Formulation and Evaluation of Polyherbal Gel for Wound Healing Potential

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. 161522416251)

Under the Supervision of

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Date: **(Amit Kumar)**

Place: Shivpuri (M.P.)

LIST OF ABBREVIATIONS

Abbreviations	Indication
g	Gram
mol	Molecular weight
w/v	Weight by volume
w/w	Weight by weight
mm	Millimeter
°C	Degree centigrade
M.p.	Melting point
h	Hour/s
ml	Mili litre
pH	Negative Logarithm of hydrogen ion concentration
min	Minute/s
%	Percentage
nm	Nanometer
ml	Mili litre
µg	Microgram
cm	Centimeter
mg	Milligram
w/v	Weight by volume
q.s	Quantity sufficient
rpm	Revolution per minute
mV	Millivolt
keV	Kilovolt

TEM	Triethanolamine
MP	Methylparaben
PP	Propylparaben
PVP	Polyvinylpyrrolidone
NaBH4	Sodium borohydride
AgNO3	Silver nitrate
H2SO4	Sulfhuric acid
NaOH	Sodium hydoxide
HCl	Hydro chloric acid
DMSO	Dimethyl sulphoxide
MIC	Minimum inhibitory concentration
FT-IR	Fourier Transform Infrared
SEM	Scanning electron microscope
EDS	Energy dispersive spectrometer
XRD	X-ray Diffraction
Uv	Ultra-violet
SI. No.	Serial Number
ICH	International conference onharmonization
RH	Routh-Hurwitz
SD	Standard deviation
CDR	Cumulative percentage drug release

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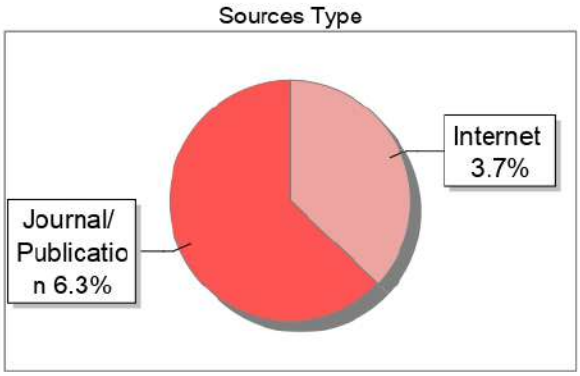

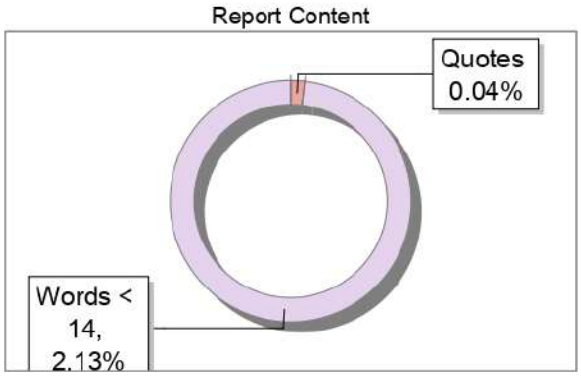

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ABSTRACT

Wounds are caused by skin injuries that disrupt the other soft tissue. The lengthy and intricate process of tissue remodelling and repair in response to injury is what is known as wound healing. Many plant-based treatments have been used to treat wounds over the years. A literature review and ethano-botanical usage led to the selection of *M. oleifera, O. sanctum, and A. indica*. Standard protocol was followed to do a preliminary physiochemical and phytochemical investigation on the crude medication. Additionally, the DPPH & FRAP method was used to check the antioxidant effectiveness, which demonstrated the effectiveness of ethanolic extracts of the chosen plant for strong antioxidant activity. Polyherbal formulations with phytopharmaceuticals for treating skin wounds through both anti-inflammatory and antibacterial action have been developed using ethanolic leaf extract from *M. oleifera, O. sanctum, and A. indica*. Plants are a rich source of plant phenolics and flavonoids, it has also been found. The formulation of the polymer carbopol 940 showed satisfactory compatibility. This study shows that polyherbal formulations with varying amounts of plant active ingredients are feasible. Though F1 & F3 have shown the best results when compared to other formulations. The antioxidant capacity of ethanolic leaf extracts was assessed using the DPPH technique and the ferric reducing antioxidant power test. The results of these techniques validate the antioxidant action. The anti-inflammatory effect of several formulations was assessed using the traditional Oleogel paw oedema method, and their potential for wound healing was assessed using a number of factors, including as wound contraction, tensile strength, and hydroxyproline content. A further proof of the scientific validity of the aforementioned activity was provided by *in-silico* molecular docking for the explanation of the postulated mechanism of action.

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CHAPTER - 1
INTRODUCTION

A sizeable section of the Indian population relies on traditional medical practices to maintain their physical and mental health. These practices are essential. Whether it is due to the pharmacological properties of medicinal plants or just folklore, the preservation of medicinal plants and the traditional applications of these plants have become the subject of significant research. Herbalism has been the subject of various medicinal claims made by Ayurveda, which is an ancient and conventional medical system that originated in India. On the other hand, it is of the utmost importance to conduct methodical research on medicinal plants that have been used in the past, with a particular focus on the traditional medical system. The past ten years have seen an increase in the use of plants as medicinal substances. Poultices, tinctures, powders, and several other herbal preparations were the prevalent forms of herbal medicines in traditional Chinese medicine. Techniques for treating particular illnesses with particular plants were passed down from generation to generation through word of mouth. In the realm of herbal medicine, there is a wealth of information regarding plants that have medicinal properties. In order to initiate the herbal course, it is common practice for a naturalist, botanist, pharmacologist, or plant ecologist to collect and identify the plants gathered. There is a possibility that collections of such plants contain species that have been identified as having proven biological activity but from which the active ingredient has not been removed. When gathering valuable plants in that country, it is imperative that you adhere to the regulations that govern intellectual property at that time. As a first step in the process of isolating and characterizing the active compounds, phytochemists, also known as natural product chemists, generate extracts from the plant materials and then subject these extracts to biological screening utilizing a variety of pharmacological tests. Ayurvedic and other traditional medical practices in India make use of around 1300 different plants for medicinal purposes in order to produce therapeutic drugs. According to the findings of previous studies, a herbal drug is an essential component of all Western medical systems. The year 2002, Mukharjee Plants provide humans with the fundamental need for sustenance, clothing, and shelter. (Baqar, 2001) Not only do medicinal and aromatic herbs have a significant commercial worth, but they have

also proven extremely important in alleviating human suffering. According to Girach et al. (2003), since ancient times, both organized cultures (such as Ayurveda and Unani) and disorganized cultures (such as folk, tribal, and aboriginal) have utilized plants as medicinal agents. It is common practice to find the pharmacological qualities of medicinal plants through a process of trial and error; but, in order to develop new medications that are up to the standards of modern medicine, it is necessary to meticulously investigate these properties. This area of research is mostly focused on the following:

- Determining the medicinal plants' active ingredients and researching the extracts to make sure they're secure, efficient, and always active.

- Isolation of active principles and identification of their structures to enable their synthesis and structural modification to have an efficient pharmacological impact. 2002's Mukhajee

In the process of preparing extracts from plant materials, phytochemists, also known as natural product chemists, put such extracts through biological screening to determine whether or not they have any pharmacological effects. It is next necessary to isolate the active compounds from one another and characterize them according to the chemical composition of their constituent parts. The traditional medical system asserts that a great number of medicinal plants has qualities that include antibacterial, anti-inflammatory, and wound-healing action.

However, despite the fact that only a limited number of these plant therapies have been subjected to a comprehensive investigation by scientific researchers regarding their mechanisms of action and effectiveness, they have been utilized since ancient times in both single plant and polyherbal mixtures. In spite of the fact that there are a great number of synthetic pharmaceuticals available for the treatment of wounds, herbal medications are more widely used since they are less expensive and have fewer or no bad effects.

1.1 Predominance of wound, inflammation and microbial Infection

According to Sandhya et al. (2011), the incidence of acute wounds in the community is approximately twice as high as the prevalence of chronic wounds, which is 4.5 per 1,000 people. Acute wounds occur 10.5 times for every 1,000 individuals. The use of plant-based

items as wound healers is popular because of the broad availability of these goods and the power they possess in their raw form.

A lack of cleanliness in impoverished countries is one of the primary reasons why wound infections are among the most prevalent illnesses. Skin epithelial injuries have the potential to alter the form and function of the tissues that lie beneath the skin. There are a number of potential causes of wounds, including abrasions, rips, contusions, and hematomas. Based on the severity of the injury, the healing process for a wound can take anything from a few days to several weeks. An inflammatory phase, a proliferative phase, and a regenerative phase are the three stages that make up the healing process. Each of these stages has an impact on the thickness of the scar tissue as well as its appearance. Wounds are injuries that occur in the skin and soft tissues. According to Harsh Mohan (2006), an inflammatory reaction takes place after a traumatic event. According to Robin et al. (2005), damage to tissues can lead to necrosis and destruction of the tissue.

Burns caused by chemical, thermal, or electrical stimulation are just a few examples of the many different types of exposure that can cause harm.

The following are examples of common causes of ulcers: 2002 David (David)

Bites from animals or stings from insects; trauma (new or recurring); burns to the scalp or body; pressure; surgical intervention—vascular, arterial, venous, or mixed; and burns to the head or upper body.

- Contamination by microbes.

Two categories of wounds exist:

1. Acute wounds

2. Persistent wound.

The causes of acute wounds, the lengths of time it takes for wounds to heal, and the environments that surround wounds are tremendously different from those of chronic wounds (Childress, 2002).

(1) Acute Wounds

According to Soni et al. (2009), acute wounds often go through four distinct stages that are continuing and overlapping with one another as they heal. These stages are hemostasis, inflammation, hyperemia generation, and regeneration that occur simultaneously.

Acute wounds are defined as cuts that are sharp or wounds that occur suddenly and quickly on the skin. In the aftermath of an injury, damaged or wounded cells and platelets secrete cytokines and growth factors, which initiate an inflammatory response.

(2) Chronic Wounds

Ongoing irritants, such as ischemia or repeated tissue injury, are the key factors that lead to the development of chronic wounds. In the event that the wound does not go through the regular stages of healing, it may become fibrous during one of those stages, which can lead to the development of a chronic wound. An injury that has occurred "has not progressed in an ordered and timely manner to achieve anatomical and functional integrity, or has undergone repair without producing long-lasting anatomical and functional consequences, is thus classified as a chronic wound."(Douglas V 2002).

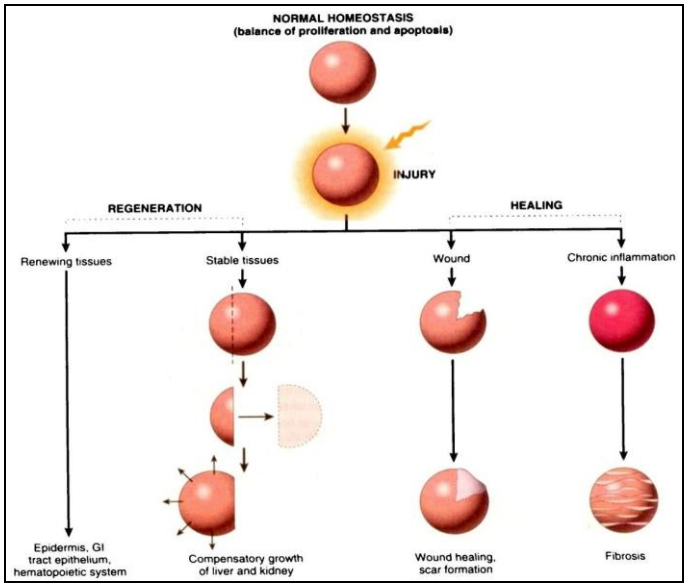

Fig. 1: Normal Responses to Tissue Injury (Robins, 2005)

The probable responses occur during following tissue injury.

1. Normal Repair
2. Regeneration
3. Excessive Healing
4. Deficient Healing.

Normal Repair	The equilibrium between scar remodelling and scar formation is restored by this reaction. This is a typical response that most victims of injuries exhibit. Regeneration of heated, harmed, or dead cells is what is happening here.
Regeneration	Complete rebuilding or replacement of the original tissues is what it entails (Harsh Mohan, 2006).
Excessive Healing	Extraordinary Healing Too much connective tissue is deposited as a result of excessive healing, which alters its structure and renders it ineffective. Over healing manifests as stenosis, fibrosis, contracture, and adhesions. Fibrosis is defined as the excessive and non-functional deposition
	of scar tissue that replaces normal structural tissue components. This is perhaps the most important fibrosis biomarker. Excessive scarring is associated to a variety of clinical conditions. Consider skin hypertrophy and keloid scarring, tendon adhesions, transmission blockages after nerve injury, scleroderma, Crohn's disease, urethral strictures, esophageal strictures, capsule strictures around breast implants, liver cirrhosis, atherosclerosis, and fibrous nonunion of bones.
Deficient Healing	Fibrosis is the exact opposite of poor healing. This happens when the connective tissue matrix is not correctly deposited. Incomplete healing is exemplified by ulcers that persist for an extended period of time.

For an extremely extended period of time, people from all over the world have utilized various plant species as a source of medicine. The study of plants has been one of the most intensive disciplines of natural product research for a very long time, but it is still a long way from being completed entirely. It is possible to significantly enhance the management and treatment of wounds by making use of plants and extracts from those plants. There is a widespread belief that herbal medicines for wound healing are not only inexpensive and easily accessible, but also safe. This is due to the fact that the use of these remedies seldom

results in hypersensitive reactions. Despite the fact that these natural treatments promote tissue regeneration and healing through a number of mechanisms, traditional medicinal herbs must first go through the process of scientific validation, standardization, and safety evaluation before they can be recommended for the treatment of wounds (Bennet, R.G. etal., 1998). The term "wound" refers to the destruction of the cellular and anatomic discontinuity that exists within a tissue. The formation of a wound may be the result of an insult to the tissue that is chemical, physical, thermal, microbiological, or immunological in nature. According to Ingold, W.M. et al. (1994), wounds are not only unpleasant but also come with an increased risk of developing infections and other undesirable effects. Diabetes, immune system problems, ischemia, starvation, ageing, local infections, and local tissue damage from burns or gunshots are some of the types of conditions that usually cause delays in the healing process of wounds. Infections are the most common adverse effects of burn injuries, and they are responsible for between fifty and seventy-five percent of deaths that occur in hospitals (Mokadas, E. et al., 1998). An organized sequence of events that restore the integrity of the damaged tissue is what constitutes the process of wound healing. As a result of the fact that many of the synthetic drugs that are now being used to treat wounds are not only expensive but also present problems such as allergic reactions and drug resistance, researchers have been looking for alternatives (Sai, K. P. etal. for 1998). In order to treat their illnesses, more than eighty percent of people around the world continue to use conventional pharmaceuticals. For wound management in particular (Kumara, P.D. et al., 2001), because they encourage the formation of an ideal environment by supplying a moist atmosphere, they are particularly useful. In spite of the fact that the mechanisms of action and efficiency of a great number of medicinal plants have not been thoroughly researched, the conventional medical system asserts that these plants can assist in the healing of wounds. A multitude of mechanisms, many of which are based on the reactions of connective tissue, are responsible for the healing process of a wound in a healthy body. The initial stage of this process is an acute inflammatory phase, which is then followed by the creation of collagen and other extracellular macromolecules, which are then changed to produce a scar (Chitra P. et al., 1998). This process is broken down into several stages. Any injury to the skin that brings to tearing, slicing, burning, or puncturing is considered a wound. There are a number of natural mechanisms that assist the body in healing wounds at

the site of injury. These include keratinocytes, white blood cell fibroblasts, and others. According to Ranonnsky AJ et al.'s research from 1980, an increase in resting energy expenditure (RES) leads to an increase in the metabolism of carbohydrates, lipids, and proteins. A number of other conditions, such as diabetes, as well as anti-cancer medications and treatments, can have an impact on the healing process of wounds. For reasons related to inadequate cleanliness, wound infections are among the most prevalent types of illnesses in nations with low incomes. When it comes to repairing broken anatomical continuity and the functional state of the skin, having a wound healing approach that is effective is absolutely necessary. The term "wound" refers to any physical injury that makes an opening or breaks the skin. According to Enoch S. et al. (2005), a wound is defined as a break in the epithelial integrity of the skin. This break can be caused by a contusion, hematoma, laceration, or abrasion, and it alters the shape and function of the normal tissue that lies beneath the skin. As a wound heals, it goes through three distinct stages: the inflammatory phase, the proliferative phase, and the remodelling phase, which determines the strength of the healed tissue as well as its appearance. According to Sumitra M et al. (2005), the healing process starts at the moment of injury and can continue for a range of times during the course of the healing process, depending on the severity of the lesion. Seventy percent of the medicines used in Ayurvedic wound therapy are derived from either plants or minerals, while ten percent come from animal components. It is believed that these medications are effective in treating a variety of conditions, such as Vrana (wounds or ulcers), Nadivrana (sinuses), Vidradhi (abscess), Visarpa (erysipelas), Upadamsha (syphilitic ulcers), and Vranajakri (Biswas TK et al., 2003).

Numerous plants, such as Aloe vera, Azadirachta indica, Carica papaya, Celosia argentea, Centella asiatica, Cinnamomum zeylanicum, Curcuma longa, Nelumbo nucifera, Ocimum sanctum, and Phyllanthus emblica, have been shown to be effective in the treatment of wounds, according to extensive research conducted by Ayurveda, Siddha, and Unani medical systems. Around the world, notably in India and China, there are a great number of traditional healers that have deep knowledge of a great number of wild plants that were previously unknown for the purpose of treating wounds and burns. The investigation of agents that promote wound healing is currently considered to be one of the most promising fields of biomedical science (Kumar B et al. 2007). Ancient medical

methods that have been utilized for generations in Asia and Africa to treat wound-related ailments are currently the subject of research that is being carried out by scientists (Krishnan P; 2006). Traditional medical techniques from all over the world have typically involved the application of a variety of medicinal herbs, extracts, or a mix of the two to wounds in order to treat them topically.

Ancient history of wound healing plants

People in the past experimented with various plants to see which ones could be used to treat certain diseases. Particularly when the animals were ill, they probably kept track of the vegetation the animals consumed. People started using thousands of plants as treatments for illnesses after centuries of trial and error. For instance, several Native American cultures employed the bark of the willow tree to treat rheumatism. Scientists have discovered that willow bark contains a painkiller identical to that found in aspirin, although it is unknown how it was selected. Many of the therapeutic plants that early man discovered are still in use today. Heart illness can be treated with the leaves of Digitalis foxglove. Malaria has long been treated with quinine, a substance that is produced from the bark of the cinchona tree in South America. Indians from South America used curare, a potent poison, to poison the tips of their arrows to treat anesthesia-causing diseases and muscle spasms. Rauwolfa is a Southeast Asian native plant whose root is used to treat high blood pressure. It has been used for a very long time to treat anxiety, sleeplessness, and fever. Belladonna and the atropine it produces are crucial in the treatment of ailments such as eye disorders, painful convulsive conditions, and others. One of the few is ephedrine, which is found in hay fever and nasal treatments.

Conifers are used to produce it. Surgical dressings contain sphagnum. The most significant drug discovery of the 20th century was the discovery of mold-produced antibiotics. Vitamin supplies are abundant in plants. Some pharmaceuticals are poisonous and violently pressed. Men had recorded knowledge of plant protective agents by the time of the great civilizations of ancient China, India, Babylon, and Egypt, which occurred around 4500 years ago. These records in writing are known as "herbals." It is likely that the original herbs were known. It has a goal value that takes up around 250 plants. The oldest evidence of the use of plants as medicine appears to be references to their healing properties in

several parts of the Rigveda in India. However, the Rigvedic plant references are relatively brief. You can use Atharva-more veda's in-depth accounts. The Rig Veda is said to have been written between 3500 and 1800 BC. There is little evidence available concerning the advancement of this science in India following the Vedas (approximately 1000 years). One of the first works on Indian medicine is the Charaksamhilla (1000 BC).

We provide usage data on more than 340 herbal products. The origin of some of these medications may not be in India. Carvings found on the walls of tombs and temples in Egypt show that the herb was utilised as medicinal.

More than 800 cures for anything from headaches to heart problems, sore throats to bug stings were included in a lengthy text that dates from as early as 3000 B.C. to 1500 B.C. About 2500 plant species are recognised in India, while 700 species and 1400 years of Nepalese history are recognised in Sri Lanka. 200 plant species are thought to be drug-producing. There are 85 medications listed in the Indian Pharmacopoeia of 1966 that use components from pharmaceutical preparations. Over 5,700 traditional medicines are listed in China's Pharmacopoeia, the majority of which have vegetable roots. It is believed that 200 persons are the sources of several weapons that have animal origins among India's 2,000 pharmaceuticals used to treat human diseases. The remainder, or around 1500, are plant-based. This number is not particularly high considering the size of our nation and the variety of flora that may be found there (Ayyanar M B etal., 2009).

Anatomy of skin

Among the many critical activities that the skin is responsible for, some of the most important ones include protection, thermoregulation, immunological response, biochemical synthesis, sensory perception, and social and sexual communication. For the purpose of treating dysfunction in one of these processes, it is possible to make use of chemical agents that can be applied topically, intralesionally, or systemically, as well as physical agents that can be applied to the skin, such as ultraviolet and ionizing radiation. The protection it offers against heat, light, damage, and illness is another beneficial feature. The characteristics of the skin, such as its thickness, color, and texture, differ depending on the bodily component. If we take the soles of the feet as an example, we can see that they do not

contain as many hair follicles as the head does. The palms and soles of the feet are also significantly thicker than the rest of the shoe. The two-way flow of water and electrolytes prevents either their absorption or loss. Individuals in the past conducted tests with a broad variety of plants in attempt to determine which of these plants may be utilized to treat various diseases. They probably kept a list of the plants that the animals consumed, especially when the animals were sick. This was especially true when the animals were sick. Over the course of several centuries, people started using thousands of different plants as treatments for a wide range of illnesses. For instance, the bark of the willow tree was utilized by a variety of Native American civilizations as a therapy for rheumatism due to its anti-inflammatory properties. A painkiller that is identical to that which is contained in aspirin has been identified by scientists in willow bark; however, the process by which this painkiller was picked is unknown. Scientists have discovered that willow bark contains a painkiller. Early humans were responsible for the discovery of a great number of plants that are in use today for medical purposes. By utilizing the leaves of the Digitalis foxglove plant, it is possible to cure illnesses that affect the heart. Quinine, which is derived from the bark of the cinchona tree in South America, has been utilized for the treatment of malaria for a very long time. Quinine is extracted from the bark of the tree. They employed curare, a potent poison, to poison the points of their arrows in order to heal diseases that caused them to go asleep and to have muscle spasms. The South American Indians were the ones in question. Utilizing the root of the Rauwolfa plant, which is indigenous to Southeast Asia, is a method of treating hypertension. For a very long time, it has been known to be effective in treating a variety of diseases, including anxiety, inability to sleep, and fever. Belladonna and the atropine that it produces come together to offer a substantial contribution to the treatment of a wide range of ailments, including eye disorders, painful convulsive syndromes, and other conditions. When it comes to the treatment of hay fever and nasal congestion, ephedrine is one of the few drugs that should be considered.

Utilization of conifers is required in order to produce it. One of the components that makes up surgical dressings is scrophnum. The most significant discovery that had place in the realm of medicines during the 20th century was the discovery of antibiotics that were produced by mold. Plants contain a large quantity of vitamin supplies compared to other sources. Quite a few pharmaceuticals are not only poisonous but also subjected to high

levels of pressure. People had already documented their knowledge of plant-based compounds that provide protection by the time the great civilizations of ancient China, India, Babylon, and Egypt emerged, which occurred roughly 4500 years ago. These civilizations were devoted to the cultivation of plants. In this context, the phrase "herbals" refers to the written and documented records. There is a good chance that the folks were already familiar with the original herbs. It takes approximately 250 plants to accomplish the goal of achieving the objective value of this item. It would appear that the earliest evidence of the use of plants as medicine can be found in the Rigveda, which is a text from India. Different sections of the Rigveda contain references to the therapeutic advantages of plants, and these references may be found in various sections. The Rigvedic references to plants, on the other hand, are only a few sentences long combined. The Atharva-more Veda offers you the opportunity to make use of the more in-depth accounts that it provides. Handwritten versions of the Rig Veda are believed to have been created between the years 3500 and 1800 B.C. Very little information is currently accessible with regard to the development of this science in India following the Vedas, which occurred roughly one thousand years ago. This is because there is very little information available. It is generally agreed that the Charaksamhilla, which was composed in the year 1000 B.C., is one of the earliest written works on Indian medicine.

We provide information on the utilization of more than 340 different herbal products. There is a potential that one or more of these drugs did not originate in India. This is a possibility. Carvings that were found on the walls of tombs and temples in Egypt provide evidence that the herb was utilized for therapeutic purposes. These carvings were discovered in Egypt.

In a lengthy text that dates back to as early as 3000 B.C. to 1500 B.C., there are over 800 therapies for a wide variety of maladies. These treatments include, among other things, remedies for headaches, heart problems, sore throats, and bug stings. Although there are roughly 2500 plant species that are recognized in India, there are additional 700 species and 1400 years of Nepalese history that are acknowledged in Sri Lanka. In India, there are approximately 2500 plant species. According to estimates, there are over 200 different plant species that are capable of producing medications. Regarding pharmaceutical preparations, the Indian Pharmacopoeia from 1966 has a total of 85 medications that comprise

components from pharmaceutical preparations. These medications are classified as pharmaceutical preparations. A reference to more than 5,700 different traditional medicines may be found in the Chinese Pharmacopoeia. The vast majority of these treatments have their roots in vegetables themselves. It is believed that 200 people are responsible for the creation of numerous weapons that have animal origins. This determination is based on the fact that there are 2,000 drugs that are used to treat human disorders. Plants are the source of the remaining portion, which is roughly 1500. According to Ayyanar M. B. et al. (2009), this number is not very high when one takes into consideration the vast region that our nation encompasses and the diverse array of plant species that may be found within it.defense mechanism that the skin possesses. Indicating that the barrier is located in the epidermis' outermost layer, the stratum corneum, chemical substances are able to enter either the isolated stratum corneum or the entire skin with approximately the same level of penetration. Because they are lacking their nucleus and cytoplasmic organelles, the keratinocytes that make up the stratum corneum are unable to hold on to their existence. In keratohyalin granules, the major protein known as filaggrin is responsible for flattening the cells and arranging the fibrous keratin into macrofibrils that are disulfide-bonded. By forming a keratinized envelope through the process of crosslinking its involucres with keratohyalin, each cell generates an insoluble exoskeleton that serves as a rigid framework for the inner keratin filaments. Lamellar lipids that are hydrophobic and come from the membrane-covering granules are responsible for filling the intercellular gap.

Epidermis	The epidermis is the skin's thin outer layer, and it is comprised of up of three parts: • ***Stratum corneum (horny layer)*** Fully developed keratinocytes that contain fibrous proteins make up this layer (keratins). The top layer is constantly being removed. Most foreign substances cannot enter the body through the stratum corneum, and it also stops fluid from leaving the body. • ***Keratinocytes (squamous cells)*** This layer located beneath the stratum corneum contains living keratinocytes (squamous cells) that grow into the stratum corneum. ***Basal layer*** Basal cells make up the innermost layer of the epidermis, known as the basal layer. The old keratinocytes removed from the skin's surface are constantly replaced by new ones produced by basal cells, which divide indefinitely. Melanocytes, which produce melanin, are also found in the epidermis (skin pigment).
Dermis	The dermis is the middle layer of the skin. The dermis contains the following: • Blood vessels • Lymph vessels • Hair follicles • Sweat glands • Collagen bundles • Fibroblasts • Nerves Fibroblasts produce collagen, the protein that holds the dermis together. This layer provides skin flexibility and strength. It also has pain and touch receptors.

