacological research and the development of natural therapeutic agents.

Future Plan

1. Isolation and Characterization of Bioactive Compounds

- Further fractionation and purification of the extract will be carried out to isolate individual phytoconstituents responsible for the observed biological activities.
- Advanced analytical techniques such as HPLC, LC-MS, FTIR, and NMR spectroscopy will be employed for structural characterization.

2. In-Vivo Pharmacological Studies

- The antioxidant and anti-inflammatory potential of the extract will be evaluated in suitable animal models to confirm the in-vitro findings and assess its therapeutic efficacy.

3. Mechanistic Studies

- Investigations will be conducted to elucidate the molecular mechanisms involved in the antioxidant and anti-inflammatory actions of the extract, including the modulation of inflammatory mediators and oxidative stress markers.

4. Safety and Toxicity Evaluation

- Acute and sub-chronic toxicity studies will be performed to establish the safety profile of the extract for future pharmaceutical applications.

5. Formulation Development

- Based on the pharmacological outcomes, suitable herbal formulations such as capsules, tablets, or topical preparations may be developed and evaluated for stability and efficacy.

6. Clinical Research

- After successful preclinical evaluation, clinical studies may be planned to assess the safety and effectiveness of the developed formulation in human subjects.

Expected Outcomes

- Identification of important phytochemical constituents present in *Cassia spectabilis* extract.
- Scientific validation of the antioxidant and anti-inflammatory properties of the plant.
- Discovery of potential bioactive compounds that may serve as lead molecules for drug development.
- Development of a safe and effective herbal therapeutic agent for the management of oxidative stress and inflammatory disorders.
- Contribution to the existing knowledge on medicinal plants and promotion of evidence-based herbal medicine.

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Research Article

**EXPLORING THE PHYTOCHEMISTRY AND
PHARMACOLOGICAL SIGNIFICANCE OF CASSIA
SPECTABILIS****Seema Wadhwani*, I. K. Yadav, Vivek Gupta**

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Abstract:

Cassia spectabilis, a member of the Fabaceae family, is a tropical ornamental plant known for its vibrant yellow flowers and a wide range of therapeutic properties. This review highlights the phytochemical composition and pharmacological potential of C. spectabilis extracts. Various studies have identified the presence of flavonoids, alkaloids, tannins, saponins, and phenolic compounds that contribute to its biological activities. The plant exhibits multiple pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer effects. These activities are attributed to its bioactive constituents that interact with cellular pathways and free radical scavenging mechanisms. Despite extensive preliminary findings, further studies are required to isolate, characterize, and validate the therapeutic potential of its active compounds through advanced pharmacological and clinical evaluations. This review aims to provide comprehensive insight into the phytochemistry and pharmacological importance of Cassia spectabilis, emphasizing its potential as a source of novel bioactive agents in drug discovery.

Keywords: Cassia spectabilis, phytochemical screening, pharmacological activity, antioxidant, medicinal plants, bioactive compounds.

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INTRODUCTION:

Medicinal plants have long served as an essential source of therapeutic agents for treating a wide range of diseases. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines, primarily of plant origin, for their primary healthcare needs (WHO, 2013). Among the diverse medicinal flora, the genus *Cassia* (family: Fabaceae) comprises more than 500 species distributed in tropical and subtropical regions and is well known for its pharmacological importance (Kumar et al., 2020).

Cassia spectabilis DC., commonly known as Golden Shower or Yellow Cassia, is an ornamental tree widely distributed in tropical Asia and South America. Traditionally, various parts of *C. spectabilis*, including leaves, flowers, bark, and seeds, have been used for their medicinal properties, such as antibacterial, antifungal, antioxidant, anti-inflammatory, antimalarial, and antidiabetic activities (Nirmal et al., 2015; Rahman et al., 2018). The plant is rich in a variety of phytoconstituents such as flavonoids, alkaloids, tannins, phenolic compounds, saponins, and glycosides, which are largely responsible for its diverse pharmacological effects (Arunachalam et al., 2014).

Phytochemical screening of *C. spectabilis* extracts has revealed the presence of bioactive compounds that contribute to its potent antioxidant potential. These natural antioxidants play a vital role in neutralizing free radicals, thereby preventing oxidative stress-related disorders such as diabetes, cardiovascular diseases, neurodegenerative conditions, and cancer (Ali et al., 2020). Recent studies have demonstrated that the ethanolic and aqueous extracts of *C. spectabilis* possess significant free radical scavenging activity against DPPH, hydrogen peroxide, and nitric oxide radicals, suggesting its potential application in natural antioxidant therapy (Jaiswal et al., 2022).

Given the growing global interest in plant-based therapeutics, this review aims to provide a comprehensive overview of the phytochemical composition and pharmacological significance of *Cassia spectabilis*. It also highlights recent advances in the understanding of its bioactive constituents, mechanisms of action, and therapeutic potential, thereby emphasizing its value as a promising candidate for future drug development.

Phytochemical Constituents of *Cassia spectabilis*

The therapeutic efficacy of *Cassia spectabilis* is largely attributed to its diverse array of bioactive phytoconstituents. Phytochemical investigations of

various parts of the plant including leaves, flowers, seeds, and bark have identified the presence of flavonoids, alkaloids, glycosides, phenolics, tannins, saponins, and terpenoids (Rahman et al., 2018; Arunachalam et al., 2014). These compounds play crucial roles in the plant's biological activities, acting as antioxidants, antimicrobial agents, and enzyme inhibitors.

Flavonoids and phenolic compounds are among the most abundant secondary metabolites in *C. spectabilis*. They are known for their potent free radical scavenging activity, metal chelation ability, and role in preventing oxidative stress-related diseases (Ali et al., 2020). Ethanolic and aqueous extracts of *C. spectabilis* have demonstrated significant levels of total phenolic and flavonoid contents, suggesting their potential as natural antioxidants (Jaiswal et al., 2022).

Alkaloids and glycosides identified in *C. spectabilis* contribute to its antimicrobial, antimalarial, and antidiabetic properties (Nirmal et al., 2015). Studies have reported the isolation of specific bioactive compounds such as spectraline, spectralinone, and cassiarin A a potent antimalarial alkaloid structurally related to acridone derivatives (Viegas et al., 2004). Cassiarin A has been shown to exhibit strong activity against *Plasmodium falciparum*, highlighting the plant's potential as a source of novel antimalarial agents (Abe et al., 2010).

Furthermore, triterpenoids and saponins present in the plant are associated with anti-inflammatory and cytoprotective effects. These compounds stabilize biological membranes, modulate oxidative pathways, and enhance immune responses (Kumar et al., 2020). The presence of tannins and diterpenes has also been linked to the plant's antimicrobial and hepatoprotective actions (Arunachalam et al., 2014).

Phytochemical profiling using chromatographic and spectroscopic techniques including TLC, HPLC, and GC-MS has provided valuable insights into the chemical diversity of *C. spectabilis*. Recent GC-MS studies of ethanolic extracts revealed the presence of bioactive molecules such as phytol, linoleic acid, and stigmaterol, which contribute to the plant's antioxidant and anti-inflammatory activities (Akinmoladun et al., 2021).

Collectively, these findings emphasize that *Cassia spectabilis* is a rich reservoir of pharmacologically active compounds, which warrant further exploration for the development of plant-derived therapeutic agents.

Pharmacological Activities of *Cassia spectabilis*

Cassia spectabilis possesses a wide spectrum of pharmacological activities due to its rich phytochemical composition. Various preclinical and experimental studies have demonstrated its antioxidant, antimicrobial, anti-inflammatory, antimalarial, antidiabetic, and hepatoprotective potential (Nirmal et al., 2015; Rahman et al., 2018).

1. Antioxidant Activity

Reactive oxygen species (ROS) are major contributors to oxidative stress, which plays a key role in the pathogenesis of several chronic diseases. The ethanolic and aqueous extracts of *C. spectabilis* have shown strong free radical scavenging activity in DPPH, hydrogen peroxide, and nitric oxide assays, with IC₅₀ values indicating significant antioxidant potential (Jaiswal et al., 2022). The presence of flavonoids and phenolic compounds is mainly responsible for this activity, as these compounds neutralize free radicals and inhibit lipid peroxidation (Ali et al., 2020).