Subcutaneous	The middle layer of skin is called the dermis. The following are found in the dermis: vascular system lymph nodes Follicles for hair Sweat ducts bundles of collagen Fibroblasts Nerves The glue that holds the dermis together is collagen, a protein generated by fibroblasts. This layer provides the skin with strength and flexibility. It also includes touch and pain receptors. The sub cutis is the skin's lowest layer. The subcutis, a network of collagen and fat cells, acts as a "shock absorber," minimizing injury and maintaining body heat.

Layers of skin

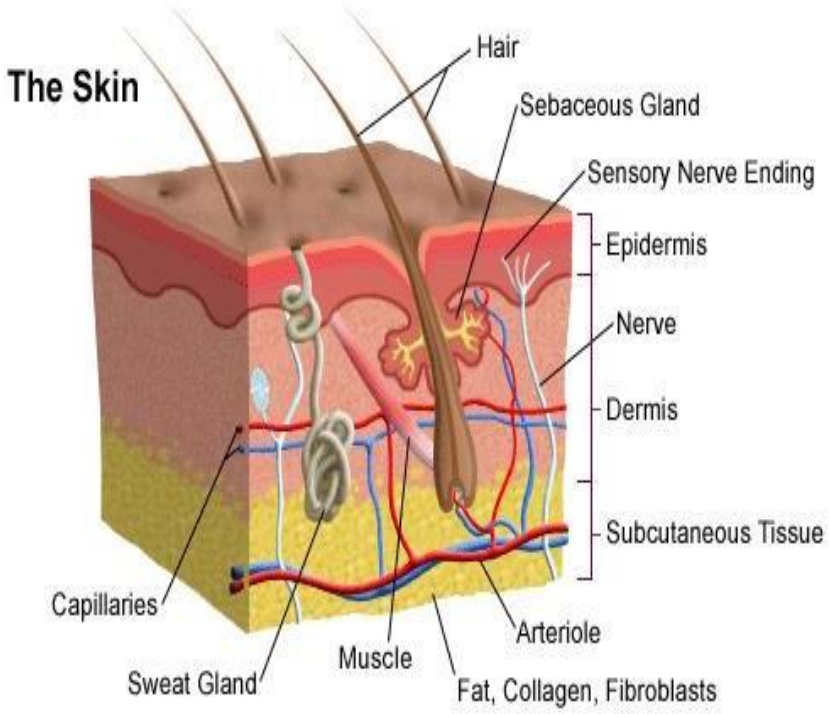

Fig. 2: Structure of Skin

Functions of skin

The stratum corneum, which is the epidermis's outermost layer, is where the barrier is located. This is demonstrated by the fact that the rates of chemical penetration through isolated skin and entire skin are essentially identical. Due to the fact that they have lost their nuclei and cytoplasmic organelles, corneocytes coming from the stratum corneum are no longer viable. Flattening the cells and arranging the fibrous keratins into disulfide cross linked microfibers are both functions of filaggrin, which is the primary protein component of the keratohyalin granule. The formation of a protective envelope around each cell is accomplished through the cross-linking of involucrin and keratohyalin, which results in an insoluble exoskeleton that functions as a rigid scaffold for the keratin filaments that are found inside the cell. Membrane coating granules are the source of hydrophobic lamellar lipids, which are responsible for filling the intercellular spaces. The combination of hydrophilic keratinocytes with hydrophobic intercellular material has the effect of inhibiting both hydrophilic and hydrophobic molecules. (Ebling, F.J.G; 1993).

Pathophysiology of wound

There are several steps involved in the process of wound healing, including coagulation, inflammation, the creation of granulation tissue, matrix generation, connective tissue remodeling, collagenization, and evaluation of the wound's force. The process of wound healing is a dynamic and complex process that restores the continuity and function of the anatomical structure. Hemostasis, inflammation, proliferation, maturation, and vascular response phase remodeling are what make up this precisely planned succession of events that overlap with one another (P Chithra et al., 2006).

Wound healing involves processes such as inflammation, cell type migration, and cell proliferation. Coagulation, which begins as soon as an injury occurs, results in a coordinated flow of neutrophils to the wound site. These cells naturally produce free radicals throughout their respiratory burst process. Certain non-phagocytic wound cells produce radicals via the non-phagocytic NAD(p)H oxidase pathway, resulting in an increase in oxygen and nitrogen near the wound site. The system's exposure to these free radicals causes lipid peroxidation, DNA damage, and enzyme deactivation, including free

radical scavenging enzymes. There are four steps to mending a wound:

(i) Coagulation, which prevents blood loss.

(ii) Inflammation and debridement of wound.

(iii) Repair, including cellular proliferation, and.

(iv) Tissues remodeling and collagen deposition.

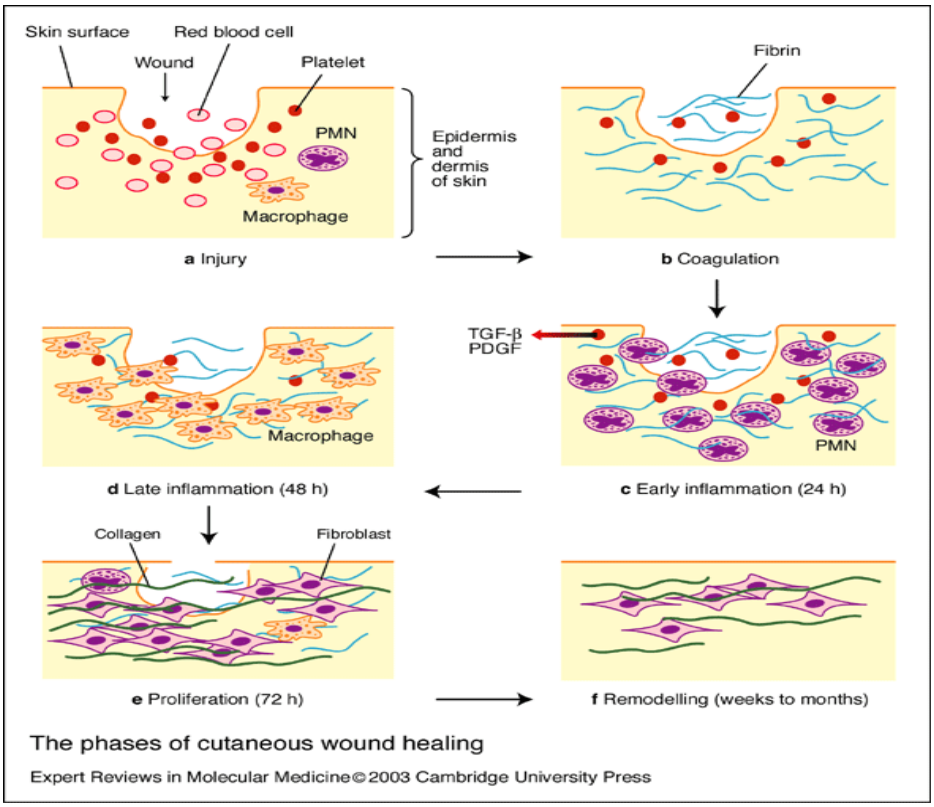

Fig.3: Process of wound healing

Wound healing is divided into three overlapping phases: inflammation, cellular proliferation, and remodelling, all of which are facilitated by continual cell-cell and cell-matrix interaction.

Phase 1-

In this coagulation and inflammatory phase, which lasts for 0–3 days, neutrophils migrate to the fibrin clot at the incision margin.

Phase 2-

During this proliferative phase, which can last anywhere from three to twelve days, the macrophages are the overwhelming majority of the cells that replace the neutrophils. The incision space is gradually encroached upon by granulation tissue, and the incissional space is filled exclusively by granulation tissue. Additional collagen fibrils are formed, and they begin to cover the incision as they spread outward.

Phase 3-

Ongoing collagen accumulation and fibroblast proliferation are both components of this remodelling phase, which can continue anywhere from three to six months and is characterized by its duration. Both the edema and the leukocyte infiltration have markedly diminished respectively. The production of collagen fibers, which results in an increase in the tensile strength of the skin, takes place during this period. For healing to occur, it is necessary for a number of different tissues and cell lineages to collaborate. It is characterized by the formation of fibrin, an inflammatory response to injury, changes to the ground substances, angiogenesis, and re-epithelization, in addition to the aggregation of platelets and the coagulation of blood. Collagen must first form a strong link between the shattered surfaces before the healing process can be considered done. When a wound heals, the wounded tissue returns to its pre-injury state as precisely as possible. When a wound contracts, the size of the wound diminishes. Both of these processes are necessary for the healing process. Substrate phases, proliferative phases, and remodelling phases are the three individual stages that make up the physiologic process of wound healing. A great number of cytokines, including growth factors, are responsible for the exact regulation of all of these activities. It has been discovered that wounds that heal themselves contain a number of these growth factors. These growth factors include, among others, platelet-derived growth factor, transforming growth factor B, fibroblast growth factor, and epidermal growth factor. In order to hasten the healing process in chronic wounds, it is essential to make use of growth-promoting agents or substances that have the ability to enhance the production of these growth factors in situ. (Suresh Reddy J etal., 2001).

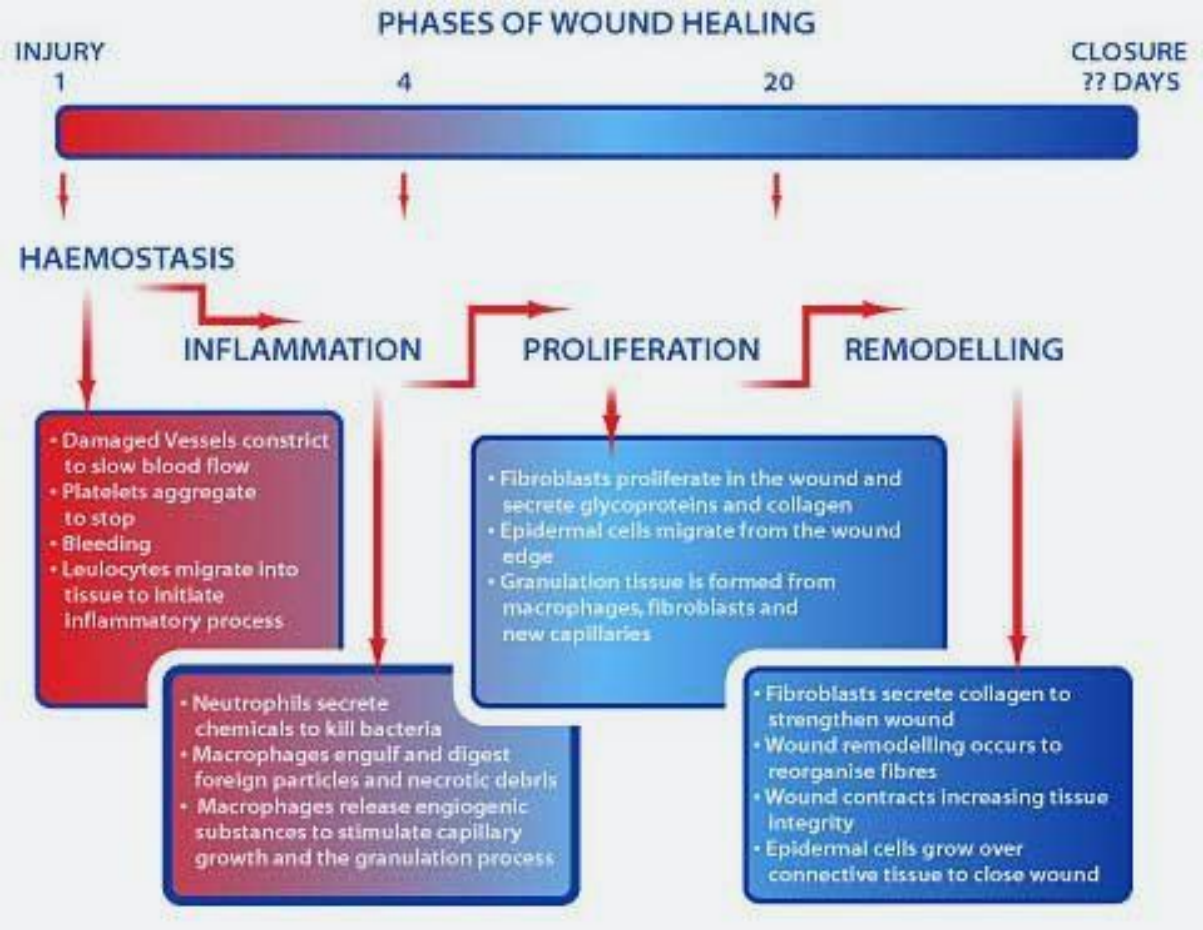

Fig. 4: Phases of wound healing

1.2 Role of Cell Signaling in Tissue injury

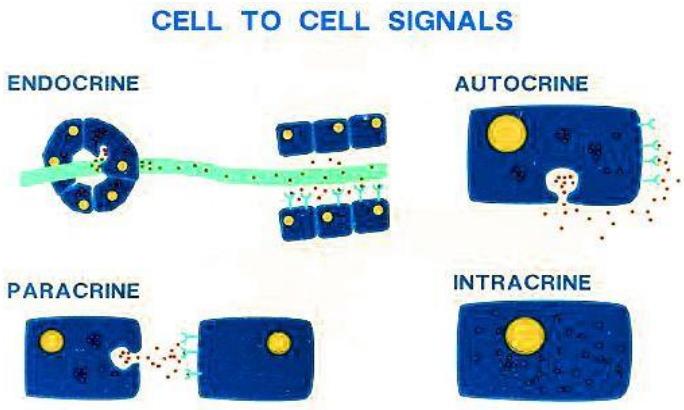

Fig. 5: Cell signaling by cytokine

Fig. 6: The four possible responses following tissue injury

The Healing Cascade

As soon as platelets come into touch with exposed collagen following an injury, the healing cascade begins to take place. The formation of fibrin clots at the site of an injury is a consequence of the release of clotting factors, which occurs when platelets clump together. In addition to providing temporary support, fibrin clots also serve to set the groundwork for subsequent healing processes. Platelets, in addition to the clotting factors that are required to stop bleeding and limit the loss of fluid and electrolytes, also release a series of chemical signals that are known as cytokines or growth factors. These signals are responsible for initiating a healing response. Platelet-derived growth factor (PDGF) and transforming growth factor beta (TGFß) are the two growth factors that have the most significant impact. PDGF is a growth factor that activates macrophages, neutrophils, smooth muscle cells, and fiber acetopes. In addition to this, it promotes the mitogenesis of either smooth muscle cells or fiber aceto cells.

PDGF, TNFA (alpo necrosis), PDGF, TNFA (Alpha necrotic tumor), and IL1 are some of the other factors that are involved in the cascade healing process. TGF is responsible for initiating the cascade healing process and stimulating it to highlight other factors. Additionally, TGF works to improve the chememain of aceta and smooth muscle cells, as well as to modify the expression and synthesis of collagen. This overwhelming signal has a net effect of causing the matrix production cells to respond energetically in order to ensure that new coupling tissues are swiftly deposited at the injured location during subsequent

proliferation. When it comes to cellular markers, neutrophils are the second most prevalent in wounds within the first twenty-four hours after damage. A significant portion of the responsibility for removing foreign materials, infections, dysfunctional host cells, damaged matrix components, and other potential pollutants from the wound site falls on the shoulders of neutrophils. The process of phagocytosis involves the process of bacteria consuming neutrophils, which is a chemical signal that attracts neutrophils to bacteria. The production of bacterial proteins results in the formation of a waste product known as f-MetLeuPhe tripeptide, which is capable of attracting cells that are involved in inflammation. It is possible for the neutrophils in the wound to increase to the point where they are bursting with bacteria. Forty-eight hours after an injury, the formation of wound macrophages is achieved by stimulating fixed tissue monocytes. These specialized macrophages that are involved in wound healing are probably the most important inflammatory cells that are involved in the normal healing response. Restricting the activity of macrophages causes a delay in the healing process. These wound macrophages, when activated, release PDGF and TGFß, which help to attract fibroblasts and smooth muscle cells to the wound site. This process is responsible for the healing process. These highly phagocytic macrophages are also important for removing any bacteria that may still be present at the wound site, as well as non-functional host cells, neutrophils that are loaded with germs, damaged matrix, and foreign debris.

Fig.7: The sequence of events during normal wound healing

Phases of wound healing

PHASES OF INVOLVED HEALING	***DAYS POST INJURY***	***CELLS IN PHASES***
Hemostasis	Immediate	Platlets
Inflammation	Days 1-4	Neutophils
Proliferation Granulation	Days 4-21	Macrophages Lymphocyte Angiocytes Neurocytes
Contracture		Fibroblasts Keratinocytes
Remodelling	Days 21-2 yrs	Fibrocytes

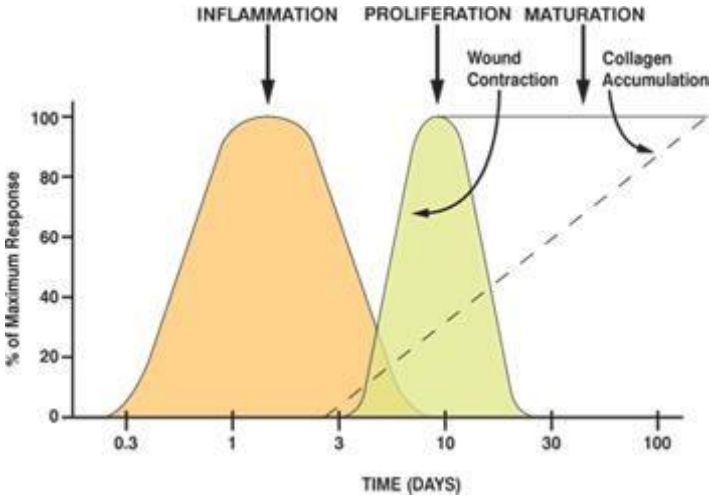

Fig. 8: Graphical representation of wound healing phases

Wound Classification

Wounds can be classified in a variety of ways, including injury type, location, presenting symptoms, depth and tissue loss, and clinical appearance. There are several grading tools available for pressure ulcers (EPUAP), burns (Rule of Nines), diabetic foot ulcers (Wagner / San Antonio), and general wounds.

General injuries are categorized as:

- ❖ Superficial (loss of epidermis only)
- ❖ Partially thick (involve the epidermis and dermis)
- ❖ entire thickness (involve the dermis, subcutaneous fat and sometimes bone)

The most common procedure for classifying wounds is to determine the primary tissue types present on the wound bed, such as black and necrotic tissue, and then compute the proportionate amount of each as a percentage. This classification system encourages continuous review, facilitates appropriate assessment and planning, and is easily visible.

1.3 Types of wound based on injury

1) ***Lacerations*** – Injury involving ripped or cut tissue. To begin the healing process, the tissue is debribed, irrigated, and cleaned of any blood clots and other objects. In order to minimise further crush injury to tissues, a local anaesthetic is supplied, and an atraumatic approach of wound closure is used. For complex extremities wounds, sterile dressings are placed, and immobilization is advised.
2.) ***Abrasions*** – Injury in which only the outermost layer of tissue is lost, as in first-degree burns. Within the first day of the injury, the wound should be cleaned of any foreign objects, often using a scrub brush to avoid painful tattooing by dirt and gravel. While the pain may be managed with local anaesthesia, the wound is treated non-surgically with moist dressings and a topical antibiotic to promote healing.
3.) ***Contusions*** – injuries to the skin and soft tissue that are the result of a hard hit, yet the layer of skin that is on the surface of the body is unaffected. It is often only necessary to provide minor medical attention to these accidents because there is no open wound. It is important to inspect contusions, however, to look for any potential deep-surface hematomas or other types of tissue injury that could indicate a more significant morbidity. It is necessary to remove a growing hematoma since it has the potential to cause damage to the skin underlying.
4.) ***Avulsions*** –Injuries in which a tissue portion is completely or partially torn off. The tissue is raised but still linked to the body in partial avulsions. Total avulsion describes a tissue that has been fully severed from the body and has no remaining attachment. The tissue is carefully cleaned and irrigated in the event of a partial

avulsion where the torn tissue is still well-vascularized and alive, and the flap is reattached to its anatomical place using a few stitches. A skin transplant or local flap is frequently used to close the incision if the ripped tissue is not viable. Total avulsion tissue is frequently exceedingly thick, necessitating debulking and fattening procedures before it can be regrafted. Major avulsions refer to amputation of an extremity, finger, ear, nose, scalp, or eyelid and call for replantation surgery (SP Zinn;2004).

Categories of wounds

A wound can be classified as acute or chronic according to how rapidly it heals. Acute wounds heal smoothly (without complications) and within the expected time frame. Chronic wounds take longer to heal and can have serious effects. An injury might be open or closed. Open wounds occur when the underlying tissue or organs are visible and accessible to the surrounding environment (similar to penetrating wounds). Damage to closed wounds occurs behind the underlying tissue and organs (non-penetrating wounds).

Factors effecting wound healing

One of the many potential causes of impaired wound healing is a variety of different circumstances. In general, two sorts of factors influence repair: local and systemic. Local variables affect the physical aspects of the wound immediately, whereas systemic factors have a detrimental impact on a person's overall health or ability to recover from a condition. There is a close connection between several of these factors, and systemic variables have an effect on wound healing through their local implications.

1. Oxygenation

There are a number of processes that are involved in the healing of wounds, and oxygen is essential for the generation of ATP, which is the source of energy for cells throughout the metabolic process. It reduces the number of wound infections, stimulates angiogenesis, boosts fibroblast proliferation and collagen formation, and promotes wound contraction. Additionally, it promotes keratinocyte differentiation, migration, and re-epithelialization. In addition, the concentration of oxygen has a significant impact on the capacity of polymorphonuclear leukocytes to produce superoxide, which is essential for the oxidative

death of pathogens.

In conclusion, the presence of the optimum amount of oxygen is essential for the speedy healing of wounds. In spite of the fact that oxygen is necessary for the healing process to continue, hypoxia is beneficial to wound healing because it stimulates the generation of growth factors and is associated with angiogenesis. One of the therapeutic methods that has the potential to sometimes reverse the effects of tissue hypoxia is through the use of hyperbaric oxygen therapy. The hyperbaric oxygen therapy (HBOT) treatment is an excellent treatment for hypoxic wounds; nevertheless, it is not widely available.

2. Infections

As a result of an injury to the skin, bacteria that are normally located on the surface of the skin are able to enter the tissues that lie beneath it. In accordance with the degree of infection and the multiplication of microbes, the wound is categorized as having contamination, colonization, local infection/critical colonization, and/or spreading invasive infection. In contrast to contamination, which refers to the presence of organisms that do not replicate on a wound, colonization refers to the presence of bacteria that replicate on a wound but do not cause damage to the tissue. The process of crucial colonization, which is also known as local infection, takes place between the beginning of local tissue reactions and the spread of germs. The presence of replicating organisms that enter a wound and cause damage to the host is an example of an invasive infection.

Inflammation, which is a normal component of the process of wound healing, is necessary for the elimination of microorganisms that have polluted the wound. In the lack of efficient cleaning, however, microbial clearance is limited, which means that inflammation may persist for a considerable amount of time. Both endotoxins and bacteria have the ability to cause pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-), to surge over an extended period of time, hence prolonging the inflammatory phase. If the situation persists, the wound may develop into a chronic condition and cease to heal. As a result of this protracted inflammation, an increase in the synthesis of matrix metalloproteases (MMPs) occurs. MMPs are a type of protease that has the ability to rip down the extracellular matrix (ECM). As the amount of protease in the body increases, the amount of naturally occurring protease inhibitors decreases. Growth factors that are present

in chronic wounds may be eliminated in a short amount of time as a consequence of this change in the equilibrium of proteases. In the same way that other infectious processes do, the bacteria that are present in wounds that are infected produce biofilms. Biofilms are complex communities of aggregated bacteria that are wrapped in a matrix of extracellular polysaccharides that they have secreted themselves. The development of mature biofilms results in the formation of protected microenvironments and a higher level of resistance to conventional antibiotic treatment. It is common to find microorganisms such as Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa), and hemolytic streptococci in wounds that are either clinically infected or clinically not infected. The presence of Staphylococcus and Pseudomonas aeruginosa appears to have a significant impact on the severity of bacterial wound infections. Due to the fact that P. aeruginosa biofilms shield the bacteria from the phagocytic activity of invasive polymorphonuclear neutrophils (PMN), it is highly improbable that many chronic ulcers would heal. This kind of approach could be able to shed some light on the reasons why antimicrobial medicines were not successful in treating chronic wounds.

3. Age

The elderly population, which includes those over the age of sixty, is expanding at a rate that is greater than that of any other demographic. Age is a primary risk factor for poor wound healing. There have been a great number of cellular and molecular research conducted on both humans and animals that have investigated age-related alterations and delays in wound healing. When it comes to healthy senior people, it is common knowledge that the effects of age merely temporarily slow wound healing, and they do not alter the healing process itself. There is a correlation between delayed wound healing in the elderly and an altered inflammatory response. This response includes delayed T-cell infiltration into the wound area, alterations in chemokine synthesis, and a decreased macrophage phagocytic capacity. The processes of re-epithelialization, collagen synthesis, and angiogenesis are considerably slower in aged mice in comparison to younger mice. Younger people and older adults heal wounds in somewhat different ways. A review of age-related changes in healing capacity found that common age-related changes occur at every stage of the healing process. These changes include increased platelet aggregation, increased secretion of inflammatory mediators, delayed infiltration of macrophages and

lymphocytes, impaired macrophage function, decreased secretion of growth factors, delayed re-epithelialization, delayed angiogenesis and collagen deposition, reduced collagen turnover and remodelling, and other similar changes.

4. Sex Hormones in Aged Individuals

As people get older, they experience problems with wound healing, and there is a correlation between sex hormones and these processes. There is a difference in the rate at which males of advanced age heal from acute wounds compared to females of the same age. It would appear that the androgens testosterone and 5-dihydrotestosterone (DHT), in addition to the steroid precursor dehydroepiandrosterone (DHEA), have a significant impact on the process of wound healing. This may be one of the factors that helps to explain this phenomena in particular. Oestrogen is responsible for almost all of the differences in gene expression that are detected between wounds from young people and those from older males, according to research that was carried out not too long ago. Research was undertaken to investigate this phenomenon. Estrogen has a role in the process of wound healing by regulating a number of genes that are engaged in multiple processes, including regeneration, matrix synthesis, protease inhibition, epidermal function, and genes related to inflammation. According to the findings of study, androgens have a negative impact on the process of healing cutaneous wounds, whereas oestrogen has the ability to assist both men and women in overcoming the age-related reduction in healing. This has the potential to be beneficial for both genders.

5. Stress

Personal health and social behavior are both profoundly influenced by stress to a significant degree. The illnesses of diabetes, cancer, impaired wound healing, and cardiovascular disease are some of the conditions that are related with stress or anxiety. A great number of research have demonstrated that stress has a detrimental effect on the homeostasis of the neuroendocrine and immune systems. The pathophysiology of stress, which is predominantly mediated by the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal medullary axis, also known as the sympathetic nervous system, is responsible for the dysregulation of the immune system.