2. Antimicrobial and Antifungal Activity

Extracts from the leaves and flowers of *C. spectabilis* have demonstrated potent antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as antifungal activity against *Candida albicans* (Rahman et al., 2018). The observed antimicrobial effect is attributed to bioactive constituents such as alkaloids, flavonoids, and saponins, which disrupt microbial cell membranes and inhibit enzyme synthesis (Akinmoladun et al., 2021).

3. Anti-inflammatory and Analgesic Activity

The anti-inflammatory potential of *C. spectabilis* has been confirmed through in vivo studies using carrageenan-induced paw edema and formalin-induced inflammation models. Ethanolic extracts reduced inflammation significantly, suggesting inhibition of cyclooxygenase and prostaglandin pathways (Arunachalam et al., 2014). The analgesic activity of the plant may be linked to its ability to modulate nociceptive mediators through the presence of flavonoids and triterpenoids (Kumar et al., 2020).

4. Antimalarial Activity

One of the most remarkable pharmacological effects of *C. spectabilis* is its antimalarial activity. The alkaloid Cassiarin A, isolated from the leaves, has demonstrated strong inhibitory action against *Plasmodium falciparum* with activity comparable to chloroquine (Viegas et al., 2004; Abe et al., 2010). This makes *C. spectabilis* a promising source for the development of new antimalarial agents.

5. Antidiabetic and Hepatoprotective Activity

In streptozotocin-induced diabetic models, ethanolic extracts of *C. spectabilis* have been reported to

reduce blood glucose levels and improve lipid profiles (Rahman et al., 2018). The plant's hepatoprotective potential has also been documented, where extracts significantly decreased elevated liver enzymes and restored normal histopathological architecture in toxin-induced hepatic injury models (Arunachalam et al., 2014).

Collectively, these studies confirm that *Cassia spectabilis* exhibits multifaceted pharmacological actions, validating its traditional medicinal uses and encouraging its further exploration as a source of therapeutic compounds.

CONCLUSION:

Cassia spectabilis has emerged as a promising medicinal plant with diverse pharmacological properties and a rich phytochemical profile. Numerous studies have confirmed the presence of bioactive compounds such as alkaloids (Cassiarin A and B), flavonoids, phenolics, glycosides, and terpenoids, which contribute to its wide range of therapeutic actions, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, and antimalarial effects. Among these, the antimalarial alkaloid Cassiarin A represents a particularly valuable natural compound with significant pharmacological potential.

The antioxidant potential of *C. spectabilis*, primarily associated with its flavonoid and phenolic content, highlights its capacity to combat oxidative stress and related degenerative disorders. Likewise, the plant's antimicrobial and anti-inflammatory activities support its traditional uses in treating infections and inflammatory conditions. Despite these encouraging findings, most studies have been limited to preliminary in vitro or in vivo evaluations.

Future research should focus on the isolation, structural elucidation, and pharmacokinetic evaluation of specific bioactive constituents to establish detailed mechanisms of action. Advanced analytical tools such as LC-MS/MS, NMR, and molecular docking studies could help in correlating the phytochemical composition with therapeutic activity. Furthermore, toxicological assessments and clinical investigations are essential to confirm the safety and efficacy of *C. spectabilis*-based formulations.

Cassia spectabilis represents a valuable source of natural compounds with significant pharmacological relevance. Continued exploration through systematic pharmacognostic, pharmacological, and biotechnological studies may pave the way for the

development of novel phytopharmaceuticals and standardized herbal formulations derived from this plant.

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PHYTOCHEMICAL SCREENING, QUANTITATIVE ESTIMATION, AND
ANTIOXIDANT ACTIVITY OF *CASSIA SPECTABILIS* EXTRACTS

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ABSTRACT

The present study aimed to evaluate the phytochemical composition and antioxidant potential of various extracts of *Cassia spectabilis*. Physicochemical parameters such as ash values and moisture content were determined to assess the purity and quality of the crude drug. The extractive value analysis revealed that the aqueous extract showed the highest yield, indicating the predominance of polar constituents. Preliminary phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, phenols, saponins, tannins, and carbohydrates, especially in the ethanolic and aqueous extracts. Quantitative estimation demonstrated that the ethanolic extract possessed the highest total phenolic (0.75 mg GAE/100 mg) and flavonoid (0.96 mg QE/100 mg) content. The antioxidant potential of the extracts was evaluated using DPPH, hydrogen peroxide, and nitric oxide radical scavenging assays. The ethanolic extract exhibited significant free radical scavenging activity with IC₅₀ values of 71.51 µg/mL (DPPH), 278.39 µg/mL (H₂O₂), and 68.25 µg/mL (NO), as compared to the standard ascorbic acid. These findings suggest that *Cassia spectabilis* possesses potent antioxidant activity, which may be attributed to its high content of phenolic and flavonoid compounds, supporting its traditional use as a medicinal plant with therapeutic potential against oxidative stress-related disorders.

Keywords: *Cassia spectabilis*, Phytochemical screening, Antioxidant activity, DPPH, Hydrogen peroxide, Nitric oxide, Phenolic content, Flavonoid content, Ethanol extract.

INTRODUCTION

Medicinal plants are rich sources of bioactive compounds that serve as potential therapeutic agents for the treatment and prevention of various diseases. The growing interest in plant-based medicine is largely due to their pharmacological efficacy, low toxicity, and cost-effectiveness when compared to synthetic drugs (Pandey *et al.*, 2013). Among these, plants belonging to the genus *Cassia* (family Fabaceae) have been extensively studied for their diverse phytochemical constituents and wide spectrum of biological

activities, including antimicrobial, anti-inflammatory, antidiabetic, and antioxidant effects (Gupta *et al.*, 2016; Singh *et al.*, 2017). *Cassia spectabilis* DC., commonly known as “Golden Shower Tree,” is a tropical ornamental plant distributed widely in Asia, Africa, and South America. Traditionally, various parts of this plant, such as leaves, bark, and flowers, have been used in folk medicine for treating skin diseases, constipation, fever, and infections (Ramesh *et al.*, 2018). Phytochemical studies have revealed that *C. spectabilis* contains a variety

of secondary metabolites, including flavonoids, alkaloids, saponins, tannins, and phenolic compounds, which are known for their antioxidant potential and pharmacological properties (Olayinka *et al.*, 2020; Ahmed *et al.*, 2021).

Antioxidants play essential role in neutralizing free radicals and preventing oxidative stress-related cellular damage, which contributes to the pathogenesis of chronic diseases such as diabetes, cancer, and cardiovascular disorders (Halliwell *et al.*, 2015). Natural antioxidants derived from plants are particularly valued for their safety and efficacy in maintaining redox homeostasis (Birben *et al.*, 2012). Hence, investigating the antioxidant properties of *C. spectabilis* extracts, alongside qualitative and quantitative phytochemical profiling, is vital for understanding its therapeutic potential and justifying its traditional use.

The present study aims to conduct a comprehensive phytochemical screening, quantitative estimation of major phytoconstituents (such as total phenolics and flavonoids), and evaluation of the antioxidant activity of *Cassia spectabilis* extracts using standard in vitro assays. This investigation will provide scientific insight into the bioactive composition and pharmacological relevance of this medicinally important plant.

MATERIALS AND METHODS

Materials

The fresh leaves of *Cassia spectabilis* were collected, shade-dried, and coarsely powdered for extraction. Analytical grade solvents such as chloroform, ethyl acetate, ethanol, and distilled water were used for successive extraction by Soxhlet apparatus. All chemicals and reagents employed, including

ferric chloride, Folin–Ciocalteu reagent, aluminum chloride, quercetin, and gallic acid, were of analytical grade and procured from standard suppliers. Ascorbic acid was used as the standard antioxidant for comparative evaluation. All glassware and instruments used were of laboratory grade and properly calibrated before use.

Methods

Procurement of plant materials

Stem of *Cassia spectabilis* were collected from the Bhopal governing region in February 2023. To prevent the phytochemicals from deteriorating, the plants were cleaned after being collected.

Drying

Stem of *Cassia spectabilis* were shade-dried at room temperature. Using an electric grinder, the dried plant material was ground into a fine powder, sieved, and stored in plastic bags until it was needed.