6. Diabetes

Across the globe, diabetes is a condition that affects hundreds of millions of individuals. There is a correlation between diabetes and a slower healing rate for acute wounds. Furthermore, this group has a higher risk of developing diabetic foot ulcers (DFUs), which are ulcers that do not heal and are chronic in nature. It is believed that 15% of all diabetics experience these ulcers. In 84 percent of instances, diabetic foot ulcers (DFUs) come before lower limb amputations connected to diabetes, which indicates that the illness has a significant impact. A multitude of intricate pathophysiological pathways are responsible for the poor wound healing that diabetes patients experience from both diabetic foot ulcers (DFUs) and acute cutaneous wounds. DFUs, venous stasis syndrome, and pressure-related chronic non-healing wounds are all types of wounds that are invariably connected with hypoxia. Insufficient angiogenesis and perfusion can lead to prolonged hypoxia, which is deleterious to the healing process of wounds. Through an increase in the concentration of oxygen radicals, hypoxia has the potential to enhance the early inflammatory response and to increase the duration of the healing process. Hyperglycemia can lead to an increase in oxidative stress when the formation of reactive oxygen species (ROS) exceeds the capacity of the anti-oxidant. A lack of wound healing occurs in diabetic mice as a result of the production of advanced glycation end-products (AGEs) and the interaction of these AGEs with receptors (RAGEs). Metalloprotease levels are at an elevated level in diabetic foot ulcers, and they are approximately sixty times greater in chronic wound fluid than they are in acute lesions. An increase in the activity of proteases leads to the degradation of tissues while simultaneously suppressing the natural healing mechanisms.

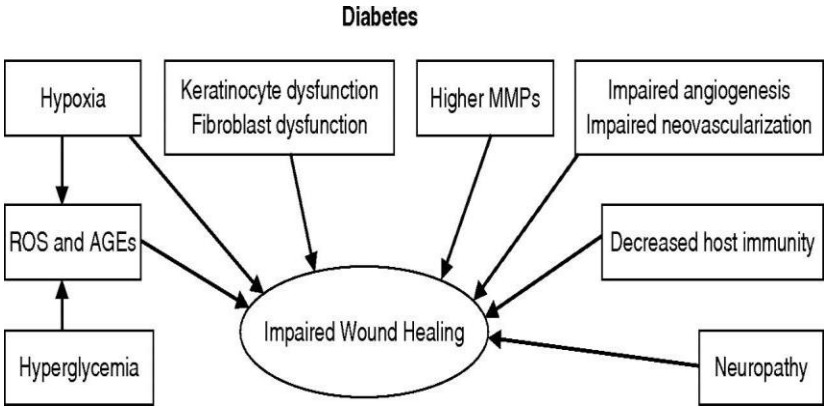

Fig.9: The potential effects of diabetes on wound healing

There are a number of factors that contribute to delayed healing in diabetics. These factors include hypoxia, malfunction of fibroblast and epidermal cells, impaired angiogenesis and neovascularization, higher levels of metalloprotease, damage caused by reactive oxygen species and advanced ageing, diminished host immunological response, and neuropathy.

7. Medications

A wide variety of drugs, including those that interfere with platelet function, clot formation, inflammatory responses, and cell proliferation, have the potential to influence the rate at which wounds heal. The only medications that are discussed in this article are those that are regularly used and have a substantial influence on the healing process. These medications include chemotherapy treatments, glucocorticoids, and nonsteroidal anti-inflammatory prescription drugs.

(i) Glucocorticoid Steroids

It is widely recognized that systemic glucocorticoids (GC), which are commonly used as anti-inflammatory medicines, hinder wound healing by lowering cellular wound responses such as fibroblast proliferation and collagen formation. Additionally, systemic glucocorticoids have been shown to exert broad anti-inflammatory effects. During the healing process, systemic steroids lead to insufficient granulation tissue growth and a reduction in the amount of wound contraction.

(ii) Non-steroidal Anti-inflammatory Drugs

Pain management, inflammation reduction, and the treatment of rheumatoid arthritis are all typical uses for nonsteroidal anti-inflammatory medicines (NSAIDs), which include ibuprofen and other similar medications. The anti-platelet activity of low-dose aspirin makes it a popular choice for usage as a therapy for the prevention of cardiovascular disease; nevertheless, it is not an anti-inflammatory medication. There is a lack of evidence to suggest that taking nonsteroidal anti-inflammatory drugs (NSAIDs) for a short period of time has a detrimental effect on the healing process. The subject of whether or whether the use of nonsteroidal anti-inflammatory drugs (NSAIDs) for an extended period of time slows down the healing process of wounds is still up for debate. There is evidence that ibuprofen can suppress cell growth during the healing process of wounds in animal models. As a consequence of this, there are fewer fibroblasts, slower wound contraction, delayed

epithelialization, and inadequate angiogenesis. Additionally, the breaking strength of the wound is decreased.

(iii) Chemotherapeutic Drugs

The majority of chemotherapeutic drugs seek to inhibit angiogenesis, rapid cell division, and cellular metabolism, so impeding many of the processes required for efficient wound healing. These medications inhibit DNA, RNA, or protein production, reducing fibroplasia and promoting neovascularization in wounds. Chemotherapeutic drugs slow cell entrance into the wound, reduce collagen production, inhibit fibroblast proliferation, and prevent wound contraction. Furthermore, these compounds weaken patients' immune systems, delaying the inflammatory phase of healing and increasing the risk of wound infections.

8. Obesity

There has been a rise in the prevalence of obesity among all age groups in the United States, including adults, children, and adolescents. The findings of a recent poll indicate that thirty percent or more of adults, as well as fifteen percent of adolescents and younger people, are overweight or obese. An increased risk of a wide variety of illnesses and health problems has been linked to obesity. These include coronary heart disease, type 2 diabetes, cancer, hypertension, dyslipidemia, stroke, sleep apnea, respiratory disorders, and delayed wound healing. Obesity has also been linked to an increased risk of delayed wound healing. But these are just a handful of the many examples. Wound complications, including venous ulcers, pressure ulcers, the formation of hematomas and seromas, skin wound infection, and dehiscence, are more likely to occur in obese individuals. Venous ulcers and pressure ulcers are two types of wound complications.

9. Alcohol Consumption

Clinical evidence and animal research show that alcohol usage slows wound healing and increases the risk of infection. Alcohol exposure, whether acute or chronic, is a factor in more than half of all trauma patients treated in emergency rooms, so the influence of alcohol on repair is quite clinically relevant. Alcohol use lowers host resistance, and being intoxicated when injured increases the likelihood of acquiring an infection in the wound. Although the particular effects vary depending on the type of alcohol exposure (chronic vs. acute, amount consumed, period of consumption, time from alcohol exposure, and alcohol

withdrawal), studies have demonstrated that alcohol has a significant impact on host-defense mechanisms.

10. Smoking

Those who smoke have a higher likelihood of developing cardiovascular and vascular diseases, stroke, chronic obstructive pulmonary disease, and a number of different malignancies. To a similar extent, it has been recognized for a long time that smoking has a detrimental effect on the rate at which wounds heal. Following surgical procedures, smokers experience a slower wound healing process, as well as an increase in a number of problems, including infections, wound rupture, anastomotic leakage, wound and flap necrosis, epidermolysis, and a loss in wound tensile strength. Smokers also have a higher risk of developing anastomotic leakage. It has been established that smokers have a shorter healing process, which has been observed in both conventional oral surgery and the installation of dental implants. Plastic and reconstructive surgeons have a tendency to avoid doing cosmetic procedures on smokers since it appears that smokers have lower cosmetic outcomes.

11. Nutrition

It has been known for more than a century that nutrition is an important aspect that plays a role in the healing process of wounds. The most obvious example is how nutritional deficiencies, such as hunger or vitamin deficiencies, can hinder the healing process of wounds following surgery or trauma. Certain types of nourishment are frequently required for patients who are malnourished and who have wounds that are chronic or that heal slowly. All of the following can have an impact on the healing process: the metabolism of vitamins, minerals, proteins, lipids, carbohydrates, and energy.

(i) Carbohydrates, Protein, and Amino Acids

carbs and lipids are the key sources of energy that are depleted throughout the process of wound healing. This is because carbs are the primary sources of energy. Glucose is the primary source of fuel for the production of ATP, which is responsible for delivering energy for angiogenesis and the deposition of new tissues. This process takes place at the cellular level. For the purpose of preventing the depletion of other amino acid and protein substrates, glucose is utilized as a source in order to generate ATP. This is done in order to

accomplish the utilization of glucose. Protein is one of the nutrients that has the most significant influence on the rate at which wounds heal. This is because protein breaks down proteins in the body. It is necessary to consume arginine, an amino acid, during periods of maximum growth, periods of high stress, and periods of damage. A semi-essential amino acid is what it is thought to be. There are a number of distinct ways in which the body reacts to arginine, some of which include the regulation of the immune system's reaction, the healing of wounds, the secretion of hormones, the tone of the blood vessels, and the function of the arterial walls. Since arginine is a precursor to proline, it is necessary for the production of collagen, the process of angiogenesis, and the contraction of wounds. As an additional point of interest, arginine is necessary for the production of collagen. In both healthy and sick persons, arginine not only enhances the performance of the immune system but also has the ability to speed up the healing process of wounds. Arginine supplementation has been shown to be an effective adjuvant therapy for wound healing. This is connected to the fact that the metabolic demand for arginine increases when psychologically stressful events are present. This is the reason why arginine supplementation has been shown to be helpful.

(ii) Fatty Acids

It is common practice to use lipids as a nutritional supplement in order to provide patients who are undergoing surgery or who are very unwell with the ability to meet their energy requirements and to provide the essential building blocks for scar healing and tissue regeneration. Patients will receive support as a result of this situation. As far as polyunsaturated fatty acids (PUFAs) are concerned, there are two distinct forms of polyunsaturated fatty acids that constitute the bulk of the total. These are fatty acids with omega-6 and omega-3 structures. Soybean oil contains omega-6, which is one of the many types of polyunsaturated fatty acids. There are several other types of polyunsaturated fatty acids. On their own, mammals are unable to generate omega-3 polyunsaturated fatty acids. This is because production of these acids is not possible. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are the two forms of omega-3 fatty acids that can be found in fish oil. There are both of these components present in the fish oil's makeup. Recently, there has been a lot of curiosity in the possible health advantages that they can give, and this interest has been steadily expanding over the past several years. Regarding the idea that

omega-3 fatty acids have any kind of impact on the process of wound healing, there is no data to back up this speculation. This is due to the fact that there is the absence of any proof to discover. On the other hand, it has been established that they have an effect on the processes of angiogenesis, cell metabolism, gene expression, and the production of cytokines that reduce inflammation in wound sites. These processes are all influenced by them. The results of the research have provided evidence that supports this assertion. The ability of omega-3 fatty acids to improve the systemic immune response of the host, which in turn enables a reduction in the risk of infectious complications and a rise in the likelihood of survival, may be the most significant benefit of these fatty acids. Omega-3 fatty acids have a variety of positive effects on the body.

(iii) Vitamins, Micronutrients, and Trace Elements

When compared to the antioxidant and anti-inflammatory properties of other vitamins, the antioxidant and anti-inflammatory properties of L-ascorbic acid (vitamin C), retinol (vitamin A), and tocopherol (vitamin E) are greatly boosted due to their high levels of antioxidant and anti-inflammatory qualities. There are a number of detrimental effects that a lack of vitamin C can have on the process of tissue repair. Vitamin C also plays a number of important roles in the process of wound healing. Vitamin C is a crucial component in the process of wound healing. It has been discovered that a lack of vitamin C is linked to a decrease in the production of collagen and the proliferation of fibroblasts, as well as a decrease in angiogenesis and an increase in capillary fragility. These findings point to the fact that vitamin C shortage is associated with these outcomes. There is a lack of vitamin C, which has been related to these negative repercussions, and this shortfall continues to exist. There is evidence that a deficiency in vitamin C can have an impact on the immune system, which in turn can lead to an increase in the prevalence of wound infections. Additionally, a deficiency in vitamin A can have an impact on the rate at which wounds heal themselves. This is similar to the previous point. Antioxidant activity, increased fibroblast proliferation, modulation of cellular differentiation and proliferation, increased production of collagen and hyaluronate, and a reduction in MMP-mediated breakdown of extracellular matrix are some of the biological effects of vitamin A (Soni H., etal;2011). Vitamin A also has the ability to inhibit the effects of a number of other cellular processes. Vitamin A is also capable of inhibiting the impacts of a variety of other cellular processes, thanks to its

antioxidant properties. Vitamin A has a wide range of actions, one of which is the ability to prevent the creation of hyaluronate and collagen itself.

Wound Healing and Phytopharmaceuticals (Soni H.*, etal*;2012).

Tannins

Tannins, which have astringent and antibacterial effects, are a vital phytonutrient in the healing of wounds.

Flavonoids

The antibacterial and antioxidant properties of flavonoids make them advantageous to the healing process of wounds. Flavonoids are beneficial to the healing process of wounds because of both of these features. In the phenolic structure of each of the three distinct types of flavones, flavonoids, and flavonols, there is a single carbonyl group that distinguishes them from one another. There are three different types of chemical compounds: flavones, flavonoids, and flavonols. The research that was conducted in vitro about the compounds has demonstrated that the compounds that plants produce as a response to microbial infection are potent antibacterial agents against a wide spectrum of pathogens. This was discovered through the research that was conducted on the compounds. Tannins are a class of chemicals that are characterized by their polymeric and phenolic properties, as well as their astringent properties. These compounds are soluble in water, alcohol, and acetone, and one of the products of their interaction with proteins is the formation of a precipitate. The reaction that takes place when benzene is coupled with pyrone rings that are already present in the molecule results in the production of coumarins, which are phenolic compounds. In addition, each of them has a perfume that is instantly recognizable, and some of them contain properties that inhibit the growth of microorganisms.

Mucilage of the Tragacanth

The means by which tragacanth mucilage facilitates the healing of wounds is the subject of discussion in this article, which also provides a potential explanation for the phenomenon in question. Furthermore, the considerable changes that were detected in the groups that were treated with tragacanth mucilage suggest that it may be effective throughout the

phases of wound healing that include proliferation and re-modeling on several occasions. These phases include the healing of wounds that have been treated with tragacanth mucilage. As a consequence of this, it is highly feasible that it possesses the ability to stimulate myofibroblasts to contract, which would hasten the process of wound healing process. Tragacanth mucilage typically has two active components: bassorin and tragacanthin. These two components are known as the active components. When it comes to the therapeutic properties that mucilage possesses, it is probable that these two components are the ones responsible for those results. In the case that tragacanthin is broken down into arabinose and glucoronic acid, it is likely that this process will lead to the coagulation of surface proteins and the prevention of infections, both of which will speed up the process of wound healing. Moreover, this process will also contribute to the prevention of infections.The means by which tragacanth mucilage facilitates the healing of wounds is the subject of discussion in this article, which also provides a potential explanation for the phenomenon in question. Furthermore, the considerable changes that were detected in the groups that were treated with tragacanth mucilage suggest that it may be effective throughout the phases of wound healing that include proliferation and re-modeling on several occasions. These phases include the healing of wounds that have been treated with tragacanth mucilage. As a consequence of this, it is highly feasible that it possesses the ability to stimulate myofibroblasts to contract, which would hasten the process of wound healing process. Tragacanth mucilage typically has two active components: bassorin and tragacanthin. These two components are known as the active components. When it comes to the therapeutic properties that mucilage possesses, it is probable that these two components are the ones responsible for those results. In the case that tragacanthin is broken down into arabinose and glucoronic acid, it is likely that this process will lead to the coagulation of surface proteins and the prevention of infections, both of which will speed up the process of wound healing. Moreover, this process will also contribute to the prevention of infections.

Current scenario

There are some situations in which there is a clear correlation between a local use and a biomedical application; yet, the concept of producing medications from plants that are

utilized in indigenous medical systems is considerably more ancient. It is possible that there are additional circumstances in which the relationship is significantly more complicated. Particularly concerning is the fact that chronic wounds are an issue that affects a significant number of patients and significantly lowers the quality of life that they enjoy. Furthermore, as a consequence of this, they are the focus of substantial concern for both patients and medical professionals. The most recent estimates suggest that there are approximately 6 million people all over the world who are afflicted with chronic wounds. According to Principe P et al. (1996), it is extremely rare for researchers in India to carry out studies on the epidemiology of chronic wounds. As a point of contrast, according to the statistics, there are 10.5 acute wounds for every 1000 individuals in the community, whereas there are 4.5 chronic wounds for every 1000 people in the community. In contrast to the approximately one to three percent of medications in the Western pharmacopoeia that are meant for use on the skin and wounds, at least one third of herbal therapies are designated for this purpose. This is because herbal remedies are believed to be more effective than pharmaceuticals. The prevalence of ulcers among hospitalized patients in the United States is estimated to range from three to five percent, and the prevalence of ulcers among patients with spinal cord injuries ranges from twenty-five to eighty-five percent. Both of these figures are based on estimates. Although there are no data available for Indian institutions, it is believed that the cost of institutional care for the same subject in the United States is one thousand dollars per day. This is despite the fact that there are no numbers available for Indian institutions. However, the same demographic study predicted that the worldwide market for the provision of wound healing properties will cost more than seven billion dollars in the United States. This was based on the fact that the United States is the largest market for such products. There is a shortage of funding and awareness among medical personnel, which makes it difficult for both conventional and Western medical procedures to repair wounds. The requirements for performing relevant research on issues demand advocacy and increasing publication, both of which are necessary in order to meet the standards. Utilization, safety, and efficacy are the categories that should be taken into consideration, as stated by Sussman, G. et al. (2007).

Botanical used as Wound Healer

Aloe vera

It is still the case that burns, ulcers, and surgical wounds are the conditions that require aloe vera treatment as the primary treatment choice. An extensive range of organic bioactive chemicals can be found in the aloe vera plant. These compounds include pyrocatechol, saponins, acemannan, anthraquinones, glycosides, oleic acid, phytol, and polysaccharides that are water-soluble and either simple or complex. Extracts of aloe vera leaf that contain acetone have substantially more potent antibacterial effects than extracts that contain alcohol or water. This contrasts with the qualities of extracts that contain water. As a result, it would appear that Gram-positive bacteria are more vulnerable to the damage that is induced by aloe vera than Gram-negative bacteria. Antibacterial properties have been demonstrated to be possessed by a variety of substances, including saponins, acemannans, and derivatives of anthraquinones, as stated by B. Salehi, S. et al. (2018).

Fig10: Aloe Vera

The consumption of acemannan, which is a main mucopolysaccharide (mesoglycan) derived from aloe vera, results in the transcription of a number of proinflammatory mRNAs. These include IL-1, IL-1, IL-6, TNF-, PGE2, and nitrous oxide, to name just a few more. Mesoglycan molecules are responsible for binding and capturing reactive oxygen species as well as endogenous mitogen inhibitors, which makes phagocytosis easier to accomplish. It just so happens that glycans extend the duration of activity of cytokines, growth factors, and other bioactive substances that have been produced. The application of acemannan topically has been demonstrated to significantly reduce the amount of time required for wound closure in a rat wound healing model. This is accomplished through the

action of cyclin D1 and AKT/mTOR signal pathways during the healing process. It is also believed that the glycans found in aloe vera have the ability to significantly boost the production of granulation tissue from scratch through a mechanism that has not yet been identified.

Calendula officinalis

The plant Calendula officinalis, sometimes known as pot marigold, is frequently used to treat a number of skin ailments, including burns, dermatitis, and wounds. Calendula officinalis is said to have a variety of pharmacological properties, including anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, and anticancer properties (C. Nicolaus etal;2017).

Fig. 11: Calendula officinalis

Currently, there is a lack of complete comprehension about the specific mechanisms that are accountable for its effects on the healing process of wounds. Through the utilization of cultures of human and mouse fibroblasts, researchers have established that extracts of Calendula officinalis possess the capability to induce fibroblast migration and proliferation in a manner that is dependent on PI3K. Extracts from the flower of the Calendula officinalis plant have been shown to enhance the development of granulation tissue in wounds that have been excisionally removed from BALB/c mice. In order to achieve this goal, it is necessary to modify the expression of connective tissue growth factor (CTGF)

and smooth muscle actin (-SMA) in living organisms. Through the use of a cutaneous wound healing model in rats and the chicken chorioallantoic membrane (CAM) experiment, it has been established that calendula officinalis increases angiogenesis in living creatures. This was accomplished by using the chicken chorioallantoic membrane (CAM) experiment.

Camellia sinensis

In Asia, green tea, which is an aqueous extract that is derived from Camellia sinensis leaves, is highly respected due to the purported health benefits that it gives. Green tea is a beverage that is consumed in abundance. From a scientific point of view, it has been demonstrated that Camellia sinensis possesses antioxidant, anti-inflammatory, antibacterial, anticarcinogenic, anti-aging, antiobesity, cardioprotective, and neuroprotective properties. This allows for the experimental validation of anecdotal evidence that has been accumulated over the course of several centuries. It is the catechins, which are polyphenolic substances that are present in Camellia sinensis, that are the major agents that are responsible for these pharmacological activities. The catechin known as (-)-Epigallocatechin-3-gallate (EGCG), which is the major catechin, is responsible for facilitating both the proliferation and differentiation of keratinocytes. Klass and his colleagues were the ones who pioneered the discovery that EGCG functions as an inhibitor of TGF- receptors. A modification of the TGF-signaling pathway, a reduction in the expression of MMP-1 and MMP-2, and a reduction in the creation of collagen type 1 in human dermal fibroblasts were all necessary approaches to achieve this goal. In light of the fact that these characteristics are present, it is possible that EGCG possesses the potential to function as an anti-scarring agent. EGCG has also been shown to suppress the STAT3 signaling pathway, which leads to a reduction in the size of keloids, the prevention of the development of pathogenic keloids, and the proliferation of these keloids. This has been established through a number of studies. (L. M. Parente et al., 2012).

Fig. 12: Camellia sinensis

According to the findings of research, methanol extracts of Camellia sinensis are able to stimulate both the production of collagen and the proliferation of fibroblasts. In addition, tests conducted in living animals have showed that Camellia sinensis helps the healing of wounds by considerably increasing the process of angiogenesis in rats. It has also been established that extracts of Camellia sinensis can speed up the healing process of wounds in a diabetic mice model. Additionally, this has been demonstrated.

Carthamus tinctorius

In order to obtain cooking oil, a substantial number of nations make use of safflower seeds, which are also known as Carthamus tinctorius. Safflower seeds are a source of oil. In Traditional Chinese Medicine (TCM) formulations, Carthamus tinctorius has been used for a long time to treat blood disorders. Despite the fact that it is not as well-known as other medicines, it has been used for a long time. Recent research has linked it to a wide variety of biological effects, such as vasodilation, immunological modulation, anticoagulation and thromboprophylaxis, antioxidation, antihypoxic, antiaging, antifatigue, anti-inflammation, antihepatic fibrosis, anticancer, and analgesia. These effects have been related with each other. It is a well-known fact that safflower seed oil has the ability to lessen the quantity of melanogenesis that takes place in B16 melanoma cells. This makes it an intriguing possibility for skin whitening. It was revealed in 2004 that the principal water-soluble monomer of safflower yellow pigments, which is referred to as hydroxysafflor yellow A

(HSYA), had antioxidant, anti-inflammatory, proangiogenic, and apoptosis-inhibitory effects. This was established by research that was carried out by J. S. Roh and colleagues. The presence of these qualities raises the possibility of providing protection against ischemia of the heart and brain.

Fig. 13: Carthamus tinctorius

Topical treatment of HSYA at a low dose of 4 mg/mL stimulates neovascularization, reepithelialization, and granulation tissue growth in rats that have been forced to develop diabetes by streptozotocin. In contrast, when the concentration is high (10 mg/mL), it slows down the healing process of wounds.

Celosia argentea

It is used in traditional medicine systems for the aim of treating mouth ulcers, eruptions, ulcers, and other skin problems. Celosia argentea, which is more often known as silver cock's comb, is the plant that is used. In addition to having antibacterial properties, hepatoprotective properties, antioxidant properties, and diabetic properties, the leaf extracts of this plant also have diabetic properties. According to the findings of Priya and colleagues, an alcohol extract of Celosia argentea has the ability to hasten the healing process of burn wounds in rats. Increasing the amount of collagen and hexosamine that is present in the wounds caused by granulation tissue is the method that facilitates this outcome. Furthermore, it was discovered that the extract induced an increase in the proliferation and motility of primary rat dermal fibroblasts. (K. S. Priya et al., 2004).

Fig. 14: Celosia argentea

Centella asiatica

Over the course of several centuries, folks have been making use of Centella asiatica, which is also known as Asiatic pennywort, with the intention of hastening the process of wound healing. It has been established that extracts obtained from the aerial sections of Centella asiatica can speed the healing process of chronic ulcers in terms of width, depth, and length. This was accomplished through the utilization of Sprague-Dawley rats. After administering extracts of Centella asiatica to rats that were suffering from acute radiation dermatitis, it was shown that the wounds healed more quickly than when the rats were given a placebo of the same substance. Using a punch wound model in the guinea pig, it has been demonstrated that asiaticoside, which is derived from Centella asiatica, possesses the capacity to stimulate the deposition of collagen and the epithelialization of the wound. (R. U. Hamzah etal;2018).

Fig.15: Centella asiatica

In a rat wound model, triterpenes extracted from Centella asiatica promote collagen remodelling and glycosaminoglycan production. In a mouse wound model, oral treatment of madecassoside from Centella asiatica was demonstrated to promote collagen synthesis and angiogenesis.

Cinnamomum cassia

The bark of the Cinnamomum cassia tree is utilized not only as a spice and flavoring component, but also as an analgesic and to enhance blood circulation. This is despite the fact that it is frequently used for these purposes. Among the seven botanical components that make up the Shexiang Baoxin pill (SBP), which is a well-known Traditional Chinese Medicine (TCM) treatment for chest pain and discomfort caused by coronary artery disease, *cinnamonomum cassia* is reported to be present. There is a common practice among manufacturers to combine *cinnamononomum cassia* with other plants. When it comes to the treatment of coronary artery disease that cannot be revascularized, a clinical trial that is currently being carried out is a randomized, double-blind clinical trial that is utilizing SBP (X. Yuan et al., 2018). This trial is currently being carried out. In addition to its anti-inflammatory and anticancer properties, studies are also being carried out to examine the impact of selective blood pressure (SBP) on hypertension, insulin resistance, and non-insulin-dependent diabetic mellitus. Cinnamaldehyde, which is a bioactive component obtained from Cinnamomum cassia, has been shown to be a natural insecticide, antibacterial, anti-diabetic, anti-lipidemic, anti-inflammatory, and neuroprotective agent, according to research conducted both in vitro and in vivo. The activation of PI3K/AKT and MAPK signaling pathways, which leads to an increase in VEGF production and induces angiogenesis in human umbilical vein endothelial cells, is another effect of this substance. There have also been reports that cinnamondehyde can speed up the healing process of wounds in zebrafish.

Fig. 16: Cinnamomum cassia

Commiphora myrrha

Myrrh is known to have a number of beneficial properties, including anti-inflammatory, antibacterial, antioxidant, and analgesic actions. These properties are well known before. Commiphora myrrha is the name of the plant that produces myrrh, which is a resinous exudate that is named after the plant. One of the many medical applications of myrrh is the treatment of parasite infections, obesity, arthritis, fractures, and problems connected to the gastrointestinal tract. Other medical applications include the treatment of fractures and arthritis. And in addition to that, it possesses anticoagulant qualities. The topical application of myrrh has proven to be effective in treating wounds, reducing swelling, and providing relief from pain (often referred to as analgesia). When making a variety of remedies, it is usual practice to combine myrrh with other active ingredients. Among the research that were carried out by Galehdari and others, it was revealed that the utilization of myrrh, Adiantum capillus-veneris, Aloe vera, and Lawsonia inermis resulted in a considerable acceleration of the wound healing process in diabetic mice. As stated by A. J. Fatani et al. (2016), the application of myrrh to human beings for a brief length of time has the capacity to successfully treat pain and prevent it from returning in the future. In vitro research has shown that myrrh has the ability to change the expression of TGF-1 and VEGF in mice dermal fibroblasts. The fact that this discovery is analogous to the findings of a large number of other herbal preparations that are mentioned in this article suggests that these preparations all share a mechanism of action by virtue of their similarities.