Determination of physio-chemical Parameters

Determination of Ash Values

A. Determination of Total Ash: 2 g of accurately weighed stem powder was incinerated in a tarred platinum or silica dish at a temperature not exceeding 450°C until free from carbon, cooled and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water, the residue was collected on an ashless filter paper, incinerated, along with filter paper, evaporated to dryness and ignited at a temperature not exceeding 450°C. The ash thus obtained was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

B. Determination of Acid Insoluble Ash:

The ash obtained from above procedure was boiled for 5 min. with 25 ml of dilute

hydrochloric acid and the insoluble matter was collected in a Gooch crucible, or on an ashless filter paper. The insoluble matter thus obtained was washed with hot water and filter paper was ignited to a constant weight along with filter paper. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

C. Determination of Water Soluble Ash:

The ash was boiled for 5 min. with 25 ml of water, the insoluble matter collected in a Gooch crucible, or on an ashless filter paper, washed with hot water and ignited for 15 min. at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight was the water soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

D. Determination of Moisture Content (loss on drying):

About 10 g of stem (without preliminary drying and cut in parts of about 3 mm in thickness), after accurately weighing (weight to within 0.01g) was placed in a tarred evaporation dish. It was then dried at 105°C for 5 hours and weighed. Drying was continued and the stem was weighed at 1 h interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was reached when two consecutive weighing after drying for 30 min. and cooling for 30 min. in a desiccator, did not show more than 0.01g difference.

Extraction by maceration process

An established and popular extraction method for separating bioactive substances from plant sources is maceration. This technique works especially well for removing phytochemicals

from plants that include sensitive components that more aggressive extraction techniques that use heat or solvents might break down. The process described below was used to prepare the extract from the powdered and shade dried herbs (Khandelwal, 2005).

Defatting with petroleum ether

After being coarsely ground, 50 grams of stem of *Cassia spectabilis* were shade-dried at room temperature. Using an electric grinder, the dried plant material was ground into a fine powder, sieved, and stored in plastic bags until it was needed. Shade dried plant material were macerated and extracted using petroleum ether. The extraction process was carried out until the material had been defatted.

Successive extraction with different solvent

Using a 48 hour maceration method, defatted dried powder was extracted using various solvents, including chloroform, ethyl acetate, ethanol and water. By increasing the contact between the plant material and the solvent, the mixture was periodically agitated to improve the extraction efficiency. To separate the liquid extract from the solid plant remains, the mixture is filtered after the soaking period. The dissolved phytochemicals are present in the filtrate. The extract is subsequently concentrated by removing the solvent, typically with a rotary evaporator or by evaporating under lower pressure. To separate particular bioactive components, the concentrated extract can undergo additional processing or analysis.

Determination of extractive value (% yield)

In the phytochemical study of plant materials, determining the extractive value also referred to as the yield percentage is an essential step. By measuring the amount of soluble components extracted from a specific amount

of plant material, it assesses the extraction process effectiveness. This measure is essential for evaluating the extraction method efficacy and comparing the extraction efficiency of various solvents or circumstances. The following formula was used to determine each extracts % yield:

Percentage Yield

$$= \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

One essential procedure in the fields of pharmacognosy and natural products chemistry is qualitative phytochemical analysis. It entails identifying the different bioactive substances found in plant materials. Alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds are some examples of these substances, which are referred to as phytochemicals (Kokate, 1994).

Quantitative studies of phytoconstituents

Estimation of total phenol content

The modified folin-ciocalteu method was used to calculate the extracts total phenol concentration (Gaur Mishra *et al.*, 2017). Various aliquots of 5–25 µg/ml were generated by dissolving 10 mg of gallic acid in 10 ml of methanol. After dissolving 10 mg of dried extract in 10 ml of methanol, the mixture was filtered. This extract was used to estimate the amount of phenol in 2 ml (1 mg/ml). 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate were combined with 2 ml of extract and each standard. For color development, the mixture was vortexed for 15 seconds and then left to stand for 10 minutes. Using a

spectrophotometer, the absorbance at 765 nm was determined.