Fig. 17: Commiphora myrrha

Curcuma longa

It is the root of the Curcuma longa plant, which belongs to the ginger family, that contains the active component known as curcumin. The culinary and medical professions have both made extensive use of curcumin over the course of its long history. The compound known as curcumin has been utilized for a fairly extensive variety of uses. The practitioners of traditional Ayurvedic medicine employ curcumin as a therapy for a wide variety of illnesses and conditions. Inflammation, liver issues, lung problems, and diabetes are some of the ailments that fall within this category. As a common treatment for cramping and pain in the stomach, curcumin is utilized in traditional Chinese medicine. This treatment is also used to treat stomach pain. Over the course of a very long period of time, a wide range of different ethnic groups have been making use of curcumin, which is one of the nutraceuticals that has been investigated in the most comprehensive manner. Curcumin has been the subject of a huge amount of research. Numerous studies that have been carried out on the topic have demonstrated that this highly pleiotropic protein interacts with important physiological processes at the transcriptional, translational, and posttranslational stages. This is the conclusion that can be drawn from the findings of these studies. There are many different target pathways, some examples of which include pro-inflammatory cytokines, apoptosis, NF-B, 5-LOX, STAT3, C-reactive protein, prostaglandin E2, prostate-specific antigen, cell adhesion molecules, phosphorylase kinase, transforming growth factor-, triglycerides, ET-1, creatinine, heme oxygenase-1, AST, and ALT. Over one hundred

clinical trials have been conducted on curcumin as a possible treatment for epithelial cancers (D. Akbik et al., 2004). These trials have been conducted in the United States. The results of some of these experiments have been favorable. The ingredient curcumin was the focus of these trials, which included investigations conducted in living organisms.

Fig. 18: Curcuma longa

Changing the pericellular and extracellular matrix is the mechanism via which curcumin exerts the majority of its beneficial effects, as demonstrated by the experimental findings obtained from these in vivo investigations and in vitro research [168]. It is therefore possible that the fact that curcumin encourages the formation of granulation tissue, collagen deposition, and fibroblasts during the healing process of cutaneous wounds should not come as a surprise.

Daphne genkwa

The daphne genkwa plant, which is believed to be one of the fifty essential herbs utilized in Traditional Chinese Medicine (TCM), can be found in China, specifically in the basins of the Yellow and Yangtze respective rivers. In addition to its usage as an anticonvulsant, analgesic, diuretic, antitussive, expectorant, and moderate sedative, daphne genkwa has a variety of other applications as well because of its versatility. Daphne genkwa has been shown to contain a number of different types of bioactive compounds, the most important of which are biflavonoids, coumarin, diterpenes, and triterpenes. They have actions that are immunoregulatory and antimelanogenesis, in addition to the analgesic and anticancer properties that they possess. Flavonoids that were extracted from the blooms of Daphne genkwa were discovered to activate the ERK/MEK pathway, which culminated in enhanced wound healing (D. Yang et al., 2017). Flavonoids were revealed to be responsible for this

improvement. The expression of collagen (COL1A1 and COL3A1) and the proliferation of fibroblasts are both under the control of this pathway, which is responsible for both of these processes.

Fig. 19: Daphne genkwa

Entada phaseoloides

Entada phaseoloides, more popularly known as St. Thomas bean, is a climbing liana that belongs to the pea family. It may be found in lowland tropical forests and coastal forests in the Western Pacific, Africa, Australia, and Asia. St. Thomas bean is a member of the pea family. Using the bark and seeds of the Entada phaseoloides plant, which are abundant in saponins and tannins, as a topical treatment for skin lesions is effective. Furthermore, throughout the course of the therapy procedure, these components are utilized as agents that suppress the growth of cancer cells, prevent bleeding, and kill bacteria. The researchers Su et al. (P. Widsten etal;2014) discovered that Entada phaseoloides extracts that were supplemented with tannins sped up the healing process for wounds that were infected in rats. This was the conclusion reached by the researchers.

Data analysis revealed that the Entada phaseoloides extracts' antibacterial, proproliferative, and promigration activity was the cause of the increased wound healing. These results have not yet been tested on actual patients.

Hibiscus rosa-sinensis

Is a shrub that is evergreen. A plant that is native to tropical South Eastern Asia is the Hibiscus rosa-sinensis, which is also often known as the shoe black plant. This plant is one of the plants that is endemic to this region. There is the possibility of consuming the blossoms of the Hibiscus rosa-sinensis orchid. There is a belief in traditional writings that

preparations prepared from plants and flowers can stimulate the growth of hair and prevent graying of the hair. According to study that was carried out in A. Bhaskaret al.,(2012), the utilization of alcoholic extracts of Hibiscus rosa-sinensis flowers is reported to give women with a greater degree of control over their fertility.

Fig. 20: Hibiscus rosa-sinensis

Furthermore, it has been found that extracts of Hibiscus rosa-sinensis have the ability to have antibacterial properties as well as wound-healing properties. By reducing inflammation, promoting fibroblast proliferation and collagen deposition, and upregulating VEGF and TGF-1 expression in rat excisional wounds, these substances are effective in treating wounds.

Ganoderma lucidum

It is usual practice in China, Korea, and Japan to refer to Ganoderma lucidum, also known as the lingzhi fungus, as "the mushroom of immortality" due to the mushroom's immense popularity in these countries. Ganoderma lucidum is an extract of a plant that is used in Traditional Chinese Medicine (TCM) to strengthen the immune system of patients. Ganoderma lucidum is taken from the plant. Furthermore, it possesses anti-inflammatory, anti-infective, antioxidant, cardioprotective, and anti-hyperlipidological effects, in addition to the acts described above. The results of clinical study indicate that consuming Ganoderma lucidum on a consistent basis leads to positive outcomes. The number of tumors in people who have colorectal adenomas, the amount of viral particles that are circulating in people who have hepatitis B, and the symptoms of hypertension have all been shown to decrease as a result of its effect. (B. Boh etal;2013).

Fig21: Ganoderma lucidum

Research carried out in the laboratory reveals that components produced from *Ganoderma lucidum* interact with and exert an influence on important enzymes that are responsible for the processes that are involved in the metabolism of lipids. The clinical evidence, on the other hand, is conflicting and suggests that *Ganoderma lucidum* is most beneficial when used in conjunction with other treatments including chemotherapy and radiation therapy. In diabetic rats, it has been demonstrated that polysaccharide extracts from the fruiting body of Ganoderma lucidum have the capacity to speed up the healing process of wounds they have sustained. The suppression of oxidative stress, the stimulation of fibroblast migration and proliferation, and the promotion of angiogenesis are all potential methods for accomplishing this goal. Furthermore, it is probable that these reactions are partially the result of the known enhancement of humoral immunity that *Ganoderma lucidum* possesses. This is something that should be considered.

Ligusticum striatum

Traditional Chinese Medicine (TCM) makes use of fifty different herbs, and the rhizome of the Ligusticum striatum plant is one of the herbs that is utilized in TCM. Both the circulatory system and the brain have benefited from its utilization for a very long time. This has been done in order to improve the overall health of the body. In addition to its practical application in the treatment and prevention of headaches, menstrual irregularities, and difficulties connected with ischemia, it is also widely utilized for the treatment of these conditions. Phthalide lactones and alkaloids are the types of chemical components that are discovered most commonly and are active from a pharmacological standpoint. Ligusticum

striatum is the organism that is responsible for the formation of these chemical components, as has been established. The number of distinct chemical components that have been found up to this point is roughly 174. By using a rabbit ear scar model, researchers were able to demonstrate that the essential oils that are derived from the Ligusticum striatum plant had the ability to reduce the formation of cutaneous scars (J.G. Wu et al., 2011).

Fig. 22: Ligusticum striatum

Lonicera japonica

Fig. 23: Lonicera japonica

In the countries of Japan, Korea, and China, where it is native, honeysuckle, which is also known as Lonicera japonica, plays a key part in the practice of traditional medicine. Since the beginning of time, honeysuckle has been used as a remedy for the treatment of infectious diseases. The Chinese State Ministry of Health conducted a substantial amount

of pharmacological and clinical research on Lonicera japonica during the 1980s. The results of these investigations showed that the plant has a wide variety of qualities, including those that were antibacterial, anti-inflammatory, antipyretic, antioxidant, anti-cancer, hepatoprotective, and antihyperlipidemic. This is more recent:

During the process of cutaneous wound healing, Chen et al. demonstrated that the ethanol extracts of the blooming aerial sections of Lonicera japonica were able to increase the processes of angiogenesis, reepithelization, the development of granulation tissue, and contraction on the skin. It is possible to consume the plant as a "health food," which provides some protection against ulcers that occur in the gastrointestinal tract (X. Shang et al., 2014). This is in spite of the fact that even in the presence of large quantities, it has the potential to cause a wide range of neurological diseases.

CHAPTER - 2
REVIEW OF LITERATURE

Herbal remedies and aromatic plants have made major contributions to the improvement of human health. Since the dawn of time, people have recognised the potential therapeutic value of experiencing suffering. In this particular field, research has been conducted to ascertain the active components of medicinal plants and to investigate the extracts in order to guarantee their safety, efficacy, and consistency. Plant materials are considered to have high levels of biological activity and are utilized in a variety of ways for the benefit of humans. There are a number of plants that have flavonoids as their primary component that are utilized for their antibacterial, wound healing, and antioxidant properties alike.

In 2023, Hong YK et al. provided a summary of the most recent understanding of the immunopathogenesis of pathological scars as well as the treatments that are currently accessible from that perspective. As a result of the development of new technologies and the utilization of in vitro and in vivo wound models, it has been established that inflammatory cells have the ability to influence pathological scarring and wound healing in both direct and indirect ways. By way of the transforming growth factor-1 signaling pathway, the increase of pro-fibrotic immune cells, which includes the expansion of M2 macrophages, dendritic cells, mast cells, and Th2 cells, leads to the differentiation of fibroblasts into myofibroblasts. In clinical management, it is of the utmost importance to regulate these inflammatory reactions in an optimal manner during the healing process of wounds. Controlling the inflammatory response that occurs during the healing process of wounds is one of the potential therapeutic strategies that might be utilized to avoid or reduce the formation of pathological scars.

NETs have been associated with the release of neutrophil extracellular traps and histone citrullination by protein arginine deiminase 4 (Padi4 in mice). Diabetes has been linked to both of these processes. There is currently a lack of clarity regarding the particular factors that enable diabetes to impede wound healing even when NETs are engaged. The findings presented in this paper by Yang S. and colleagues 2023 reveal that diabetic wound conditions increase the vulnerability of neutrophils to NETosis. Through the use of in vitro

studies and in vivo wound healing models utilizing wide-type and Padi4 -/- mice, we demonstrate that NETs have the ability to activate PAK2 by way of the membrane receptor TLR-9. Phosphorylation of the intracellular protein Merlin/NF2 by PAK2 is the subsequent step in the process of blocking the Hippo-YAP pathway. It is necessary for YAP to bind itself to the transcription factor SMAD2 and then transfer from the cytoplasm into the nucleus in order to facilitate the endothelial-to-mesenchymal transition (EndMT). This ultimately reduces the rate at which wounds heal and prevents angiogenesis from occurring. It is possible to inhibit the Merlin/YAP/SMAD2 pathway in order to reduce the amount of NET-induced EndMT. A faster rate of wound healing is achieved through the suppression of NETosis, which reduces EndMT and promotes angiogenesis. Through the activation of EndMT via the Hippo-YAP pathway, these findings suggest that NETosis is responsible for the slowing of the healing process of diabetic wounds. In order to find cellular targets that are amenable to therapeutic intervention for DFUs, it will be of major value to have a more in-depth understanding of the molecular mechanisms that regulate NETosis and EndMT.

Mesenchymal stem cells, also known as MSCs, are multipotent stem cells that have the capacity to self-renew, develop in a number of different ways, and be regulated by paracrine stimuli. Exosomes are vesicular subcellular particles that can range in size from 30 to 150 nanometers. They serve as one-of-a-kind intercellular messengers that regulate the biological activities of skin cells. When compared to MSCs, MSC-derived exosomes (also known as MSC-exos) have a lower immunogenicity, are easier to retain, and have an exceptionally high level of biological activity. Multipotent stem cells (MSC-exos), which are primarily derived from adipose-derived stem cells (ADSCs), bone marrow-derived MSCs (BMSCs), human umbilical cord MSCs (hUC-MSCs), and other types of stem cells, have the ability to influence the activity of fibroblasts, keratinocytes, immune cells, and endothelial cells in diabetic wounds, inflammatory wound repair, and even wound-related keloid formation. As a consequence of this, Zhou C et al. 2023 focused their attention on the various functions and mechanisms of various MSC-exos utilized in the process of wound healing, in addition to the existing limitations and contrasting points of view. It is vital to have a thorough understanding of the biological properties of MSC-exos in order to design a cell-free therapeutic tool that has the potential to be used for skin regeneration and wound repair.

Collagen and extracellular matrix (ECM) are the building blocks of wound healing, and myofibroblasts are responsible for their creation and organization into scar tissue. Scar tissue is used to restore injured tissues. The structural integrity of the tissue that has been destroyed is swiftly and effectively replaced by scar tissue, albeit at the sacrifice of the functionality of the replaced tissue. It is possible for fibrotic diseases to lead to organ failure. These conditions can be brought on by excessive or extended myofibroblast activity. In this review, the nomenclature of myofibroblasts, as well as their phenotypic characteristics and functions, are outlined. The major function of the cell, extracellular matrix, and tissue mechanics in regulating tissue repair through the regulation of myofibroblast activity will be the focus of Schuster R et al.'s research in 2023. In addition to this, we will discuss the ways in which mechanical intervention-based therapies have the potential to improve wound healing results. In spite of the fact that the physiology and pathology of myofibroblasts have an effect on every organ, we will concentrate on hypertrophic scarring and the healing of cutaneous wounds as examples of normal tissue repair as opposed to fibrosis. One of the most important things that can be learned from this analysis is that myofibroblasts can be produced from a wide variety of cell sources, depending on the local environment and the type of injury. This can be done in order to either restore tissue integrity and organ function or to give an unsuitable mechanical environment.

Wound healing is the typical response that the body has when it is injured. The three components of the wound healing response that are shared by all organs are inflammation, which serves to prevent infection and prompt the elimination of dead cells; active anti-inflammatory signaling, which serves to stop the inflammatory response; and a repair phase that is highlighted by the formation of scar tissue in the extracellular matrix. The size of the scar that is produced as a result of the scarring process is determined by the capacity of the endogenous cells that make up each organ to repair. There is a capacity for regeneration in the keratinocytes that make up the skin, and wounds normally go through the process of complete reepithelialization. On the other hand, cardiac myocytes in the heart have a limited or nonexistent capacity to regenerate, and these myocytes are totally replaced by scars when they become necrotic. Even though there are differences in tissue regeneration, the skin and the heart share substantial similarities in the way they repair wounds. These

similarities can be utilized to predict how the heart would react to a disease. In this review article, Lindsey ML et al. 2023 provide an overview of our current understanding of how the skin's response to a traumatic event can provide insight into the myocardium's ability to respond to a myocardial infarction.

Wounds on the skin trigger a chain of healing processes that are coordinated in both space and time throughout the body. In this type of endogenous wound repair, fibroblasts perform a number of functions, including the activation and attraction of innate immune cells, the production and deposition of scar tissue, and the movement of fascial connective tissue into wounds in a manner that is analogous to a conveyor belt. Within the context of the healing process, a comprehensive investigation into the variety and flexibility of fibroblasts may be of assistance in gaining a better understanding of wound diseases and laying the framework for new therapeutic methods. With this in mind, Knoedler S et al. 2023 illustrate the various types of fibroblasts and describe the specific roles that each type plays in the process of wound healing. There is also discussion regarding potential future directions from a clinical-translational point of view.

The skin is responsible for a number of important tasks, including the management of temperature, the prevention of infection, the reduction of excessive fluid and electrolyte loss, and the provision of tactile feedback regarding the surrounding environment. In addition, the skin plays a significant role in how individuals perceive their appearance, as well as their self-confidence and body image. In order to determine the extent of the skin's disruption following burn injuries, it is vital to have a solid understanding of the typical anatomical composition of the skin. This is because the skin serves a wide variety of tasks. It is mentioned in Shah NR etal;2023 that the pathophysiology of burn wounds, early assessment, following progression, and healing of burn wounds are all discussed. By providing an overview of the multiple microcellular and macrocellular alterations that occur as a result of burn injury, this review also enhances the capacity of medical practitioners to deliver patient-centered, evidence-based burn treatment.

Wound healing can be improved by utilizing oxygenation strategies that are capable of effectively increasing the levels of oxygen in the wound. Han X et al. 2023 provided a summary of the stages of wound healing, the function that hypoxia plays in the healing process, and the current efforts of incorporate various oxygen delivery or producing

materials for wound dressing. Catalase, nanoenzyme, haemoglobin, calcium peroxide, or materials based on perfluorocarbon compounds are some examples of these materials. Additionally, hyperbaric oxygen therapy and photosynthetic microorganisms are also included in this category. In this article, we examine the potential benefits and drawbacks of these dressings, as well as their mode of action and the degree to which they are efficient in oxygenation. We place a strong emphasis on the relevance of optimizing the design of wound dressings in order to meet the requirements of therapeutic needs and improve clinical outcomes.

The authors of the 2023 study, Hom DB et al., provided a summary of a generalized strategy for treating a wound that is healing at a slower rate than was anticipated. During the preoperative evaluation, patients should be screened for a variety of chronic medical conditions and medications, including prior radiation exposure, use of cigarettes or electronic cigarettes, chronic use of steroids, alcoholism, diabetes, malnutrition, and other chronic medical conditions and medications. Even with the most advanced surgical healing techniques, there is still a possibility that certain wounds will exhibit signs of continuing inflammation. The facial plastic surgeon needs to be skilled at recognizing delayed healing and determining both internal and extrinsic risk factors in order to be able to carry out an intervention in a timely manner.

Fibroblasts are cells that are extremely active and are necessary for the fibrosis of tissue as well as the healing process. Despite this, there is still a lack of understanding regarding the ways in which they influence the deposition and remodelling of extracellular matrix in both normal and pathologic circumstances. As part of the current investigation, Talbott HE and colleagues (2022) conducted a review of the current state of our knowledge regarding the biology and heterogeneity of fibroblasts, with a specific focus on the role that fibroblasts play in the process of skin wound healing. The limitations of the existing approach and knowledge are also evaluated, and we take into consideration the new techniques that have been developed in the field. These techniques make it possible to have a more nuanced and contextualized understanding of these complex systems. Through this review, a wide variety of studies that concentrate on the fibroblast are highlighted. The fibroblast is a cell type that is sometimes overlooked, despite the fact that it plays vital roles in wound healing and other processes.

Within this area of research, Ordeghan Allahyar Noori and colleagues (2022) investigated the effects of high-intensity interval training (HIIT) and a collagen, nanoclay, and tadalafil hydrogel on the wound healing process of diabetic rats. Following the production of the hydrogel, testing were carried out to determine its biocompatibility and antibacterial effectiveness. Following the introduction of diabetes in the rat model, the therapeutic effect of collagen/nanoclay/tadalafil hydrogel was investigated, and the evaluation of wound healing was carried out using both macroscopic and microscopic examinations. The MTT test revealed that the collagen/nanoclay/tadalafil hydrogel did not exhibit any form of cytotoxicity that could be considered detectable. Hydrogel was found to be effective in inhibiting the growth of E. coli and S. aureus as well. After 21 days, the macroscopic findings revealed that the hydrogel/HIIT exercise and hydrogel groups had seen a significant amount of wound contraction in comparison to the HIIT exercise and control groups. The thickness of the epidermis, the amount of collagen present, the presence of fibroblasts, and the density of the epidermis were all visible when seen via a microscope.

Twenty-two, Carmen R. Silva-Correa and others. The topical preparations of T. tuberosum (gel and cream) were evaluated for their capacity to promote wound healing on wounds that were produced in rats. For the purpose of this investigation, a topical cream and gel formulation was utilized. This formulation contained one percent of the acidic ethanolic extract that was produced using the tubers of T. tuberosum ecotype black. 1.5 N hydrochloric acid and 96 percent ethanol are the components that make up the extract. Each of the four experimental groups of 32 Balb/c mice received a topical treatment on a daily basis for a period of fourteen days: Group I was the control group, which did not receive any treatment. Group II was a commercial ointment that contained neomycin, polymyxin B, and bacitracin. Group III was a 1% T. tuberosum gel, and Group IV was a 1% T. tuberosum gel followed by a 1% T. tuberosum cream preparation. A determination was made on the degree of wound closure in the mice while they were undergoing treatment. Following this, the animals were placed to sleep so that skin samples could be obtained for histological analysis.

A polyherbal formulation that contained Punica granatum and Coleus aromaticus extract was evaluated for its potential to promote wound healing using a burn wound model. Soni

H et al. (2022) also conducted this evaluation. The investigation came to the conclusion that the ointment that was created from the phenolic and flavonoid-rich extract of C. aromaticus, P. granatum, and polyherbal plants has a significant potential for healing wounds. This conclusion was reached based on the findings of the investigation.

A scientific investigation of the efficacy of the crude extract and solvent fractions of the leaves of Vernonia auriculifera Hiern was carried out by Mulatu Kotiso Lambebo et al. 2021. The investigation focused on the leaves' potential to cure wounds. During the course of the experiment, extraction was carried out by means of a maceration technique that involved 80 percent methanol. Additionally, a portion of the crude extract was fractionated by means of aqueous, chloroform, and ethyl acetate solvents. Hard paraffin, cetostearyl alcohol, white soft paraffin, and wool fat were the components that were utilized in the production of simple ointment bases in accordance with the British Pharmacopoeia. Afterwards, the extract was utilized in the production of two distinct ointment formulations, one of which was 5 percent by weight, and the other was 10 percent by weight. Mice and rats were utilized, respectively, for the purpose of conducting research on wound healing and cutaneous toxicity analyses. For the purpose of evaluating the ability for wound healing in excision, incision, and burn wound models, the criteria of wound contraction %, epithelialization time, tensile strength, and histological analysis were utilized. Following the representation of the data as mean standard error of the mean, the findings were analyzed using one-way analysis of variance (ANOVA) and the post hoc Tukey test.

Hydrogels are the subject of present research into novel drug delivery formulations that aim to provide stable and economical drug delivery systems. This is due to the fact that hydrogels are known to lessen difficulties that are associated with both conventional dosage forms and novel drug delivery systems. The objective of the research conducted by Soni H et al. in this area was to determine whether or not rutin could be effectively administered through a hydrogel drug delivery system for the purpose of evaluating wound healing activities. As a result of the fact that the drug content of formulation H2 (0.025 percent w/w) was comparable to that of formulation H3 (0.030 percent w/w), formulation H2 was considered to be the most suitable phraseology.

Research conducted by Judith Salas-Oropeza and colleagues in 2020 investigated the efficacy of B. morelensis essential oil in the treatment of wounds using a mouse model. The

production of the essential oil was accomplished by the process of hydro-distillation, and the chemical composition of the oil was analyzed through the use of gas chromatography-mass spectrometry (GC-MS). In the murine model, both the potential for wound contraction and the effectiveness of wound healing were evaluated. In vitro testing was performed to determine the cytotoxic potential of peritoneal macrophages derived from BALB/c mice. According to the findings, the essential oil included a total of eighteen compounds that were similar to terpenoids. Regardless of the dose, the essential oil demonstrated remarkable WHE, caused a rise in WC, and did not exhibit cytotoxic effects. When essential oil (EO) was added to the culture medium at a concentration of 1 mg/mL, the cell viability dropped below 80%. However, when doses of 0.1 and 0.01 mg/mL were utilized, the cell viability remained at or above 90%. As a consequence of this, EO did not have an effect on the proliferation of fibroblasts; however, it did have an effect on the migration of fibroblasts when wound-like studies were conducted in monolayer cultures. As a wound-healing agent, the essential oil was shown to have high-quality healing effectiveness, and it also produced scars that had strong tensile strength and were able to repair themselves quickly, as the findings of the study revealed. The essential oil of B. morelensis most likely functions by enabling fibroblasts to migrate to the site of the wound, thereby activating them in the process of collagen synthesis, and by facilitating the remodelling of the collagen that has already been formed.

Singh Manish Pal et al. 2019 conducted an experiment in which they evaluated the ability of fruit extracts Terminalia bellerica Roxb. and gallic acid to promote wound healing in rats that had been intentionally caused to have hyperglycemia. Both excision and dead space wound models were utilized throughout the course of the inquiry. The percentage of the contraction wound, the number of days that had passed since the re-epithelialization, the scar area, the tissue hydroxyproline level, and the weight of the tissue granuloma were all evaluated. An oral dose of gallic acid (200 mg/Kg) and an oral dose of Terminalia bellerica fruit extract (400 mg/Kg) were administered to streptozotocin-induced diabetic rats, as evidenced by the data. In the model of the excision wound, it was discovered that they considerably (p 0.05) increased the percentage of wound contraction, decreased the size of the scar region, and shortened the number of days required for re-epithelialization. Furthermore, in a model of a dead space wound, the levels of hydroxyproline in diabetic

rats were found to be higher than those seen in the diabetic control group. Vitamin C, which is a common drug, was also discovered to significantly speed up the healing process of wounds in rats. According to these data, diabetic rats in the treated groups had worse rates of wound healing than the control group.

The evaluation of wound healing activities was the primary focus of the first in-vitro macrophagic cell culture investigation that was conducted. In every stage of the wound healing process, macrophages play a significant role. The research conducted by Soni H et al. in 2019 on the effects of hydrogel containing rutin at different concentrations on a variety of haematological parameters served as the foundation for their investigation into macrophagic cell culture. In the present investigation, the effects of rutin on macrophage cell culture were investigated at three different concentrations: 0.20 M, 0.25 M, and 0.30 M and respectively. Specifically, the data indicated that the medicine, when administered at a concentration of 0.025 M, significantly stimulated the proliferation of macrophages in cell culture. As a consequence of this, it caused wound healing activity to increase.

The usefulness of the crude extract of leaves from Acanthus polystachyus Delile (Acanthaceae) for the treatment of wounds was investigated by Wubante Demilew and colleagues (2018). During the crude extraction process, methanol was utilized at a concentration of eighty percent. A comparison of the wound healing activities of the crude extract in ointments containing 5% (w/w) and 10% (w/w) was carried out on Swiss albino mice that had been subjected to excision, infection, and incision wound models. Based on the findings, it was shown that both ointments with a weight-to-weight ratio of 5 percent and 10 percent significantly accelerated the pace of wound contraction and the tensile strength when compared to the group that served as the negative control (P 0.05). In a wound model that was infected with S. aureus, the wound healing activity of the group that was treated with ointment at a concentration of 10 percent (w/w) was shown to be greater than that of the groups that were treated with ointment at a concentration of 5 percent (w/w) and nitrofurazone ointment. Based on these findings, it may be concluded that a crude extract of A. polystachyus leaves possesses the ability to cure wounds. This provide more evidence that the plant has a long-standing reputation for treating wounds caused by S. aureus that are either infected or not infected.

For the purpose of determining whether or not hydrogel formulations are effective in

treating wounds, Soni H et al. (2018) assessed a variety of metrics, such as the tensile strength and the contraction of the wound. The final product displays the wound location discount for each of the distinct companies that were operating during the fourth, eighth, twelfth, and sixteenth days of the period. It has been reported that animals that were fed formulations of H2 and H3 experienced wound healing for themselves. It was determined that the wound contraction percentages were 82.9 and 80.3 percent, respectively, on the twentieth day of the procedure. It took twenty days for the epithelization process to complete for each and every formulation. On account of the fact that its drug content is lower (0.25 percent w/w) than that of the H3 formulation (0.50 percent w/w), the H2 formulation is considered to be the optimal formulation. After further investigation, it was found that the H2 formulation has the highest tensile strength in comparison to the standard.

A study that was carried out in 2017 by Minh Can Nguyen MSc and his colleagues studied the effectiveness of an ethanolic extract of S. juventas root in the treatment of wounds. To begin, administering an ethanolic extract produced from S. juventas roots at a dose of 100 mg/kg/day dramatically accelerated wound healing in a mouse excision wound model. Mice given the extract after seven days showed a 2.3-fold drop in inflammatory cells, a 1.7-fold rise in fibroblasts, and an improvement in angiogenesis in the wound granulation tissue. Furthermore, a molecular study found that treating wounds with an ethanolic extract of the root of the S. juventas plant lowered TNF- and NF-B1 gene expression by 4.7 and 3.7 times, respectively. TGF-1 and VEGF, on the other hand, saw gene expression increase by 1.9 and 6.5 times, respectively, as compared to prior generations. Overall, the results of our testing strongly suggest that the ethanolic extract of S. juventas root has an exceptional capacity for wound healing.

Anindita Kundu et al. (2016) investigated the wound-healing properties of P. fulgens ethanol root extract (EPF) and its ethyl acetate fraction (PFEA) in experimental rats. The use of both excision and incision models resulted in wounds in animals. The injured animals were treated for sixteen days with EPF (oral: 200 -400 mg/kg and topical: 5-10% w/w) and PFEA (oral: 75 mg/kg and topical: 1.75 percent w/w). Several physical (wound contraction, epithelialization rate, tensile strength) and biochemical (hydroxyproline, hexosamine, proteins, DNA) parameters were investigated during the

study. We found the oxidant product (lipidperoxidase), antioxidant enzymes (catalase, superoxide dismutase), and reduced glutathione. The morphology and histology of skin tissues were investigated. When the animals were treated topically with PFEA (1.75% w/w) and EPF (10% w/w), wound healing resources increased significantly ($p < 0.05$). It was revealed that the amounts of hydroxyproline, hexosamine, protein, and DNA were significantly (p 0.05) increased within, reaching 59.22, 70.42, 61.01, and 60.00 percent. This effect was confirmed by histological and morphological representation, which resulted in considerable reepithelialization of the recovery area (p 0.05). In addition, EPF and PFEA demonstrated significant antioxidant activity ($p < 0.05$). The study offered clinical proof that P. fulgens, which is high in polyphenolic chemicals, has outstanding wound healing properties, corroborating previously documented claims. Léguillier et al. (2015) studied five Calophyllum inophyllum oil (CIO) extracts from Indonesia (CIO1), Tahiti (CIO2, three), the Fiji Islands (CIO4), and New Caledonia (CIO5) in 2015. Their cytotoxic, wound-recovery, and antibacterial activities were investigated, and the results were used to develop a comprehensive guide to their traditional usage and validate their safety. The Alamar blue assay on human keratinocyte cells verified the 5 CIO's protection. Human keratinocyte cells will be employed in the scratch check assay at CIO wound healing centers. CIO-inspired antibacterial innate immune response was tested by ELISA, which detected the production of defensin-2 in human byproduct macrophages. The antibacterial activity of CIO was investigated using oilogramme against 20 cardio Gram- and 20 cardio Gram+ bacterial species, as well as a multi-drug resistant strain of S. aureus and anaerobic Gram+ bacterial species such as Propionibacterium acnes and Propionibacterium granulosum. We used bioautography on Staphylococcus aureus to identify the polarity profile of the compounds responsible for antibacterial action. Using the Alamar Blue assay, we were able to confirm that CIO could effectively treat 2.7 to 11.2% of keratinocyte cells. When it comes to healing, every CIO tested improved in vitro wound closure. When keratinocytes were cultivated after scratch with CIO at 0.1 percent, the healing element was 1.3 to 2.1 times better than the control. Furthermore, our data reveal that CIO has remarkable antibacterial effects: one against Gram+ microbes via direct reduction of mitotic development, and another

substantial influence against Gram- microorganisms via increased production of -defensin 2 peptide via macrophages. It is noteworthy to note that the CIO concentrations required to promote wound healing and prevent microbial growth are lower than those required to produce cytotoxicity in keratinocyte cells. We conducted a bioautography experiment against S.aureus to evaluate the polarity profile of the additives responsible for CIO's antibacterial activity. Our findings showed that the additives responsible for bacterial growth prevention are particularly polar at the TLC chromatographic profile and are present in the oil's resinous fraction. This study examined the cytotoxicity, wound healing, and antibacterial activities of five CIO that have previously been used to treat inflammatory wounds. We used mobile and microbe cultures to illustrate CIO's pharmacological effects as a wound healing and antibacterial medicine. Furthermore, we confirmed that CIO awareness is required to demonstrate that healing effects are less common than concentrations that produce in vitro harmful results. This study provides guidelines for typical CIO uses for the first time. CIO is an effective treatment for inflammatory wounds, particularly in tropical settings, due to its antibacterial and wound healing characteristics.

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examined the cytotoxicity, wound healing, and antibacterial activities of five CIO that have previously been used to treat inflammatory wounds. We used mobile and microbe cultures to illustrate CIO's pharmacological effects as a wound healing and antibacterial medicine. Furthermore, we confirmed that CIO awareness is required to demonstrate that healing effects are less common than concentrations that produce in vitro harmful results. This study provides guidelines for typical CIO uses for the first time. CIO is an effective treatment for inflammatory wounds, particularly in tropical settings, due to its antibacterial and wound healing characteristics.

Previous work done on the proposed plants used in the investigation

Pattanayak Priyabrata *et al* 2012	*O. sanctum L.* has been shown to have anti-fertility, anticancer, antidiabetic, antifungal, antibacterial, cardioprotective, analgesic, antispasmodic, and adaptogenic properties. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), an active ingredient of O. sanctum L., has been determined to be substantially responsible for its medicinal potential.
Shetty *et al* 2006	The aqueous extract of *O. sanctum L.* was evaluated for its wound healing efficacy in rats. Wound-breaking strength in the incision wound model, epithelization duration, and percent wound concentration in the excision wound model were all investigated due to increased % wound contraction.*Ocimum sanctum L.* may help regulate aberrant healing, including keloids and hypertropic scars.
Udupa sl *et al* 2006	The ethanolic extract of *O. sanctum L.* leaves was tested for both normal wound healing and dexamethasone-depressed healing. The extract considerably boosted wound breaking strength, wound epithelialization speed, and wound contraction, as well as an increase in wet and dry granulation tissue weight and strength. In addition, the extract greatly reduces dexamethasone's anti-healing effects in all wound healing models.

Prakash.P *et al* 2005	*Ocimum sanctum,* also known as tulsi in Hindi and holy basil in English, is an upright, soft, hairy scented herb or undershurb found throughout India. Tulsi is often grown in gardens. Two types of *Ocimum sanctum* are found in cultivation: tulsi plants with green leaves known as sri tulsi and tulsi plants with purple leaves known as Krishna tulsi. *Ocimum sanctum* is revered to Hindus and is used as a medicinal plant in Indian homes to treat a variety of diseases. It demonstrates a variety of therapeutic activities. It is used to treat cancer, fertility, diabetes, and other diseases.
Godhwani S *et al* 1987	Methanolic extract and aqueous suspension of *O. sanctum* L. (500 mg/kg) inhibited acute and chronic inflammation in rats, as tested by carrageenin-induced pedal edema and cratonoil-induced granuloma and exudates. The response was comparable to that of 300 mg/kg sodium salicylate. Both the extract and the solution demonstrated analgesic effect in a mouse hot plate method, and the methanol extract increased the tail withdrawal reaction time of a sub-analgesic dosage of morphine. Both preparations reduced pyrexia generated by the typhoid-paratyphoid A-B vaccination. The antipyretic activity of methanol extract and aqueous suspension was weaker and lasted less time than that of 300 mg/kg sodium salicylate.
Singh S *et al* 1998	Linoleic acid found in the fixed oil of various species of *O. sanctum L.* has been shown to inhibit the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism, potentially contributing to its anti-inflammatory properties.

Kelm MA *et al* 2000	Compounds from O. sanctum L. extract, including Civsilineol, Civsimavatine, Isothymonin, Apigenin, Rosavinic acid, and Eugenol, were tested for anti-inflammatory and cyclooxygenase inhibitory properties. Eugenol inhibited cyclooxygenase-1 by 97% at 1000 μM (pn). Civsilineol, Civsimavitin, Isothymonin, Apigenin, and Rosavinic acid inhibited cyclooxygenase-1 by 37, 50, 37, 65, and 58%, respectively, at 1000 μM doses. These compounds had comparable activity to Ibuprofen, Naproxen, and Aspirin at doses of 10, 10, and 1000 μM..
Barua *et al.* 2010	*Azadirachta indica A. Juss*, often known as Neem, has been shown to have strong antibacterial, immunomodulatory, and anti-inflammatory properties that complement the wound healing process.
Indra Prasad Pandey, *et al.*, 2012	It has been said that every portion of this plant can be used as a herb. Neem oil has active compounds that immediately aid in the wound healing process. As a result, neem has a direct effect on the wound healing process, allowing the skin to retain its suppleness as it heals. Clinical investigations have shown that it reduces inflammation just as effectively as cortisone acetate, which promotes wound healing.
Hawkins *et al* 2006	*A.indica* is a source of terpenoids, which play an important role in wound healing. Terpenoid strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply.
Debjit Bhowmik *et al* 2010	Nimbidin, a component of Neem, has been show to posses' potent anti-inflammatory and antiarthritis activity. Nimbiden suppresses the functions of macrophages and neutrophils involved in inflammation.
Suaib Luqman *et al* 2012	Investigated effect of *Moringa oleifera* leaf and fruit extracts on markers of oxidative stress, its toxicity evaluation, and correlation with antioxidant properties using in vitro and in vitro assays. The aqueous extract of leaf was able to increase the GSH and reduce MDA level in a concentration-dependent manner. The ethanolic

	extract of fruit showed highest phenolic content, strong reducing power and free radical scavenging capacity. The antioxidant capacity of ethanolic extract of both fruit and leaf was higher in the in vitro assay compared to aqueous extract which showed higher potential in vivo. Safety evaluation studies showed no toxicity of the extracts up to a dose of 100 mg/kg body weight. Our results support the potent antioxidant activity of aqueous and ethanolic extract of *Moringa oleifera* which adds one more positive attribute to its known pharmacological importance.
Paikra *et al* 2017	*Moringa oleifera Lam.*, also known as munga, is a popular nutritional herb that contains valuable pharmacological properties such as anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-fertility, anti-cancer, anti-microbial, anti-oxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic activity. The plant is also known as the horseradish tree and the drumstick tree. This plant's entire structure offers excellent therapeutic properties. It has high levels of vitamin A, vitamin C, and milk protein. There are several types of active phytoconstituents, including alkaloids, protein, quinine, saponins, flavonoids, tannin, steroids, glycosides, fixeMoringa oleifera Lam., also known as munga, is a popular nutritional herb that contains valuable pharmacological properties such as anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-fertility, anti-cancer, anti-microbial, anti-oxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic activity. The plant is also known as the horseradish tree and the drumstick tree. This plant's entire structure offers excellent therapeutic properties. It has high levels of vitamin A, vitamin C, and milk protein. There are several types of active phytoconstituents, including alkaloids, protein, quinine, saponins, flavonoids, tannin, steroids, glycosides, fixed oil, and lipids. This plant is also found in tropical climates. Other ingredients include niazinin A, niazinin B, and niazimicin A, niaziminin B. The current review looks at the phytochemical composition,

	therapeutic applications, and pharmacological activities of this plant.d oil, and lipids. This plant is also found in tropical climates. Other ingredients include niazinin A, niazinin B, and niazimicin A, niaziminin B. The current review looks at the phytochemical composition, therapeutic applications, and pharmacological activities of this plant.

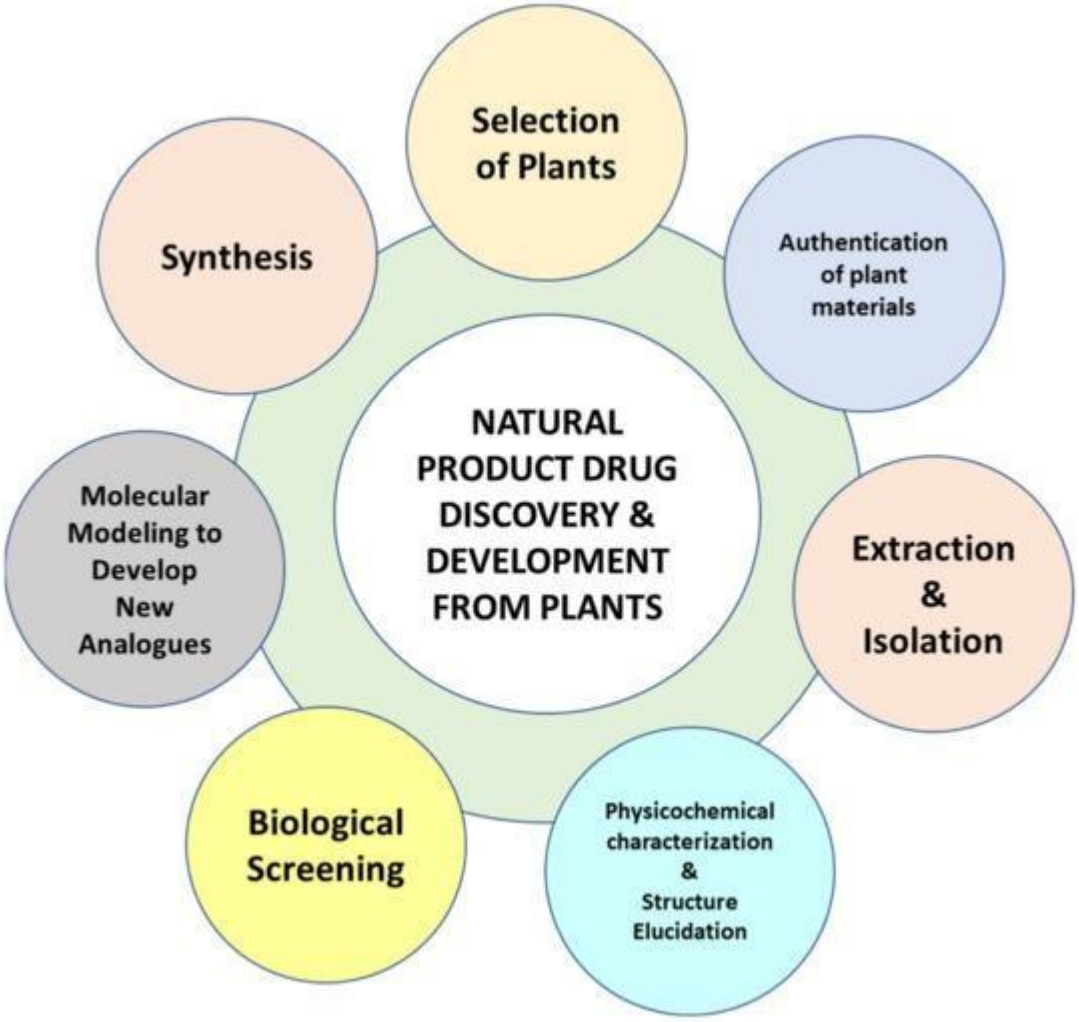


Fig 24: Previous work done on Molecular docking studies

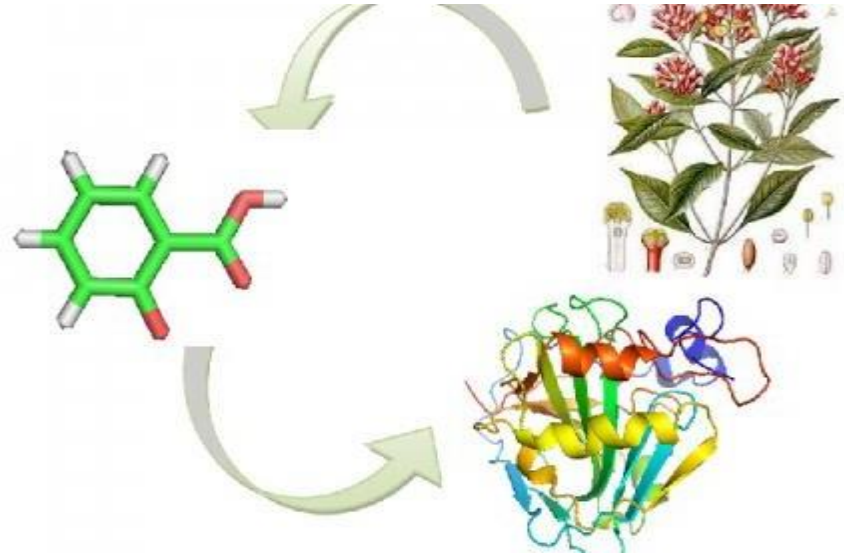


Fig 25: Basic approach of Drug designing

Adegbola PI et al. (2021) investigated the inhibitory potentials of Azadirachta indica and Xylopia aethiopica isolates against SARS-CoV-2 viral accessory proteins and the host serine protease. The protein data (SARS-CoV-2 Papain-like protease (PLpro) (PDB: 6wx4), Chymotrypsin-like main protease (3CLpro) (PDB:6YB7), SARS-CoV nsp 12 (PDB: 6nus), and Host cell protease (TMPRSS1) (PDB:5ce1) were obtained from the protein data bank (PDB), while the SDS format of each Ligand was obtained from the Pubchem database. The molecular docking analysis was carried out with Auto Dock Vina 1.5.6, and the interaction between the ligands and protein was visualised using Discovery Studio 2019. The vNN Web Server was utilised to obtain the ADMET prediction of the ligands' pharmacokinetics and toxicity characteristics. Our findings revealed that all plant isolates exhibited negative Gibb's free energies, indicating high binding affinities for both virus and host protein. Overall, 23 of the 47 isolates demonstrated good binding affinities similar to the reference drug dexamethasone. Azadironic acid, Nimbionone, Nimbionol, and Nimocinol are the only compounds with favourable pharmacokinetics and toxicity profiles, despite the fact that several of the compounds have high binding affinity for viral and host proteins. This study gives light on potential SARS-CoV-2 inhibitors and novel therapy strategies. Future research will focus on the wet laboratory validation of Azadironic acid, Nimbionone, Nimbionol, and Nimocinol against coronavirus disease.

Liman R et al. (2022) evaluated root growth, mitotic index (MI), chromosomal aberrations (CAs), and DNA damage using A. cepa ana-telophase and alkaline comet assays. Additionally, molecular docking was used to determine pethoxamid's affinity for binding to DNA and VLCFA synthases. The length of onion roots was greatly reduced in the test to limit root growth in a concentration-dependent manner. In A. cepa root cells, pethoxamid increased CAs such as interrupted ana-telophase, chromosome laggards, stickiness, anaphase bridges, and DNA damage while lowering MI in a concentration- and time-dependent way. Molecular docking revealed that pethoxamid has a high binding affinity for all synthases involved in the sequential generation of VLCFAs and preferentially binds to GC-rich locations in the minor groove of the DNA structure. It was discovered that the herbicide's varied binding capabilities with DNA and VLCFA synthases could be the cause of pethoxamid-induced genotoxicity and cytotoxicity.

Hassan HA et al. (2023) evaluated the fungal diversity of Allium cepa and discovered 11 isolates that were morphologically recognised. Penicillium sp. (LCEF10), one of the isolated fungal strains, demonstrated potential anti-infective activity against the tested microbes (Fusarium solani ATTC 25922, Pseudomonas aeruginosa (ATTC 29231), Staphylococcus aureus ATTC 27853, Candida albicans ATTC 10231), and their MICs were measured using the well diffusion method, so it was subjected to molecular identification and phylogenetic analysis. Furthermore, strain LCEF10's ITS sequence showed a consistent assignment, with the highest sequence similarity (99.81 percent) to Penicillium oxalicum NRRL 787. The crude ethyl acetate extract of Penicillium sp. LCEF10 was analysed for metabolomics using LC-HR-ESI-MS. The metabolic profiling revealed the presence of polyketides, macrolides, phenolics, and terpenoids. Furthermore, an in silico molecular docking analysis was performed to determine which chemicals were most likely responsible for the anti-infective properties.

Cosio T et al. (2023) executed an against one another, randomized controlled trial to evaluate the appearance of 64 subjects' post-surgical scars after two times daily topical application, comparing the effect of a class I pullulan-based medical device containing 5% Allium cepa extract and 5% hyaluronic acid gel to a class I medical device silicone gel on new post-surgical wounds. 2) Method: After four, eight, and twelve weeks of treatment,

objective scar assessments were conducted using the Vancouver Scar Scale (VSS), POSAS, and other ratings, followed by statistical analysis. The trial was registered with clinicalTrials.gov (NCT05412745). Parallel molecular docking simulations were used to investigate the role of Allium cepa in the TGF-/Smad signal pathway. Results (3) We discovered that in participants who used devices containing Allium cepa and HA at weeks 4 and 8, VSS, POSAS scale, itching, and redness decreased significantly. Following 12 weeks of treatment, no statistically significant differences in evaluated scores were seen. Safety was further assessed by gathering negative incidents linked with the gel's application. Both trial groups demonstrated equal levels of subject adherence to the recommended gel and safety. Molecular docking simulations reveal that Allium cepa suppresses fibroblast growth and contraction via the TGF-/Smad signalling pathway. (4) Conclusion: The topical administration of a pullulan-based medical device containing HA and Allium cepa significantly reduced local inflammation, potentially minimising the likelihood of keloids or hypertrophic scars.

Muhammad SA et al. (2015) conducted computational docking to investigate the inhibitory effects of quercetin glycosides. Quercetin glycosides, a naturally occurring metabolite, were isolated from buckwheat and onions to serve as a ligand for molecular interaction. The crystallographic structure of the molecular target angiotensin-converting enzyme (ACE) (peptidyl-dipeptidase A) was obtained from the PDB database (PDB ID: 1O86). For the purpose of comparison, the well-known ACE inhibitor Enalapril was employed as the gold standard. PyRx's AutoDock Vina functionality, which relies on scoring functions, was utilised to do computational docking research. When compared to the standard (-7.0 kcal/mol), quercetin's binding energy to angiotensin-converting enzyme was optimal at -8.5 kcal/mol. These findings suggested that quercetin glycosides could be a useful ligand for treating hypertension, myocardial infarction, and congestive heart failure.

CHAPTER - 3

PROFILE OF SOME PROPOSED PLANTS FOR RESEARCH SHEVAGA

Fig 26: Moringa oleifera

TAXONOMIC CLASSIFICATION (Ved DK *etal*;2016)	**COMMON VERNACULAR NAME (Ved DK *etal*;2016)**
Kingdom - Plantae **Sub kingdom** - Tracheobionta **Super Division** - Spermatophyta **Division** - Magnoliophyta **Class** - Magnoliopsida **Subclass** - Dilleniidae **Order** - Capparales **Family** - Moringaceae **Genus** - *Moringa* **Species** - *oleifera*	**English** - Moringa, Drumstick tree, Horse radish tree **Latin** - Moringa oleifera **Sanskrit** - Surajana **Hindi** - Sahjan (सहजन) **Tamil** - Amukira **Kannada** - Keramaddinagaddi **Gujarati** - Saragvo **Bengali** - Sojne danta **Oriya** - Sajana or Sujuna **Punjabi** - Surajana **Chinese** - La mu (辣木)

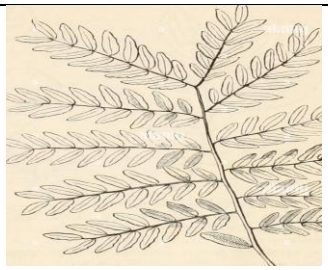
Habit: A deciduous tree, up to 12m with medium-sized.

Part used: Leaves

Description: A delicate tree of modest height with corky bark. Compound leaves have immature, light-green leaflets that darken with age and turn yellow in the fall. typically found in forests as a means of escape, but because cuttings grow quickly, they are also

employed as A fragile tree that comes to a moderate height and has bark that is corky.

Compound leaves feature leaflets that are light green and young. As the leaves mature, they take on a darker colour and eventually turn yellow. Providing a route of escape, they are generally found in forests; nevertheless, because to the rapid growth of cuttings, they are also used as a hedge. Small and delicate, flowers have a perfume that is reminiscent of honey. A capsule that ranges in length from nine to twenty inches is the fruit.a hedge. Flowers are small, delicate, and honey-scented. The fruit is a capsule that is 9 to 20 inches long.

Leaves Description	
Leaf arrangement: Alternate -spiral	
Leaf type: Tri-pinnate	
Leaf shape: Ovate or elliptic	
Leaf Apex: Rounded	
Leaf Base: Rounded	

CHEMICAL CONSTITUENTS

There are a number of major components that are found in the moringa plant. These include deic, palmitic, and stearic acid, saponins, glycoside, gum, and protein. Vitamins B1, B2, B3, C, and A (each containing 8855 international units per 100 grammes). There are four types of minerals: calcium, iron, phosphorus, and magnesium. It is possible to find significant quantities of the vitamins A, B, and C, as well as riboflavin, nicotinic acid, folic acid, pyridoxine, beta-carotene, calcium, iron, and alpha-tocopherol, in the pods, flowers, and leaves of the plant. The high flavonoid content of the Moringa genus is one of the factors that contributes to the powerful antioxidant impact that it possesses. The flavanol and glycoside types of flavonoids make up the majority of the flavonoids that are found in this genus. Routine, quercetin, rhamnetin, kaempferol, apigenin, and myricetin are the flavonoids that are found in the genus with the highest frequency of occurrence. 4-O-(-L-rhamnopyranosyloxy)-benzyl glucosinolate is the glucosinolate that is found in the greatest abundance in this species. Gallic acid is the phenolic acid that is found in the leaves of M. oleifera the most frequently. Furthermore, the leaves include elagic acid, ferulic acid, caffeic acid, o-coumaric acid, and chlorogenic acid. These acids are all constituents of the plant. According to Nur Zahirah Abd Rani et al. (2018), the leaves and seeds were utilised in the process of isolating sitosterol.

Traditional uses	**Biological Activity**
Leaf = Diarrheal, dysentery, colitis, sores, skin infection, cut, scrapes, anemia, rashes & sign of aging. **Seeds** = warts **Oil** = Gout and acute rheumatism **Flower** = Tumor, inflammation, Hysteria, enlargement of spleen & muscles diseases. **(Silver, J ;2017 & Parrotta, J. A. 1993)**	Antispasmodic, Antihypertension, Anti-inflammation,Antifertility,Antihyperglycemic, Antihyperlipidemic, and Hypocholesterolemic, Antiviral, Antileishmanial, Anti-convulsant,Anti-microbial & anticancer activity. **(Nur Zahirah Abd Rani *eta;*2018 & Anwar, F *etal*. ; 2007)**.

TULSI

Fig 27: TULSI

TAXONOMIC CLASSIFICATION (Priyabrata Pattanayak *etal*;2010)	**COMMON VERNACULAR NAME**
Kingdom: Plantae **Division:** Magnoliophyta **Class:** Magnoliopsida **Order:** Lamiales **Family:** Lamiaceae **Genus:** Ocimum **Species:** O. tenuiflorum **Scientific Name:** *Ocimum Sanctum*	**English** = Holy Basil **Sanskrit** =Tulasi **Other names** = Manjari, Krishna Tulsi, Trittavu, Tulshi and Thulsi.

Part uses: Leaf

CHEMICAL CONSTITUENTS

Minerals such as calcium, zinc, and iron are present, in addition to chlorophyll and other phytonutrients. Vitamins C and A are also present. It has also been shown to facilitate the proper digestion, absorption, and utilisation of nutrients derived from food and other herbs. Per one hundred grammes, the edible component contains 30 kilocalories of protein, 0.5 grammes of fat, 2.3 grammes of carbs, 287 milligrammes of phosphorus, 15.1 milligrammes

of iron, and 25 milligrammes of vitamin C. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), euginal (also known as eugenic acid), urosolic acid (2,3,4,5,6a,7,8,8a,,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid), and carvacrol (5-isopropyl-2-methylphenol) are all components that may be found in the volatile oil collected from the leaves. Seed volatile oil contains undec-4-ene, methyl carvicol (also known as estragol: 1-allyl-4-methoxybenzene), fatty acids, and sitosterol. Additionally, the seed mucilage contains certain sugars, and the green leaves contain anthocyans. All of these components are found in the seed vapour. According to Priyabrata Pattanayak et al. (2010), the sugars are made up of polysaccharides and xylose.