Estimation of total flavonoids content

The aluminum chloride technique was used to determine the total flavonoid content (Parkhe and Bharti, 2019). Various aliquots of 5–25 µg/ml were produced by dissolving 10 mg of quercetin in 10 ml of methanol. 10 mg of dried extract were dissolved in 10 ml of methanol, and the resulting mixture was then filtered. The flavonoids in 2 ml (1 mg/ml) were estimated using this extract. 2 ml of 2% AlCl₃ solution were added to 2 ml of extract of each standard, and the combination was then allowed to stand for 15 minutes at room temperature. The absorbance was then measured at 420 nm.

In-vitro antioxidant activity of ethanolic extract of *Cassia spectabilis*

DPPH method

With a minor modification, the revealed method was applied to assess the extracts overall ability to scavenge free radicals (Parkhe and Jain, 2018). A DPPH solution (6 mg in 100 ml methanol) was made and kept in a dark location. Standard and test concentrations ranging from 10 to 100 µg/ml were generated. 1.5 ml of DPPH and 1.5 ml of each standard and test were prepared in a separate test tube; the absorbance of this mixture was immediately measured at 517 nm. 1.5 ml of DPPH and 1.5 ml of methanol were tested as a control absorbance at 517 nm. Using the following formula, the % inhibition of free radical DPPH was determined:

$$\% \text{ inhibition} = \frac{[(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}] \times 100\%}$$

Hydrogen peroxide method

Hydrogen peroxide was used to test the extracts *in-vitro* antioxidant properties (Wasiullah *et al.*, 2023). 2.4 ml of 0.1 M phosphate buffer (pH 7.4), 1.0 ml of ethanolic sample (100-500µg/ml), and 2 ml of hydrogen peroxide (43mol) were added. After ten minutes of maintaining the resultant solution, the absorbance at 230 nm was measured. The blank was ready without hydrogen peroxide, and the control was made without any sample. It was combined with ascorbic acid as a standard compound. The percentage of free radical hydrogen peroxide scavenging activity has been computed.

$$\% \text{ inhibition} = \left[\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \right] \times 100\%$$

Nitric oxide method

The Griess reagent was measured and sodium nitroprusside was converted to nitric oxide. In aqueous solution at physiological pH, sodium nitroprusside spontaneously generates nitric oxide. It then interacts with oxygen to produce nitric ions, which may be measured using the Griess reagent. Reduced nitric oxide production is the result of nitric oxide scavengers competing with oxygen (Bawane *et al.*, 2020). Different extract concentrations were combined with sodium nitroprusside (10 mmol/L) in phosphate buffer saline (PBS), and the mixture was incubated for 150 minutes at 25°C. Specimens were treated with Griess reagent (1% sulfanilamide, 2% H₃PO₄, and 0.1% naphthylethylenediamine dihydrochloride). The absorption of standard ascorbic acid solutions prepared in the same way with Griess reagent as a positive control was measured at 546 nm using the chromophore absorbance produced during the

diazotization of sulphanilamide nitrite and subsequent coupling with naphthylethylenediamine. The following formula was used to determine the inhibition proportion:

$$\text{Radical scavenging activity (\%)} = \left(\frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100$$

Where, A_{test} is the absorption when the extract or standard is present, and A_{control} is the absorption of the control (without extract).

RESULTS AND DISCUSSION

The present study was carried out to evaluate the physicochemical parameters, phytochemical constituents, total phenolic and flavonoid content, and antioxidant potential of various extracts of *Cassia spectabilis*.

The physicochemical evaluation (Table 1) provides preliminary information regarding the purity and quality of the crude drug. The total ash value (4.86%) and acid-insoluble ash (1.52%) were within the acceptable range, indicating minimal contamination with inorganic matter such as silicates and oxides. The water-soluble ash (6.25%) suggested the presence of water-soluble inorganic salts, whereas the low moisture content (1.52%) indicated good stability and reduced risk of microbial contamination during storage.

The extractive values (Table 2) showed that the aqueous extract (11.47 % w/w) yielded the highest extraction, followed by the ethanolic extract (7.23 %). This suggests that the majority of phytoconstituents in *Cassia spectabilis* are polar in nature. The comparatively lower yield from chloroform (1.25 %) and ethyl acetate (2.62 %) extracts indicates a lesser presence of non-polar compounds.