Traditional uses	**Biological Activity**
Expectorant, analgesic, anti-cancer, anti-asthmatic, anti-emetic, anti-diarrhetic, anti-fertility, hepatoprotective, hypotensive, hypolipidmic, and antistress drugs are all examples of pharmaceuticals that are used during pregnancy. Tulsi has also been utilised as a cure for a variety of conditions, including fever, bronchitis, arthritis, convulsions, and others. A decoction of Tulsi leaves that is diluted in water is given to patients who are experiencing issues with their liver and gastrointestinal tract. The juice of the leaves of Ocimum sanctum L. is combined with Triphala in Ayurvedic eye drop formulations that are advised for the treatment of glucoma, cataract, persistent conjunctivitis, and other eye disorders that cause terrible pain. In addition, patients are given the juice of effervescent leaves in order to cure persistent fever, diarrhoea, bleeding, and dyspepsia.. **(Khanna N *etal*;2003)**	It has antidiabetic, cardiac, wound-healing, radioprotective, hypolipidemic, antioxidant, and anti-microbial effects.**(Gupta S *etal*;2006)**

NEEM

Fig 28: NEEM

TAXONOMIC CLASSIFICATION (Soni H *etal*;2012)	**COMMON VERNACULAR NAME**
Kingdom: Plantae	**Common Name-** Nimba
Division: Magnoliophyta	**Sanskrit**- Arishta,Nimba
Order: Sapindales	**English-** Margosa tree, Neem tree
Family: Meliaceae	**Hindi**- Nim, Nimb
Genus: *Azadirachta*	**Punjabi**- Nim
Species: *A. indica*	**Nigeria** - Dogoyaro

Part uses: Leaf

CHEMICAL CONSTITUENTS

Neem oil is composed of bitter components that are extracted from various plant sections. These components include meliacin, azadirachtin, gedunin, nimbidine, nimbolids, nimbin, salanin, meliacin, and valastin. Neem oil is a powerful anti-inflammatory agent. In the year 1968, Butterworth and Morgan made the initial discovery of azadirachtin. Nimbin, melantriol, salannin, and azadirachtin were the four major substances that were classified as limonoid compounds. Limonoids can kill insects and pests, making them a popular pesticide. **(Ahmad Eid *etal.*,2017)**.

Traditional uses	**Biological Activity**
The twigs of the Neem tree are commonly used in India for the purpose of cleaning teeth. In addition, despite the fact that they are relatively unattractive to patients, the branches of the neem tree are among the most practical techniques of dental therapy that are recognised by traditional medicine. Surprisingly, the neem plants present a wonderful opportunity for the development of contemporary dental care products. In addition, the leaves of the neem tree are used as a herbal remedy for persons who suffer from acne. In addition, neem leaves can be utilised as a treatment for eyes that are irritated. An infusion that is very similar to this one can also be used to treat sore throats. **(Biswas K *etal*;2002)**	In addition to having anti-inflammatory, antipyretic, analgesic, male antifertility, and antiulcer qualities, it also has antibacterial, antifungal, antimalarial, anticancer, and antiviral activities.. **(Yogesh W *etal*;2015)**

CHAPTER - 4
RATIONALE OF THE STUDY

According to a comprehensive analysis of the ethno-medical history, the plants *Moringa oleifera, Ocimum Sanctum,* and *Azadirachta indica* are known to contain a multitude of phytopharmaceuticals that possess a wide range of pharmacological properties. These properties include anti-inflammatory, anti-pyretic, analgesic, anxiolytic, hepatoprotective, immunomodulatory, and antioxidant effects. It is acceptable to draw the conclusion that the active substances in the selected plants also possess anti-inflammatory, anti-microbial, and wound-healing characteristics. This conclusion is based on the flavonoids the plants contain and the antioxidant activity they possess. Wound infection is one of the most common diseases in countries with lower levels of economic development. This is mostly due to the lack of sufficient hygiene conditions. The healing of wounds is a common problem scenario that occurs in patients and other individuals who have immune systems that are compromised. All the medications that were a part of this research project have already proven their unique efficacy in a number of different commercial formulations. The following is a list of some of the commercial formulas that are the subject of this discussion:

WOUND HEALER (*FAME PHARMACEUTICALS*)	**PURIM** (*HIMALAYA HEALTH CARE*)
COMPOSITION- Each tablet contains - *Curcuma longa* extract *Aloe vera* extract *Lawsonia alba* extract *Emblica officinalis* standard powder	**COMPOSITION** **Each tablet contain** *Curcuma longa* *Cassia fistula* *Psorelea coryfolia* *Picrorhiza kurroa*

All of the commercial formulations that have been addressed up until this point are available in tablet form, which may result in a delayed or delayed reaction. In light of this, preparations have been made to create a formulation comprising a mixture of All of the commercial formulations that have been addressed up until this point are available in tablet form, which may result in a delayed or delayed reaction. As a result, efforts have been made to develop a formulation of these three treatments in combination, with a gel foundation, that has the potential to be more effective when applied topically. This is done with the goal of treating the wound in a more expedient manner while minimising the risk of adverse effects. Furthermore, they are easily accessible, inexpensive, and don't cause any harm to the environment. These three plant extracts, which have a gel foundation, have the potential to be more successful when applied topically, as they have the ability to cure wounds more quickly and without causing any adverse effects. In addition to this, they are readily available, affordable, and have a small level of toxicity involved.

AIM AND OBJECTIVES OF RESEARCH WORK

***"The art of healing comes from nature and not from the physician. Therefore the physician must starts from nature with an open mind"*- Paracelsu**

Traditional medicine makes considerable use of medicinal herbs that have anti-inflammatory qualities in order to treat a variety of inflammatory and microbial invasion issues. There is an absolute requirement for additional research in this area since antimicrobial chemicals generated from plants are a resource that is primarily underutilised but possesses immense potential in the medical industry. In the conventional medical system, there are a number of medicinal plants that are believed to assist in the healing of wounds. Despite the fact that only a few number of these plant remedies—both single plant and multi-herbal preparations—have been subjected to a comprehensive scientific investigation into their mechanisms of action and effectiveness, they have been employed for millennia.

In spite of the fact that there are a great number of synthetic pharmaceuticals available for the treatment of wounds, herbal medications are more widely used since they are less expensive and have fewer or no bad effects.

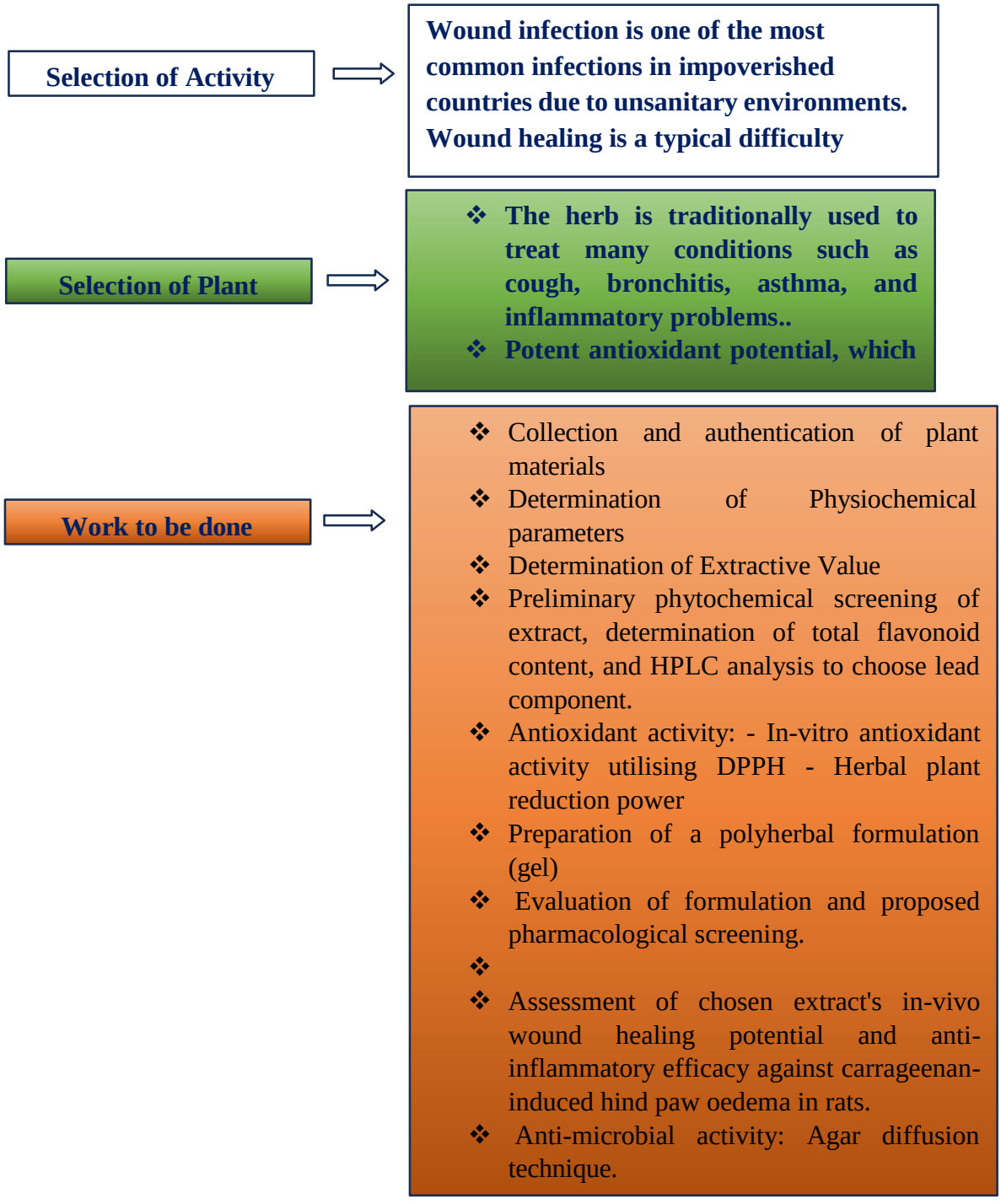
A formulation of these three pharmaceuticals that is a combination has been devised utilising a gel base in order to cure the wound more quickly and without any adverse effects. They have been combined in this formulation. When used topically, this compound is more efficacious than when taken systemically. Additionally, they are easily accessible, inexpensive, and have a low level of toxicity associated with them.

To investigate the possible effects of various agents, such as the antibacterial, anti-inflammatory, and wound-healing capabilities of plant origin, a polyherbal formulation consisting of a variety of extracts is selected. Since this was the case, the purpose of the current inquiry was to analyse the capacity of extracts of selected plants to improve wound healing and to scientifically validate this capacity using molecular docking analysis against the enzyme glycogen synthase kinase 3-protein.

CHAPTER - 5

PLAN OF WORK

Conceptual Framework of current investigation:



CHAPTER - 6

EXPERIMENTAL WORK

6.1 Preliminary Work

(a) Literature Review

After gaining enough data from noteworthy articles and journals, it was found that there was room to research the other pharmacological properties of the following plant.:

a) Moringa oleifera

b) Ocimum sanctum

c) Azadirachta indica

Hence it was selected for further studies.

(b) Collection and Authentication of Plant

In the month of February, the plant was collected from the Shivpuri area in the state of Madhya Pradesh. A herbarium file in which plant pieces were included was developed and certified.

(c) Drying and Size Reduction of Plant Material

On the basis of these findings, additional research was conducted. matter for the component was shade-dried. It was pounded into granular powder using mils. To guarantee consistency, coarse powder was forced through a #20 sieve and stored in a covered container in a cool, dry location. These findings were used for further inquiry.

d) Determination of solvent extractive value: (Mukherjee, 2002)

1) Determination of water soluble extractive value

5 gm of powdered crude medication was macerated with 100 ml of water in a closed flask for 2 hours, agitated intermittently for 6 hours, and stored safely for 18 hours. Following filtration, 25ml of the filtrate was evaporated to dryness in a shallow dish with a flat bottom coated in tar, dried at 105°C, and then weighed. The fraction of water-soluble extractives

was estimated using air-dried medicine as a baseline.

2) Determination of alcohol soluble extractive value

Tannins, alkaloids, and resins can be extracted from a wide range of substances using alcohol as a solvent. Ethyl alcohol (95 percent v/v) was employed to determine the extractive's solubility. A closed flask was used to macerate 5 gm of powdered medication with 100 mL of ethanol for 24 hours, occasionally shaking it for 6 hours, and then leaving it for 18 hours before filtering. The filtrate was evaporated till dry in a tar-coated flat-bottomed shallow dish, then dried at 105°C and weighed. The air dried medicament used as a reference point for calculating the proportion of extractives that are ethanol soluble.

3) Determination of Moisture Content: (Mukherjee, 2002)

Managing the moisture content of the crude medicine is also necessary. Moisture content should be kept as low as possible to minimise raw drug deterioration caused by chemical changes or microbial contamination.

Procedure: The proper weight and storage of 5g of powdered crude drug samples in an infrared moisture balance. The weight loss was calculated as a percentage (%) moisture loss compared to an air-dried sample of crude medication.

(d) Preparation of Crude Extract

The Soxhlet apparatus was commonly used in a series to create extracts from diverse plant materials. The following sections address distinct plant extraction techniques:

A. Extraction of Shewaga

Extraction of powder of leaves was done by solvent extraction process.

Soxhlet Extraction

The solvent extraction was done using a Soxhlet equipment. Alcohol was employed as the extraction process' solvent.

Fig 29: Soxhlet Extraction process of Shewaga

Soxhlet Extraction

100 gm. of coarsely powdered leaves

↓

Soxhlatation with ethanol for 48 hr. at 60-70^{0}c

↓

Extract was collected & dried at room temp. For 5days

↓

Extract was collected as semisolid paste & weighted

B. Extraction of Tulsi powder

Extraction of leaves powder was done by solvent extraction process.

Soxhlet Extraction

Soxhlet apparatus was used for the solvent extraction. The solvent used in the extraction process was ethanol.

Fig. 30: Soxhlet Extraction process of Tulsi

80 gm. of coarsely powdered leaves

↓

Soxlatation with ethanol for 30 hr. at 60-65^{0}c

↓ Extract was collected

Extract was dried at room temperature for 4 days

↓

Extract was collected as hard paste & weighted

C. Extraction of Neem powder

Extraction of Neem powder was done by solvent extraction process.

Soxhlet Extraction

Soxhlet apparatus was used for the solvent extraction. The solvent used in the extraction process was ethanol.

Fig 31: Soxhlet Extraction process of Neem

130 gm. of coarsely powdered leaves of neem

↓

Soxlatation with ethanol for 72 hr. at 65-70 ^{0}c

↓

Extract was collected & dried at room temp. for 5 days

↓

Extract was collected as hard paste & weighted

e) Qualitative Phytochemical analysis (Kokate, C.K, 2003 & Khandelwal K.R. 2003)

Diverse qualitative tests were performed on the obtained extracts to determine the presence or absence of common phytopharmaceutical compounds.

i. <u>Alkaloids</u>

With a few drops of diluted hydrochloric acid, a little amount of alcohol extract was mixed and filtered. The filtrate was then thoroughly examined using several alkaloid reagents, such as:

(A) Mayer's Reagent

Alkaloids precipitate after being treated with Mayer's Reagent. To 1 ml of extract, add 1 ml of Mayer's reagent (potassium iodide solution). The yellowish yellow precipitate indicated the presence of alkaloids.

(B) Dragendorff's Reagent

As a Dragendorff reagent, the alkaloid precipitates in an orange-brown colour. Dragendorff's reagent was applied to 1 ml of extract (potassium iodide bismuth solution). The orange-red precipitate indicated the presence of alkaloids.

(C) Hager's Reagent

Using Hager's Reagent, alkaloids produce a yellow precipitate. A saturated aqueous solution of picric acid in 3 ml of Hager reagent was mixed with 1 ml of extract. The presence of alkaloids was shown by the yellow precipitate.

(D) Wagner Reagent

The alkaloid works as a Wagner reagent, producing a reddish brown precipitate. To detect alkaloids, add 2 ml of Wagner reagent (iodine in potassium iodide) to 1 ml of extract and observe the formation of a reddish brown precipitate.

ii. <u>Carbohydrates and glycosides:</u>

Each extract was separately diluted in distilled water and filtered in small amounts. The filtrate is put through the following tests for carbohydrates:

(A) Molisch's test

2 mL of extract from the test tube's side was mixed with 1 mL of -naphthol solution and concentrated sulphuric acid. The presence of carbohydrates was shown by the formation of purple or purplish red where the two liquids mixed.

(B) Fehling's solution

A few drops of extract are combined with equal volumes of Fehling's A (copper sulphate in distilled water) and Fehling's B (potassium tartrate and sodium hydroxide in distilled water) reagents, which are then heated to create bricks. Red copper(I) oxide did precipitate.

(C) Benedikt-Test

If there are reducing sugars, 1 ml of the extract is treated with a few drops of the

Benedikt reagent, an alkaline solution containing a copper (II) citrate complex, and heated in a water bath until it turns reddish brown. A precipitate forms.

iii. <u>Proteins and free amino acids:</u>

Milon, biuret, and ninhydrin concentrations were determined using a small sample of alcohol extract diluted in a few millilitres of water.

iv. Gum: The extract's swelling properties were examined by gently adding 10 ml of extract to 25 ml of anhydrous alcohol, stirring continuously, filtering, and air-drying the mixture.

v. Terpenoids:

The drug flakes had Sudan III (alcohol solution) added to them. Terpenoids are present, as indicated by the colour red.

vi. Volatile oil:

By hydro-distillation method.

vii. Tannins:

Small amounts of alcoholic extracts were taken separately in water and tested for the presence of tannins and phenolic compounds using aqueous bromine solutions, a 1% gelatin solution containing 10% sodium chloride, and a 5% ferric chloride solution.Small amounts of alcoholic extracts were taken separately in water and tested for the presence of tannins and phenolic compounds using aqueous bromine solutions, a 1% gelatin solution containing 10% sodium chloride, and a 5% ferric chloride solution.

viii. **Flavonoids:**

(Shinoda test) (Magnesium hydrochloride reduction test):

Drop by drop, add strong hydrochloric acid to the test solution, along with 0.5 g of magnesium shavings, and then examine the colour.

Zinc Hydrochloride Reduction Test:

Zinc concentrate and powder are mixed into the test solution. Hydrochloric acid. Heat the mixture and then verify the colour.

Determination of Total Flavonoids Content

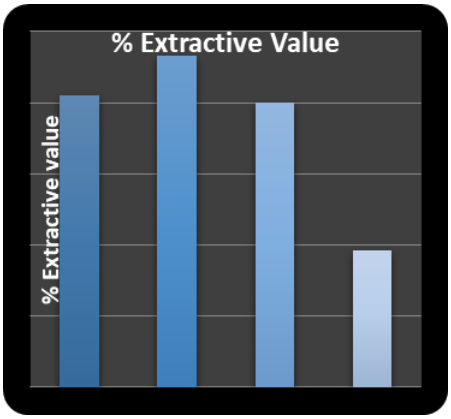
The total flavonoid concentration was calculated using the aluminium chloride colorimetric method in terms of quercetin equivalents. $AlCl_3$ (2 percent w/v) in methanol was mixed with 2 ml of plant extract (10 mg/ml) in each solvent (stock solution), which was then

added to a methanolic acetic acid solution (0.5 percent v/v) (Probe solution PS). To make 25 ml from 1 ml of SS, a methanolic acetic acid solution (CS) was used. After 30 minutes, the PS and SS absorbances were measured at 420 nm. The results were provided as a percentage of the total flavonoid content (Chen HY et al., 2007).

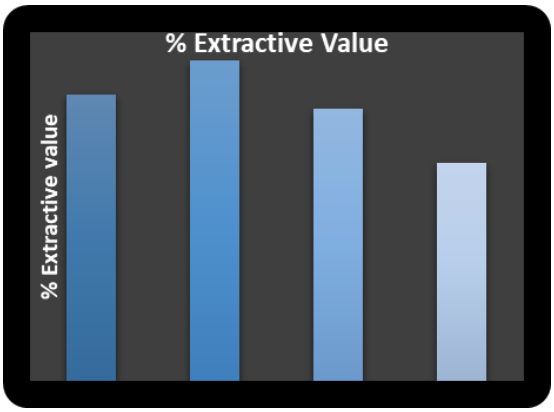
TFC = Absorbance at 420 x dilution x 100 / $E^{1\%}_{1\ cm}$ x wt. of extract in gm

Table 1: % Extractive value

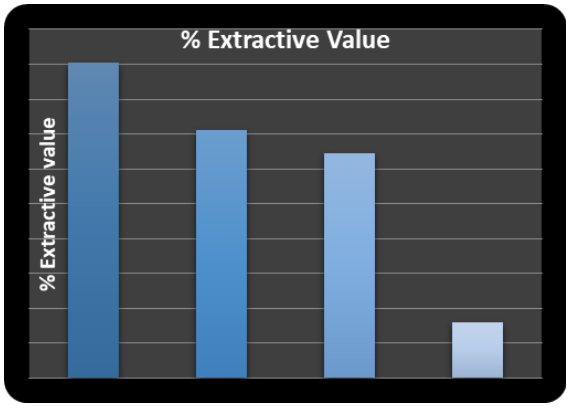
S.No.	Plant	% Extractive value			
		Water soluble	Ethanol soluble	Methanol soluble	Ether
1.	*M. oleifera*	8.2	9.32	8.01	3.86
2.	*O. sanctum*	4.1	4.6	3.9	3.12
3.	*A. indica*	18.1	14.21	12.9	3.22



Graph.1: % Extractive of *M. oleifera*



Graph.2: % Extractive of *O. sanctum*



Graph.3: % Extractive of *A. indica*

Fig 32: Different Extractives Graph

Table 2: Loss on Drying

S.No.	Plant	LOD
1.	*M. oleifera*	4.1
2.	*O. sanctum*	0.7
3.	*A. indica*	15.2

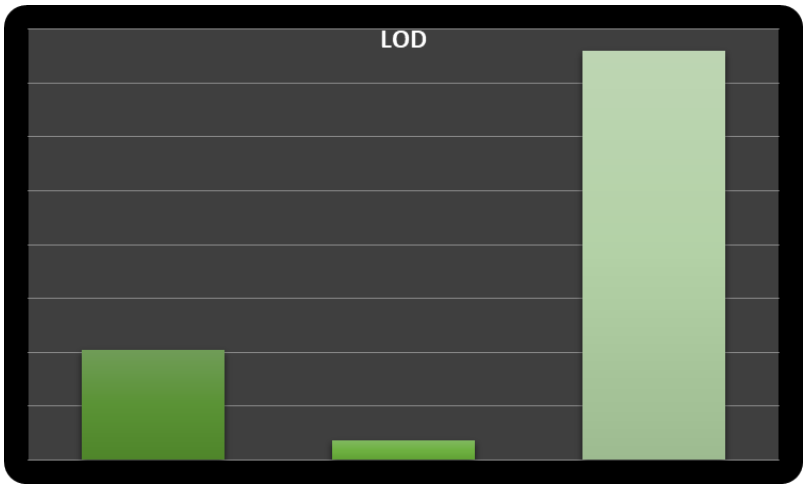


Fig 33: Graph.4: LOD

Table 3: Phytochemical Screening of Ethanolic leaves extracts

Test	*M. oleifera*	*O. sanctum*	*A. indica*
a) Carbohydrate			
Molish	(-)ve	(-)ve	(-)ve
Benedict	(-)ve	(-)ve	(-)ve
Starch	(-)ve	(-)ve	(-)ve
Hexose sugar	(-)ve	(-)ve	(-)ve
b)Tannin			
$FeCl_3$	(+)ve	(+)ve	(+)ve
c)Protein			
Biuret	(-)ve	(-)ve	(-)ve
Xanthoprotein	(-)ve	(-)ve	(-)ve
d) Amino acid			
Ninhydrin	(-)ve	(-)ve	(-)ve
e) Alkaloids			
Dragnodroff	(-)ve	(+)ve	(+)ve
Mayer	(-)ve	(+)ve	(-)ve

f) Steroid			
Salkowski	(+)ve	(+)ve	(+)ve
Libermann – Bucher	(-)ve	(+)ve	(+)ve
g) Flavonoids			
Shinoda	(+)ve	(+)ve	(+)ve
NaOH	(-)ve	(+)ve	(-)ve
Lead acetate	(+)ve	(+)ve	(+)ve
h) Coumarin	(-)ve	(-)ve	(-)ve
i)Glycosides			
Baljet	(-)ve	(-)ve	(-)ve
Legal	(-)ve	(+)ve	(-)ve
Killer-Killani	(-)ve	(-)ve	(-)ve

(+)ve = Present (-)ve = Absent

Table 4: Total Flavonoid Content

S.No	Sample	TFC(mg/gm)
1.	Ethanolic extract of *M. oleifera*	33.2
2.	Ethanolic extract of *O. sanctum*	25.38
3.	Ethanolic extract of *A. indica*	52.9

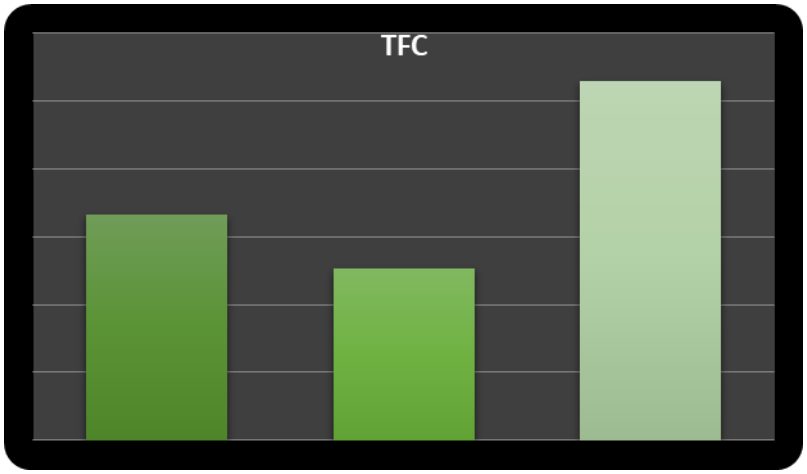
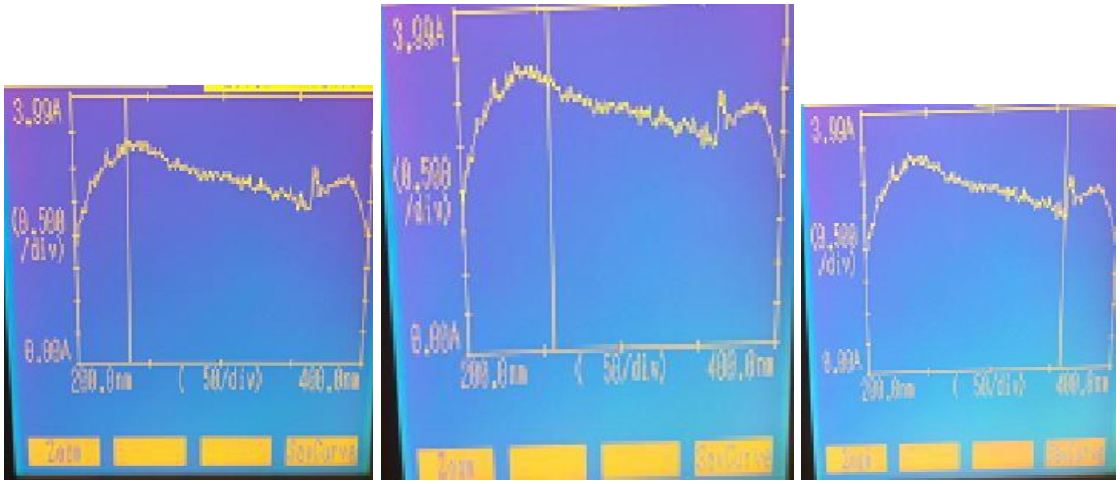


Fig 34: Graph.5 Total Flavonoid content

Spectrophotometrically analysis

The extracts were dissolved in ethanol, and Shimadzu 1700 UV spectrophotometer was used to measure the UV absorption peaks.