The phytochemical screening results (Table 3) revealed the presence of important bioactive compounds such as flavonoids, phenols, saponins, tannins, and carbohydrates in ethanolic and aqueous extracts, whereas ethyl acetate extract showed the presence of glycosides and flavonoids. Chloroform extract displayed limited chemical diversity, confirming that most of the biologically active constituents are polar. The presence of these phytochemicals is responsible for various pharmacological activities, especially antioxidant and anti-inflammatory properties. The statistical parameters related to the standard curves of Gallic acid and Quercetin (Table 4) indicate the reliability and accuracy of the calibration data obtained during the quantitative estimation of total phenolic and flavonoid content, respectively. Both standard compounds exhibited a Beer's law range of 5–25 µg/ml, demonstrating linearity within the selected concentration range.

The regression coefficient (R^2) values were found to be 0.999 for Gallic acid and 0.998 for Quercetin, which are very close to 1, confirming an excellent linear correlation between concentration and absorbance.

The regression equations obtained for Gallic acid ($y = 0.034x - 0.000$) and Quercetin ($y = 0.043x + 0.023$) describe the direct proportionality between absorbance and concentration.

Quantitative analysis (Table 5) demonstrated that the ethanolic extract had the highest total phenolic (0.75 mg GAE/100 mg) and flavonoid (0.96 mg QE/100 mg) content, followed by the aqueous extract. This higher concentration of phenolics and flavonoids in ethanolic extract correlates well with its enhanced antioxidant capacity.

The antioxidant activities were evaluated using DPPH (Table 6), hydrogen peroxide (Table 7), and nitric oxide (Table 8) radical scavenging assays. The ethanolic extract exhibited concentration-dependent scavenging activity, with IC_{50} values of 71.51 µg/mL (DPPH), 278.39 µg/mL (H_2O_2), and 68.25 µg/mL (NO), compared to the standard ascorbic acid which showed stronger inhibition at lower concentrations. Although the ethanolic extract was less potent than ascorbic acid, its significant activity indicates the presence of effective antioxidant compounds capable of neutralizing free radicals. The results (Tables 1–8) indicate that *Cassia spectabilis* possesses potent antioxidant potential, which may be attributed to the synergistic effect of its phenolic and flavonoid constituents. These findings support the traditional medicinal use of *Cassia spectabilis* and warrant further pharmacological and chemical investigations to isolate and characterize the active compounds responsible for these effects.

Table 1: Physicochemical test of *Cassia spectabilis*

S. No.	Parameters	Value (% w/w)
1	Total ash	4.86
2	Water soluble ash	6.25
3	Acid insoluble ash	1.52
4	Moisture Content	1.52

Table 2: Extractive values of *Cassia spectabilis*

S. No.	Extracts	% Yield (w/w)
1.	Chloroform	1.25
2.	Ethyl acetate	2.62
3.	Ethanolic	7.23
4.	Aqueous	11.47

Table 3: Result of phytochemical screening of extracts of *Cassia spectabilis*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanolic extract	Aqueous extract
1.	Alkaloids Wagner's Test: Hager's Test:	-ve -ve	-ve -ve	-ve -ve	-ve -ve
2.	Glycosides Conc. H ₂ SO ₄ Test:	-ve	+ve	-ve	-ve
3.	Flavonoids Lead acetate Test: Alkaline test:	-ve -ve	+ve -ve	+ve +ve	+ve +ve
4.	Diterpenes Copper acetate Test:	-ve	-ve	-ve	+ve
5.	Phenol Ferric Chloride Test: Folin Ciocalteu Test:	-ve -ve	-ve -ve	-ve +ve	+ve +ve
6.	Proteins Xanthoproteic Test:	+ve	+ve	+ve	+ve
7.	Carbohydrate Fehling's Test: Benedict's Test	-ve -ve	-ve -ve	+ve -ve	+ve +ve
8.	Saponins Froth Test:	-ve	-ve	+ve	+ve
9.	Tannins Gelatin test:	-ve	-ve	-ve	+ve
10.	Sterols Salkowski Test:	-ve	-ve	-ve	-ve

+ve= present, -ve=negative

Table 4: Statistical parameters related to standard curve of Quercetin and Gallic acid