ETOH extract of *M. oleifera* **ETOH extract of *O. sanctum*** **ETOH extract of *A. indica***

Fig. 35 : UV Spectrum

6.2 Chromatographic Finger printing of Plant extracts HPLC analysis of plant extracts

The UV Finder LC100, Cyberlab TM, Salo Torrace, Millburry, MAO 1527, USA, and the LCUV100UV were used for the HPLC testing. Using a CAPCELL (C18) HPLC (4.6 mm IDX 250 mm), type MG 5 m, number AKAD/05245, the chromatographic partition was made. A 1.5 mL/minute flow rate was employed together with a 25 °C cutting temperature. There were twenty-five liters of ethanolic plant extract injected (Soni H et al., 2012).

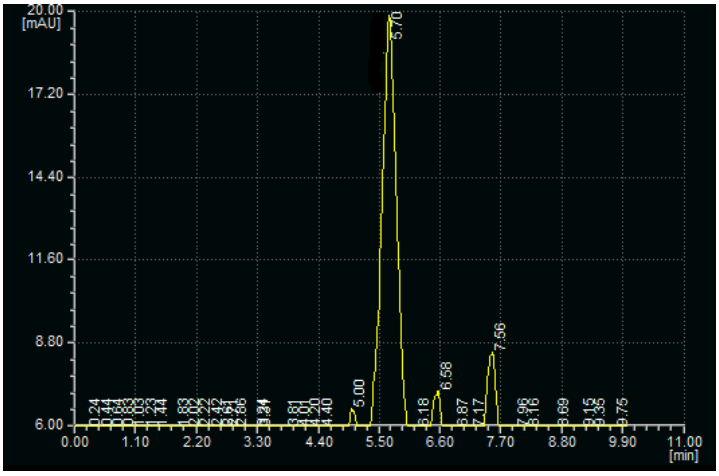


Fig.36: HPLC chromatogram of Ethanolic extract of *M. oleifera*

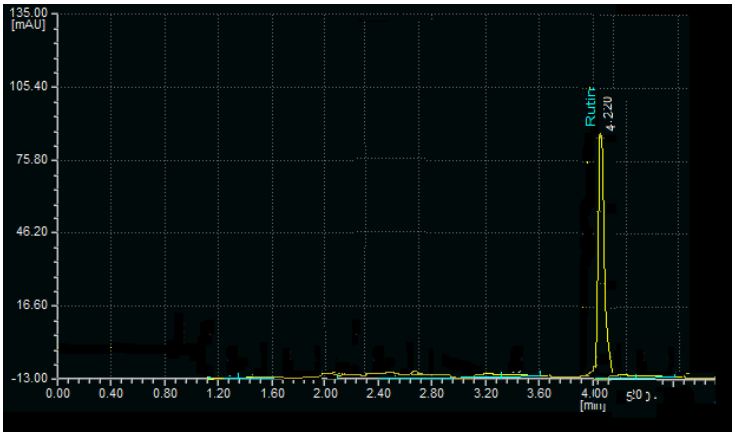


Fig.37 :HPLC chromatogram of standard rutin

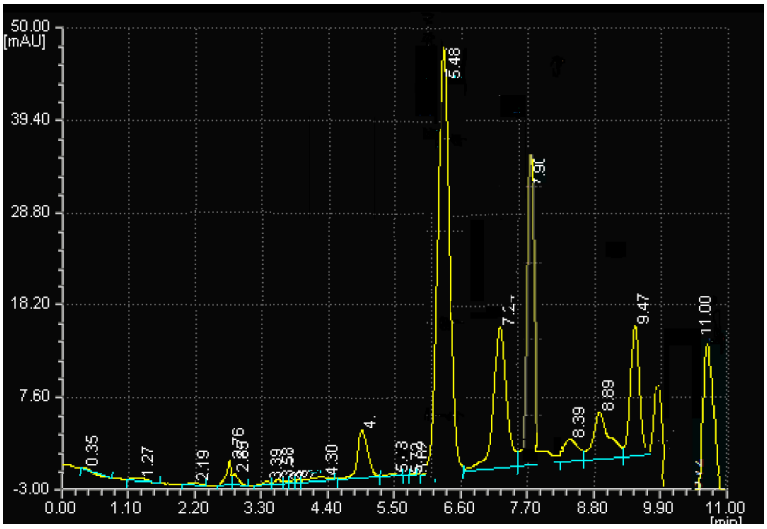


Fig.38: HPLC chromatogram of Ethanolic extract of *O. sanctum*

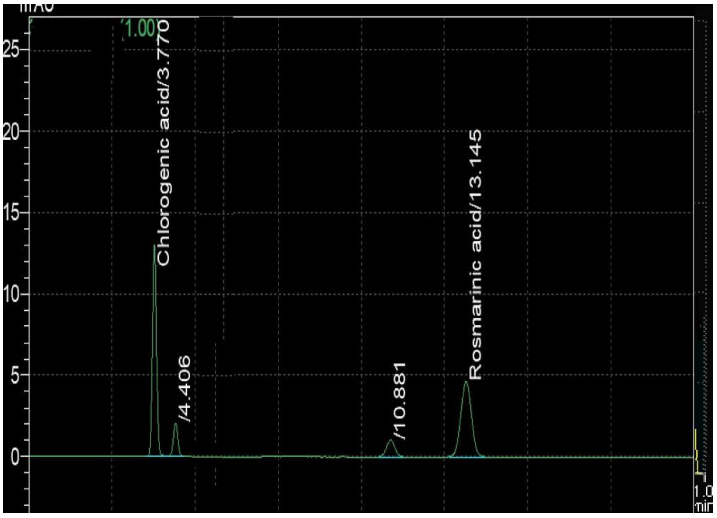


Fig.39 :HPLC chromatogram of standard Chlorogenic acid

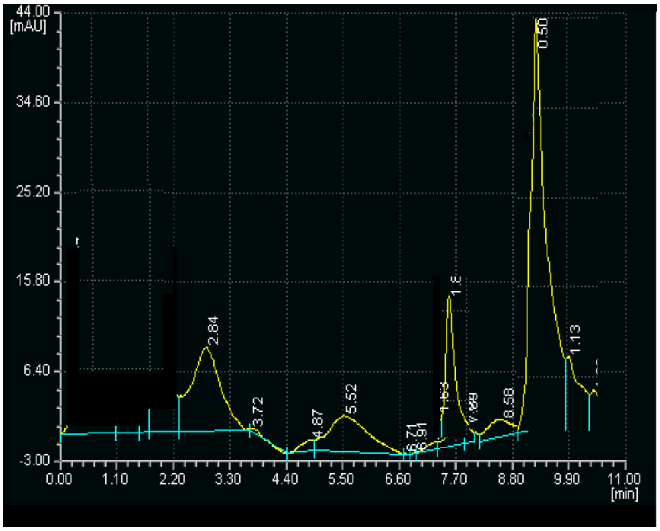


Fig.40: HPLC chromatogram of Ethanolic extract of *A. indica*

Table 5: HPLC Analysis of Plants extract

S.No	Sample	Height	Area	Conc.	RT	Inference
1.	Ethanolic extract of *M. oleifera*		1014986 1114483	61.4524 59.2523	5.70 7.70	Rutin Quercetin
2.	Ethanolic extract of *O. sanctum*	52566 42869	914980 734582	59.342 42.321	5.48 7.9	Chlorogenic acid Gallic acid
3.	Ethanolic extract of *A. indica*	163534 182521	143742 133722	84.3042 85.2116	7.70 8.80	Rutin Quercetin

6.3 *In-vitro* antioxidant activity using DPPH

Using modified Hossain et al. 2013 standards, the antioxidant activity of the ethanolic leaf extracts from each plant was assessed. Four milliliters of each concentration were placed in the working tube, and the test tube was quickly agitated and filled with DPPH (2.2diphenyl1picrylhydrazyl) and one milliliter of 0.1 mM methanol. After shaking each test tube, they were all left in the dark for 45 minutes at 27 °C. The same process was used to prepare the control sample, which was devoid of extract. Next, a UV spectrophotometer was used to assess the test samples' absorbance at 517 nm. The log dosage inhibition curve was utilized to evaluate the antioxidant activity of each sample, and the results were represented as the concentration needed to impede the production of DPPH radicals by 50% (IC50 g mL-1):

% Radical Scavenging Activity = Acontrol- Asample /Acontrol * 100

Where, Acontrol = Absorbance of control

Asample = Absorbance of sample

6.4 Ferric Reducing Antioxidant Power (FRAP) Assay

Different concentrations of BHT and ethanolic leaf extracts were diluted in 30 ml of methanol to create distinctive 100 g/ml stock solutions. The stock solution was diluted to produce lower quantities of extracts (20, 40, 60, 80, and 100 g/ml). Weigh five milligrams of BHT and dissolve it in fifty milliliters of methanol to create a stock solution containing 500 mg/ml of lesser concentration BHT (20, 40, 60, 80, and 100 mg/ml). The stock solution is then made less. Separately, 2.5 mL of pH-6.6 phosphate buffer, 1 mL of each reagent and standard concentration, and 2.5 mL of 1% potassium ferricyanide were mixed and incubated for 30 minutes at 50°C. The solutions were now mixed with 2.5 cc of 10% TCA and centrifuged for 10 minutes at 3000 rpm. Just 2.5 ml of the supernatant was taken out and combined with 0.5 ml of the recently made 0.1 irric chloride solution. At 700 nm, the absorbance was measured (Poonia et al., 2011).

Table 6: 50% inhibition (IC_{50}) for various plant extract by DPPH method

S.No	Sample	IC_{50} (µg/ml)
1.	Ascorbic acid	3.17
2.	Ethanolic leaves extract of *O. sanctum*	30.92
3.	Ethanolic leaves extract of *A. indica*	82.2
4.	Ethanolic extract of *M. oleifera*	86.1

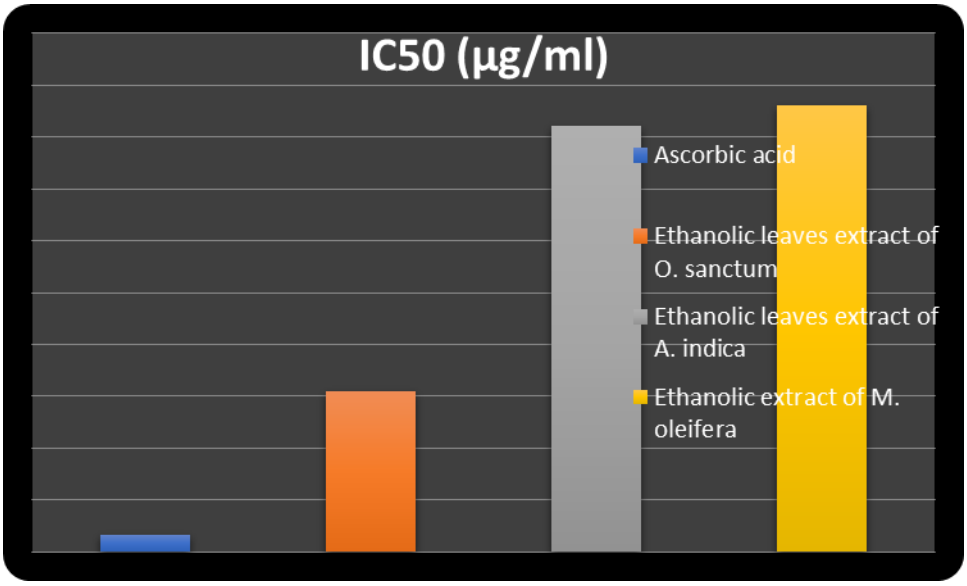
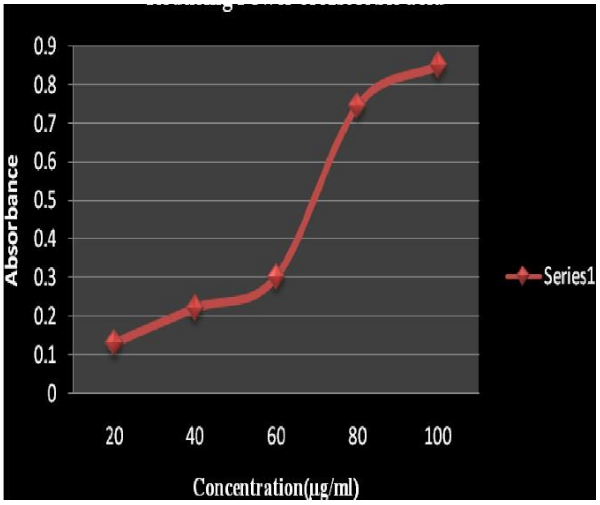


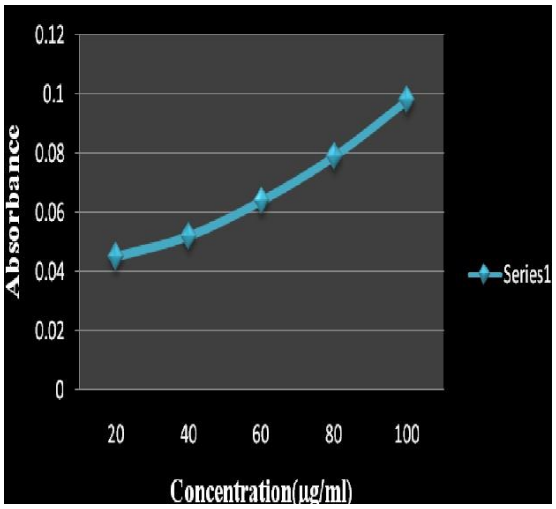
Fig 41: Graph.6 Comparative antioxidant activity of extracts

Table 7: Total reducing power of plant various extract

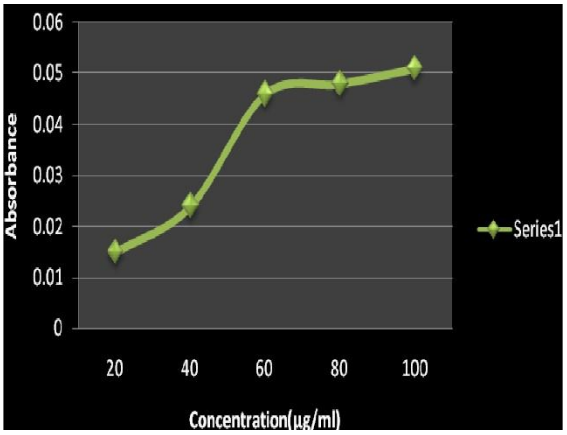
Concentration (µg/ml)	Absorbance			
	BHT (standard)	**Ethanolic extract *O. sanctum***	**Ethanolic extract *A. indica***	**Ethanolic extract of *M. oleifera***
20	0.130	0.045	0.015	0.023
40	0.221	0.052	0.024	0.026
50	0.302	0.064	0.046	0.054
80	0.745	0.079	0.048	0.059
100	0.850	0.098	0.051	0.062
FRAP	**0.769**	**1.16**	**2.89**	**3.1**



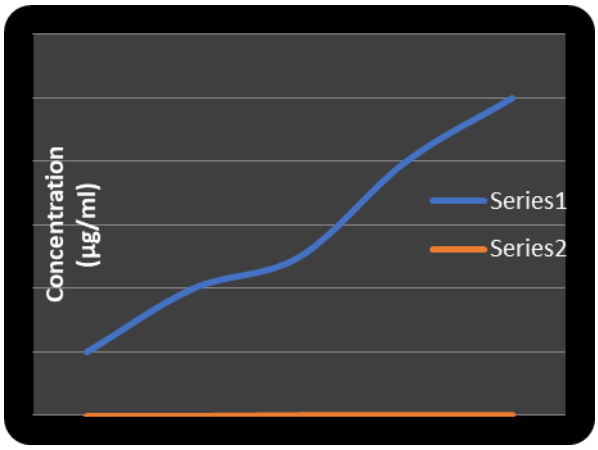
Graph 7: Reducing Power of BHT



Graph 8:Reducing Power of *O. sanctum*



Graph 9:Reducing Power of *A.indica*



Graph 10:Reducing Power of *M. oleifera*

Fig 42: Different Concentration of different plant

6.5 Proposed Mechanism studies by Molecular docking approach

In-Silico assessment of Anti-inflammatory potential of plant Phenolic and flavonoids

By breaking down cyclic adenosine monophosphate, the PDE superfamily members phosphodiesterase 4 (PDE4) and phosphodiesterase 7 (PDE7) promote inflammation in both immunomodulatory and pro-inflammatory cells. Dual inhibitors of PDE4 and PDE7 are a new family of pharmacological candidates that can control pro-inflammatory as well as T-cell function. They may be especially useful in the treatment of a variety of immune

system problems as well as inflammatory diseases with fewer unfavourable side effects. **(Grewal AS etal.,2017)**.

The present research work was planned to design the molecular docking of plant phenolic and flavonoids as dual inhibitors of PDE4 followed by evaluation of their anti-inflammatory activity and *in-silico* docking studies.

Ligand Preparation

ChemDraw was used to create the 2D structures of ligands such gallic acid, rutin, rutin, and chlorogenic acid (K.R. Cousins ;2005). Chem3D software was used to transform the two-dimensional ligand structures into three-dimensional structures with optimum 3D geometry. For AutoDock compatibility, the optimised structure was saved in PDB format **(R. Jain etal., 2020).**

Preparation of the grid file

By creating a grid box around the active sites, Autodock's regions of interest were identified by taking grid area into account. Grid boxes are essential to the docking process because they are designed to cover all amino acids other than those found in receptors that are present in active sites and required for binding. Three thumbwheel widgets on the grid box allow us to adjust the number of points in the x, y, and z dimensions. The study's chosen value for the spacing between grid points is 0.392, and the number of points studied are 40, 40, and 40 points in the x, y, and z dimensions are 44.16, -34.73, and -54.88 as x, y, and z centres (S. Mujwar ;2021).

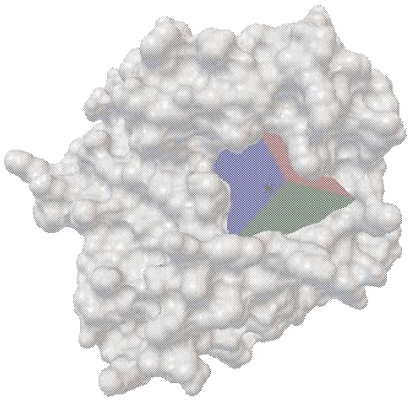


Figure 43: Grid box covering all active sites in receptor

Preparation of the docking file

The docking tool used for all computations was Autodock4.2. The visualisation and other operations required for docking study were carried out using Pymol, Chimera, DS visualizer, MMP Plus, and other software. (S. Mujwar etal.,2019).

Docking of Phosphodiesterase-4 (PDE4)

Crystal structure

Download the protein crystal structure from the Protein Data Bank website. The protein's receptor is connected to a binding ligand. The Protein Data Bank's 7F2K.pdb file, which provides the primary receptor and structural information, was utilized. Within the receptor, the bound ligand was found (A. Kaur etal.,2016).

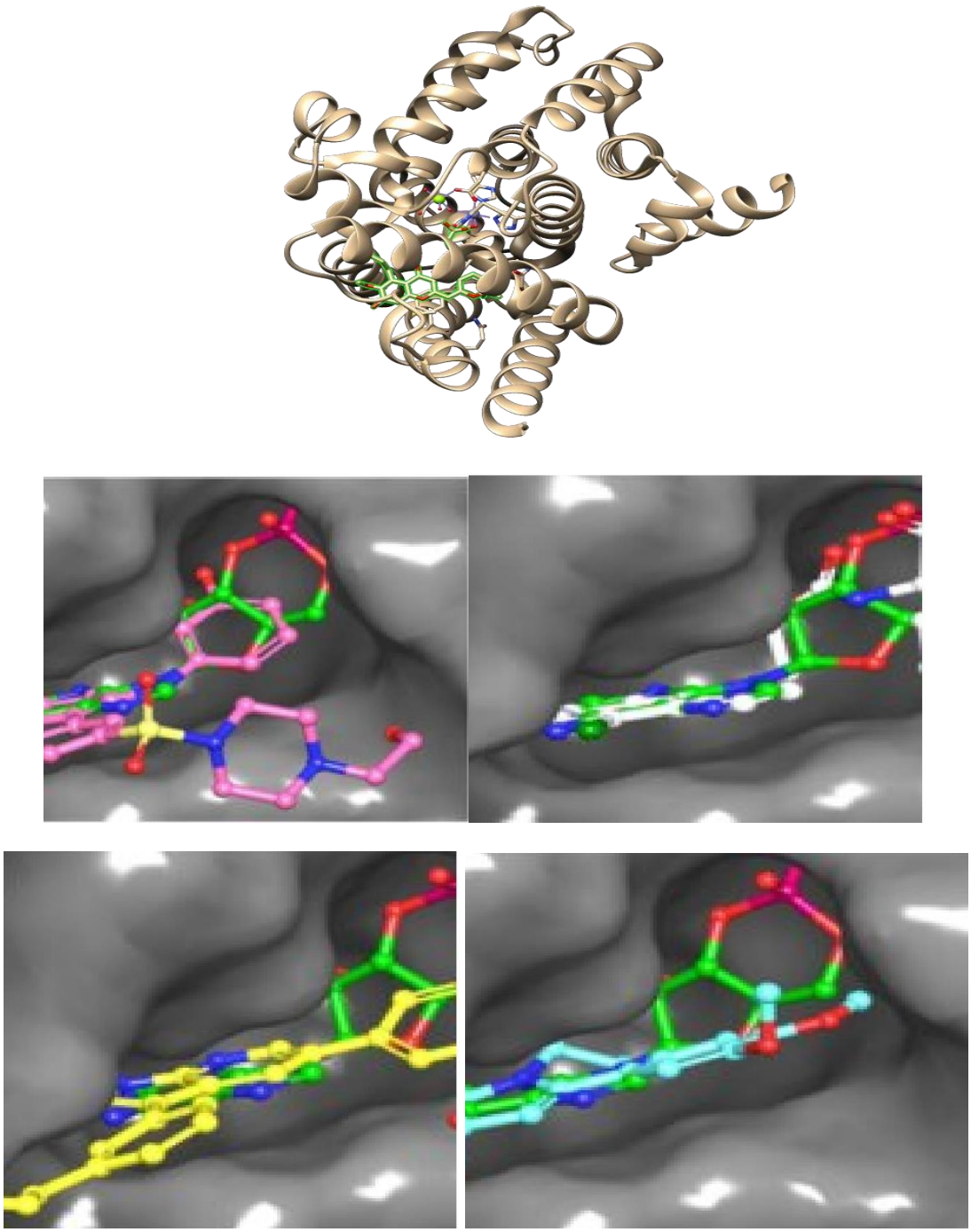


Figure 44: Crystal structure of PDE4 enzyme with bound ligand (PDB ID-7F2K)

Processing of Protein

Chain A was chosen for the experiment from the two chains that comprise the downloaded receptor protein. The Chimera program was utilized to remove the bound ligand 0X8 from the macromolecular complex(E.F. Pettersen etal., 2004).

Molecular Docking Simulation Studies

Autodock was utilized to dock ligands such gallic acid, rutin, quercetin, and chlorogenic acid to the PDE4 enzyme. While no receptor residues were rendered flexible, the ligand's connections remained flexible.

Toxicity & ADME-T Studies

Online application. OSIRIS evaluates the modified lead molecules to anticipate the presence of any hazardous groups and ADME-T features (U.P. Agrawal N etal., 2020).

6.6 *In-Silico* assessment of Anti-microbial potential of plant Phenolic and flavonoids

Antimicrobial resistance in microorganisms is currently generating headlines and poses a significant threat to human health worldwide. Methicillin-resistant Staphylococcus aureus (MRSA), penicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci, and mycobacterium contamination are particularly concerning. Many of these pathogens are resistant to various antibacterial medications, including TB. Because of the current status of chemotherapeutic resistance mechanisms, it is easier to develop innovative antimicrobial medicines that block key bacterial targets. In this context, current research into antibacterial drugs has focused on the enzyme aminoacyl-tRNA synthetase (AaRS). These enzymes play an important role in protein production because they catalyze the formation of aminoacyl-RNA. Protein production halts when these enzymes are blocked, limiting bacterial growth in both in vitro and infectious settings. These enzymes are appealing therapeutic targets for the treatment of bacteria(Julian Gregston Hurdle et al., 2020).

Ligand Preparation

ChemDraw was used to create a 2D structure of a ligand such as chlorogenic acid, rutin, quercetin, and gallic acid (K.R. Cousins ;2005).. Chem3D software was used to transform the two-dimensional ligand structures into three-dimensional structures with optimum 3D geometry. For AutoDock compatibility, the optimised structure was saved in PDB format (R. Jain etal., 2020).

Preparation of the grid file

Autodock's zones of interest were defined by drawing a grid box around the active

locations and factoring in grid area. Grid boxes are necessary for the docking process because they are designed to cover all amino acids other than those located in receptors, which are present in active sites and required for binding. The grid box contains three thumbwheel widgets that allow us to alter the number of points in the x, y, and z dimensions. Another thumbwheel can be used to adjust the spacing between grid points. In this study, the value chosen was 0.392, and 40, 40, and 40 points on the x, y, and z axes were considered (S. Mujwar;2021).

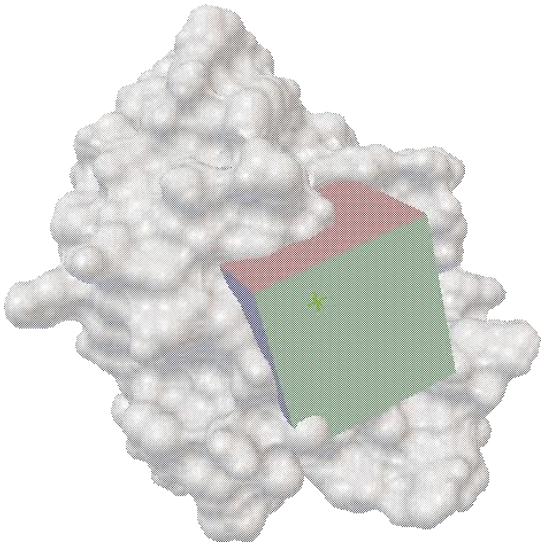


Figure 45: Grid box covering all active sites in receptor

Preparation of the docking file

Autodock4.2 was the docking application used for all computations. The tasks required for visualizing data and executing other chores for docking study were conducted using Pymol, Chimera, DS visualizer, MMP Plus, and other programs (S. Mujwar etal.,2019).

Docking of Isoleucyl-transfer RNA (tRNA) synthetase (IleRS)

Crystal structure

One can obtain the protein's crystal structure from the Protein Data Bank website. The protein's receptor is connected to a binding ligand. The Protein Data Bank's 1WNZ.pdb file, which contains the primary receptor and structural information, was utilized. The receptor

contained the bound ligand, 2'-(L-valyl)amino-2'-deoxyadenosine (2VA). (A. Kaur etal.,2016).