Parameters	Gallic acid	Quercetin
Beer's Law Range	5-25 µg/ml	5-25 µg/ml
Regression Coefficient	R ² = 0.999	R ² = 0.998
Regressed line equation (y = mx + c)	y = 0.034x - 0.000	y = 0.043x + 0.023

Table 5: Estimation of total phenol and flavonoids content of *Cassia spectabilis*

S. No.	Extracts	Total phenol content	Total flavonoids content
		Gallic acid equivalent (GAE) mg/ 100 mg	Quercetin equivalent (QE) mg/ 100 mg
1.	Ethyl acetate	-	0.21
2.	Ethanollic	0.75	0.96
3.	Aqueous	0.62	0.83

Table 6: % Inhibition of ascorbic acid and ethanolic extract of using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	46.25	29.57
2	20	51.69	32.46
3	40	62.78	40.29
4	60	67.05	43.97
5	80	75.91	54.14
6	100	89.34	61.25
IC ₅₀ value		15.91	71.51

Table 7: % Inhibition of ascorbic acid and ethanolic extract of using hydrogen peroxide method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	100	49.79	31.54
2	200	64.87	40.98
3	300	75.03	52.76
4	400	85.41	62.54
5	500	93.56	73.81
IC ₅₀ value		80.55	278.39

Table 8: % Inhibition of ascorbic acid and ethanolic extract of using Nitric oxide method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	20	40.25	27.35
2	40	65.78	37.91
3	60	70.75	49.58
4	80	79.24	56.74
5	100	86.46	60.89
IC ₅₀ value		25.08	68.25

CONCLUSION

The present investigation concludes that *Cassia spectabilis* possesses significant phytochemical richness and antioxidant potential. The physicochemical evaluation confirmed the quality and purity of the crude drug, while extractive value analysis indicated that polar solvents, particularly water and ethanol, are more efficient in extracting bioactive constituents. Phytochemical screening revealed the presence of important secondary metabolites such as flavonoids, phenols, saponins, tannins, and carbohydrates, which are known for their therapeutic benefits. Among all extracts, the ethanolic extract showed the highest concentration of phenolic and flavonoid compounds and exhibited remarkable free radical scavenging activity in DPPH, hydrogen peroxide, and nitric oxide assays.

These findings suggest that *Cassia spectabilis* is a promising source of natural antioxidants that could play a key role in preventing oxidative stress-related diseases. The study provides a scientific basis for the traditional medicinal use of this plant and encourages further research on isolation, characterization, and pharmacological evaluation of its active constituents for potential therapeutic applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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CERTIFICATE OF PARTICIPATION



THIS CERTIFICATE IS PRESENTED TO

Seema Wadhwani

From **FACULTY OF PHARMACY, P.K. UNIVERSITY SHIVPURI M.P.**

For his/her active participation in **Online Mode** in the **International Conference** on
“Recent advances in Drug Delivery Systems” (ICDDS-2025) organized by **GCRG**
COLLEGE OF PHARMACY, Lucknow (U.P.) India on **Saturday, 22ND**
February 2025. He/She also presented a paper/poster entitled

PHYTOCHEMICAL SCREENING, QUANTITATIVE ESTIMATION, AND ANTIOXIDANT ACTIVITY
OF CASSIA SPECTABILIS EXTRACTS

PROF.(Dr.) DINESH CHANDRA
PRINCIPAL & CONVENOR
GCRG COLLEGE OF PHARMACY,
BKT LUCKNOW



PROF. A.N. SINGH
DIRECTOR GENERAL, GCRG
GROUP OF INSTITUTIONS,
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**2ND INTERNATIONAL CONFERENCE ON SUSTAINABILITY AND GREEN TECHNOLOGY
(ICSGT 2025)**

Certificate of Participation

This is to certify that Dr./Mr./Ms. **Seema Wadhwani** affiliated to **Faculty of Pharmacy, PK University Shivpuri (M.P)** has presented titled **Exploring the Phytochemistry and Pharmacological Significance of Cassia Spectabilis** in the 2nd International Conference on Sustainability and Green Technology (ICSGT - 2025) organized by FS University, Shikohabad, Uttar Pradesh, India, held during 10th & 11th October 2025.

Organising Secretary
ICSGT - 2025, FS University

Conference Chair &
Director General, FS University