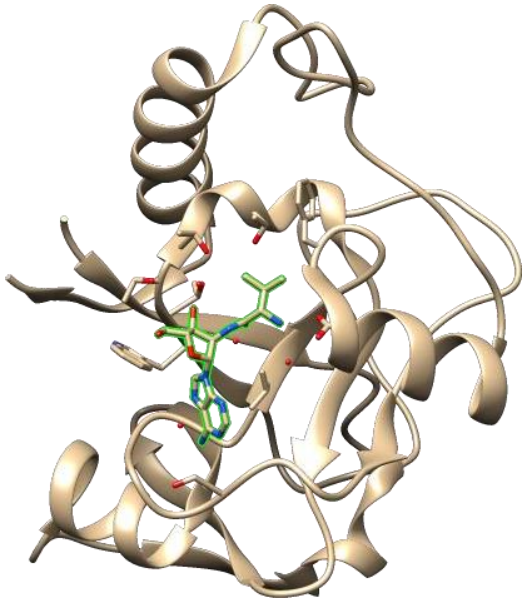


Figure 46: Crystal structure of IleRS enzyme with bound ligand 2VA(PDB ID-1WNZ)

Processing of Protein

The only chain in the receptor protein that has been downloaded is chain A, which was selected experimentally. The Chimera program was utilized to remove the bound ligand 2VA from the macromolecular complex (E.F. Pettersen etal., 2004).

Molecular Docking Simulation Studies

The IleRS enzyme was docked with ligands such as gallic acid, rutin, quercetin, and chlorogenic acid via autodock. The ligand linkages remained flexible, but none of the receptor residues were rendered flexible (U.P. Agrawal N etal., 2020).

Toxicity & ADME-T Studies

Online tool OSIRIS analyses the changed lead molecules to anticipate the presence of any toxic group, as well as the presence of any toxic group and ADME-T characteristics (T. Sander etal., 2009).

In-Silico **assessment of wound healing potential of plant Phenolic and flavonoids**

The planned and coordinated process of wound healing includes inflammation, matrix deposition, cell proliferation, tissue modeling, collagenation, and epithelialization. 2012: Soni H et al. The Wnt/b-catenin pathway promotes wound healing by inhibiting the glycogen synthase kinase-3 (GSK-3) protein, which is a key regulatory enzyme. A variety of medicinal plants have been studied to see if they may be used to make wound-treatment drugs. Rutin, Quercetin, Chlorogenic Acid, and Gallic Acid were tested in silico against GSK-3.

Ligand Preparation:

ChemDraw was used to create a 2D structure of a ligand such as chlorogenic acid, rutin, quercetin, and gallic acid (K.R. Cousins ;2005). Chem3D software was used to transform the two-dimensional ligand structures into three-dimensional structures with optimum 3D geometry. For AutoDock compatibility, the optimised structure was saved in PDB format (R. Jain etal., 2020).

Preparation of the grid file

Autodock's zones of interest were defined by drawing a grid box around the active locations and factoring in grid area. Grid boxes are necessary for the docking process because they are designed to cover all amino acids other than those located in receptors, which are present in active sites and required for binding. The grid box contains three thumbwheel widgets that allow us to alter the number of points in the x, y, and z dimensions. Another thumbwheel can be used to adjust the spacing between grid points. In this study, the value chosen was 0.392, and 40, 40, and 40 points on the x, y, and z axes were considered (S. Mujwar;2021).

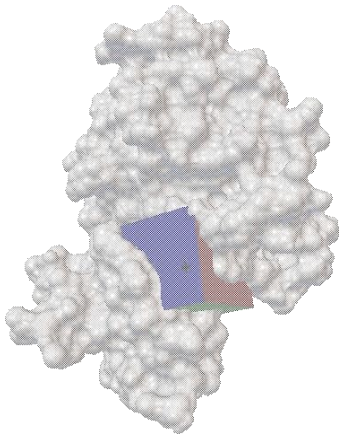


Figure 47: Grid box covering all active sites in receptor

Preparation of the docking file

The docking tool used for all the computations was Autodock4.2. Pymol, Chimera, DS visualizer, MMP Plus, and other applications were used to carry out the visualisation and other tasks required for docking research (S. Mujwar etal.,2019).

Docking of Glycogen Synthase Kinase-3β (GSK-3β)

Crystal structure

The Protein Data Bank website allows you to download the protein's crystal structure. The protein contains a receptor and a binding ligand. The Protein Data Bank's 7OY5.pdb database was used to obtain primary information on receptor and structure. (A. Kaur etal.,2016).

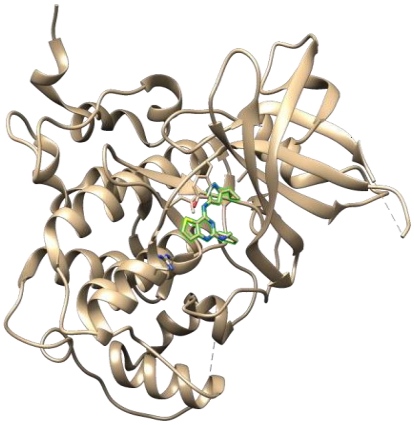


Figure 48: Crystal structure of GSK-3beta enzyme with bound ligand

Processing of Protein

The downloaded receptor protein has two chains, A and B, and the experiment will focus on chain B. Using the Chimera program, the bound ligand 39I was separated from the macromolecular complex (E.F. Pettersen etal., 2004).

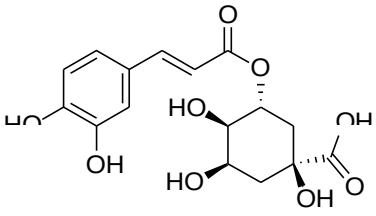
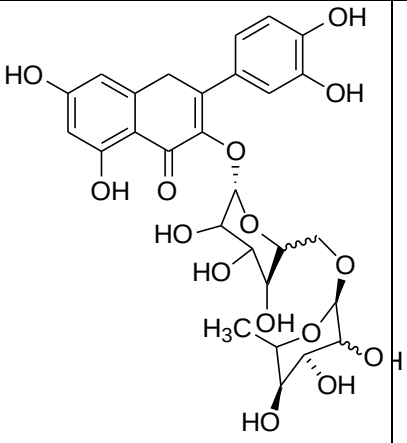
6.7 Molecular Docking Simulation Studies

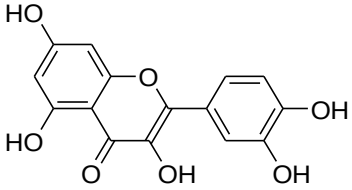
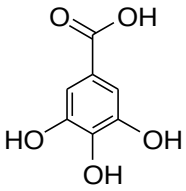
Autodock was used to dock ligands such gallic acid, rutin, quercetin, and chlorogenic acid against the GSK-3 enzyme. While no receptor residues were rendered flexible, all of the ligand's linkages were retained flexible (U.P. Agrawal N etal., 2020).

Toxicity & ADME-T Studies

Online application OSIRIS analyses the changed lead molecules to anticipate the presence of any toxic groups as well as the presence of any toxic groups and ADME-T characteristics (T. Sander etal., 2009).

Table 8: Result of docking of against PDE4 enzyme

S. No	Compound	Structure	B.E.	H-Bond	Residual Interaction	
					Pi-Interaction	Van der Waals
1	Chlorogenic acid		-4.06	Asp201, Asp318, His160, Pro322	Phe372, Tyr159	Glu230, Met273, His204, Asn209, Ser208, Asn321, Leu319
2	Rutin		-5.74	His204, His160, Met273, Glu230, Asp201, Asn321, Pro356	Asp318, Ile336, Leu319, Phe372	Gln343, Ser208, Asn209, Thr271, His200, Tyr159, Cys358, Phe340, Met357, Ser368

3	Quercetin		-6.76	Gln369, Asn321, Asp318	Ile336, His160	Tyr329, Val377, Tyr159, His164, Met273, Phe340
4	Gallic acid		-3.55	Gln369, Asn321, Asp318, Tyr159	Leu319	His160, Ser320, Pro322, Phe372

Interactions

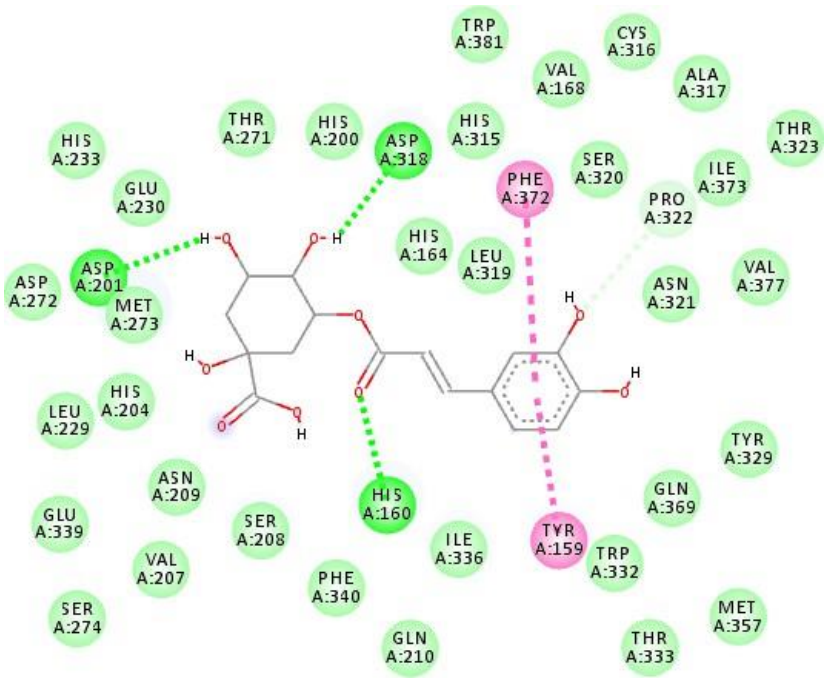


Figure 49: Binding interaction of chlorogenic acid with PDE4.

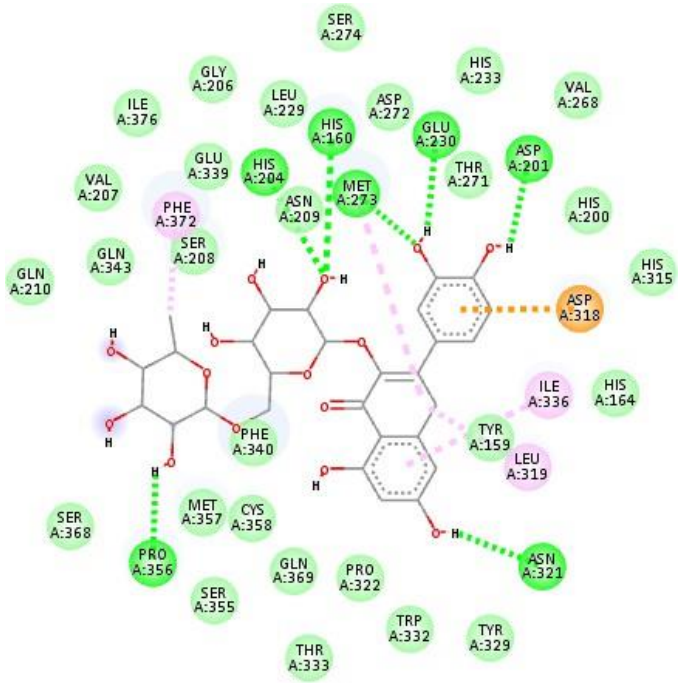


Figure 50: Binding interaction of rutin with PDE4.

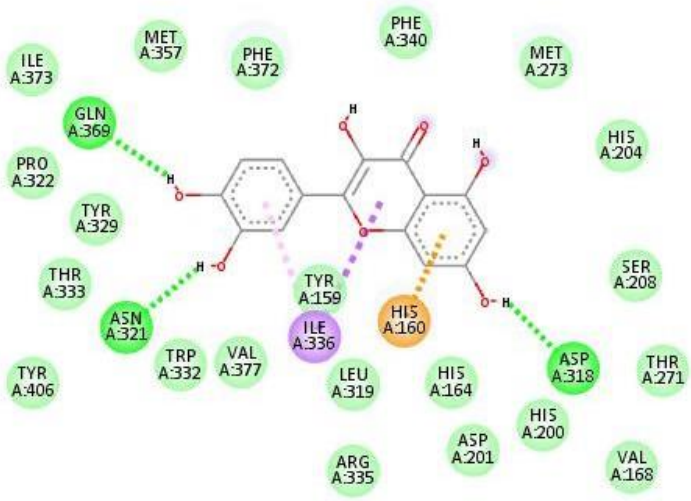


Figure 51: Binding interaction of quercetin acid with PDE4.

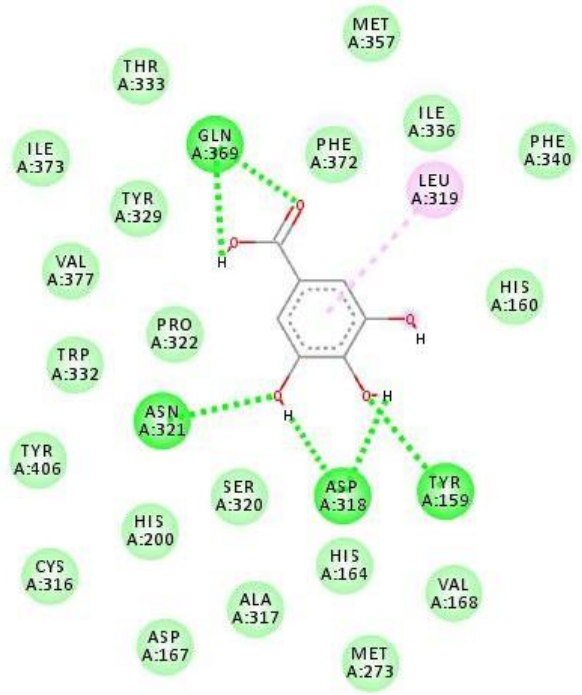


Figure 52: Binding interaction of gallic acid with PDE4.

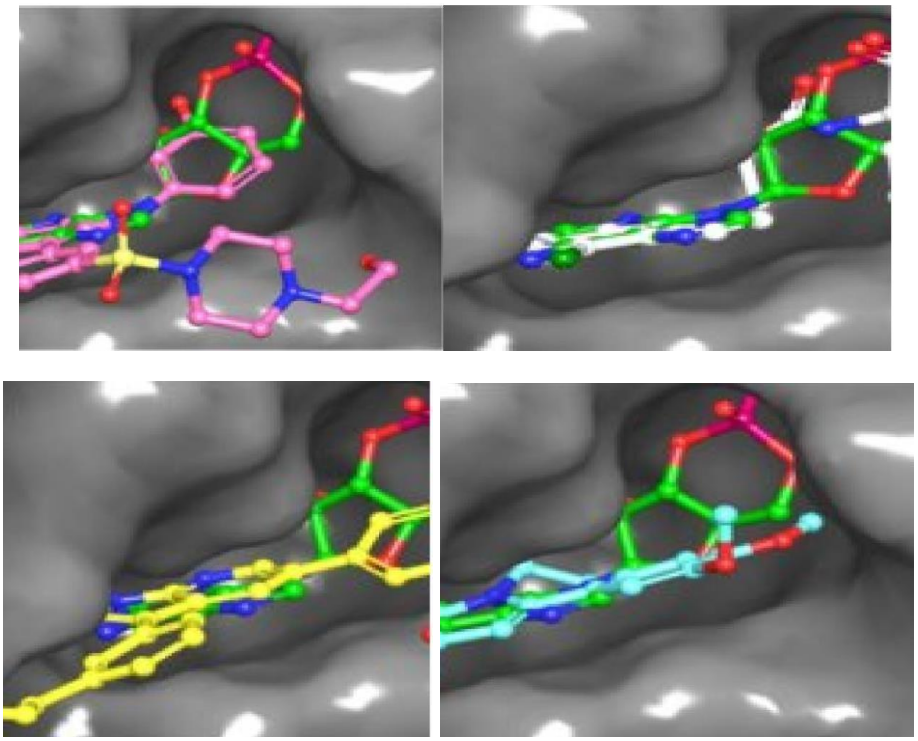
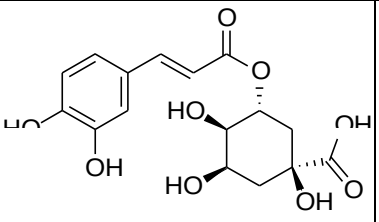
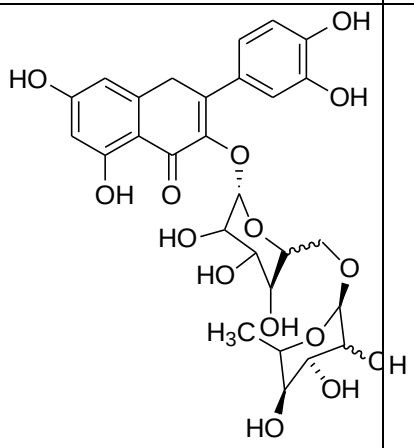
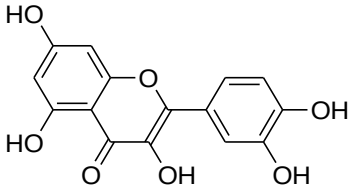
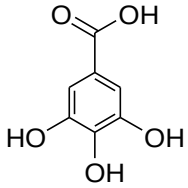


Figure 53: Binding Mode of Q,R,CA & GA.

Table 9: Result of docking of against IleRS enzyme.

S. No	Compound	Structure	B.E.	H-Bond	Residual Interaction	
					Pi-Interaction	van der Waals
1	Chlorogenic acid		-4.20	Gly316, Ser310, Thr229, His319, Asp328, Thr230	Val318	Asp313, Val309, Trp227, Thr228, Tyr308, Thr233
2	Rutin		-5.06	Thr229, Trp227, Thr315, Ile317, Ser310, Tyr308, Asp328	Trp227, Val318	Gly314, Val309, Thr230, Thr228, Glu327, Gln320, Ala321
3	Quercetin		-7.42	Thr315, Ser310, Ile317	Val318, Asp313	Gly316, Gly314, Thr228, Thr229, Thr230, Tyr308
4	Gallic acid		-4.56	Asp313, Ser310, Trp227, Ile317, Gly316	Val318, Trp227	Thr315, Thr228, Val309, Ile226

Interactions

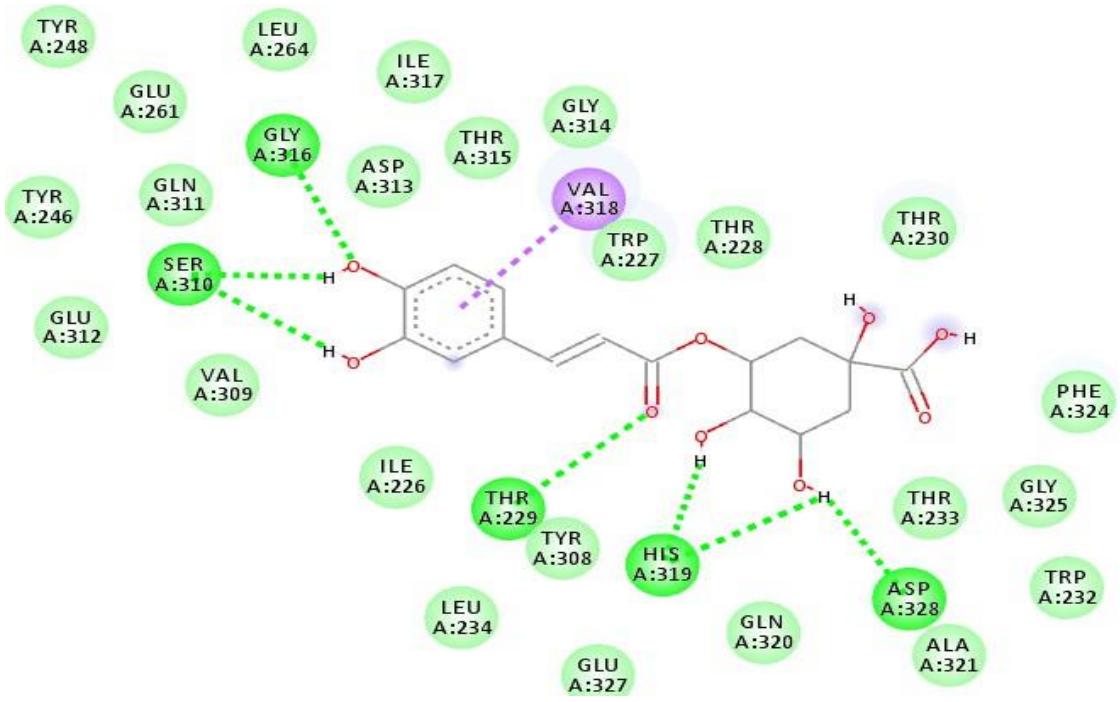


Figure 54: Binding interaction of chlorogenic acid with IleRS.

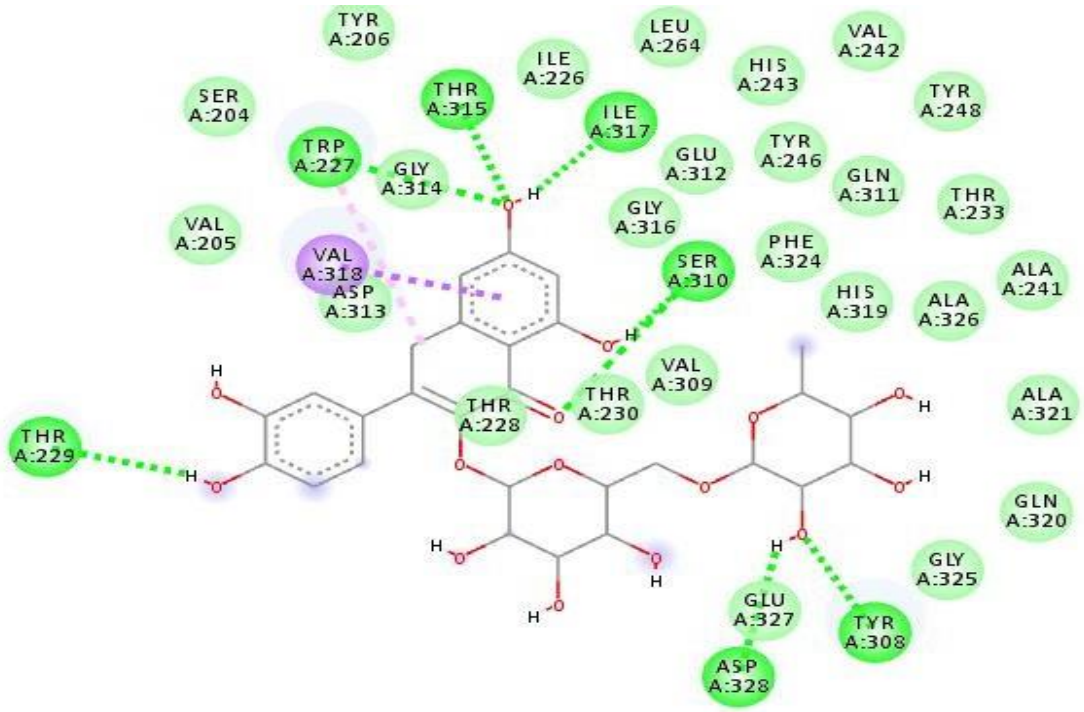


Figure 55: Binding interaction of rutin with IleRS.

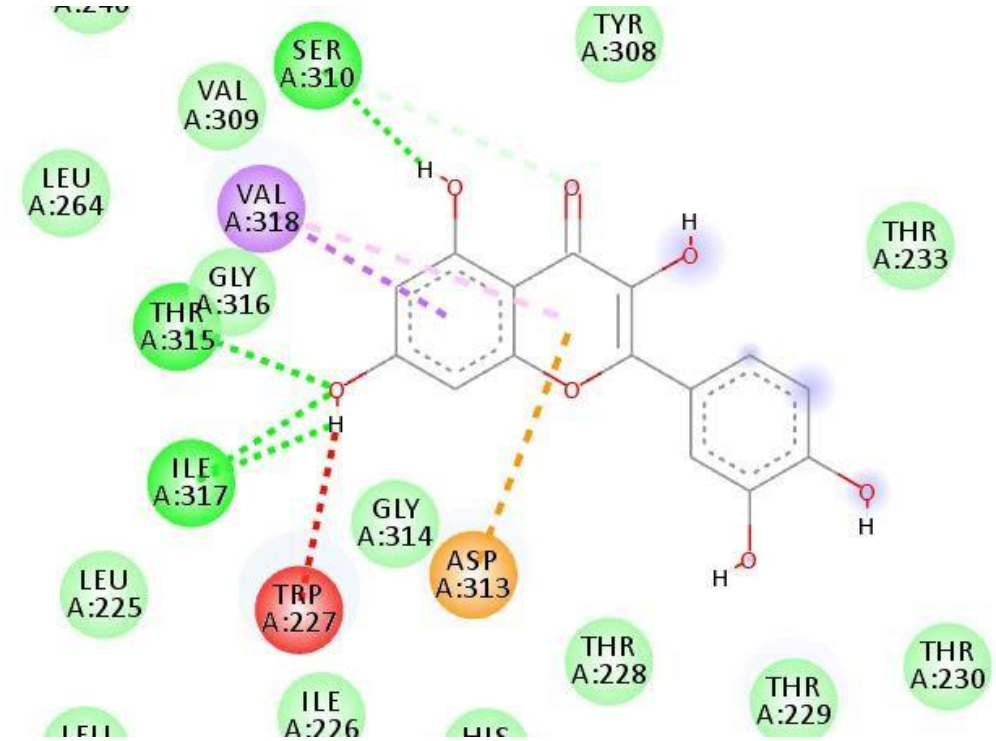


Figure 56: Binding interaction of quercetin acid with IleRS.

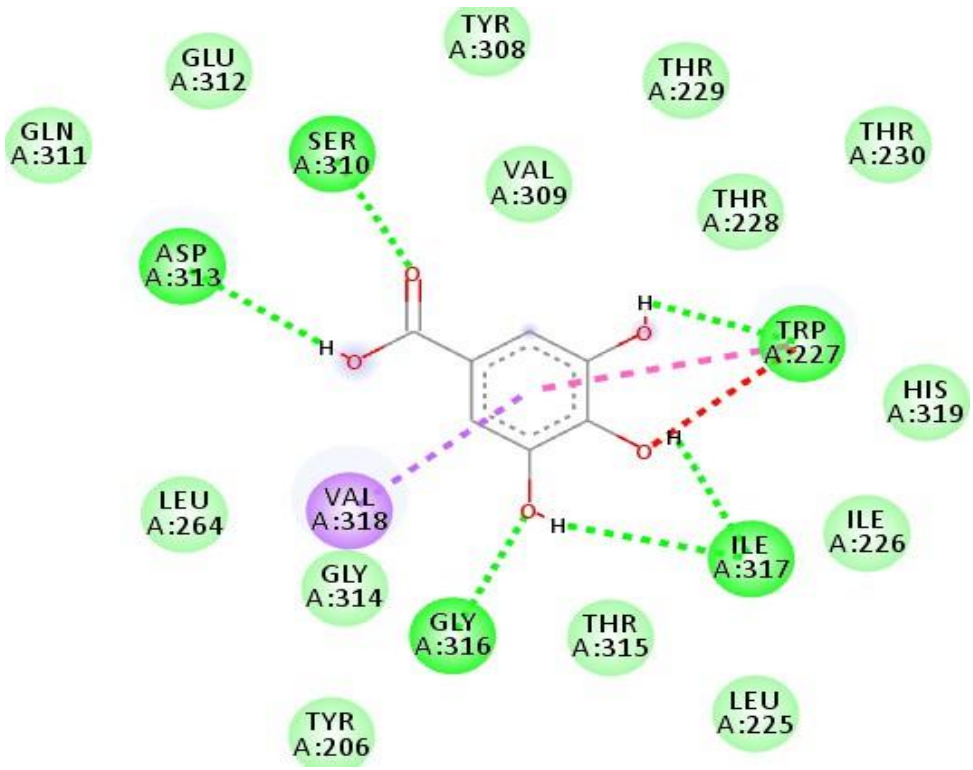


Figure 57: Binding interaction of gallic acid with IleRS.

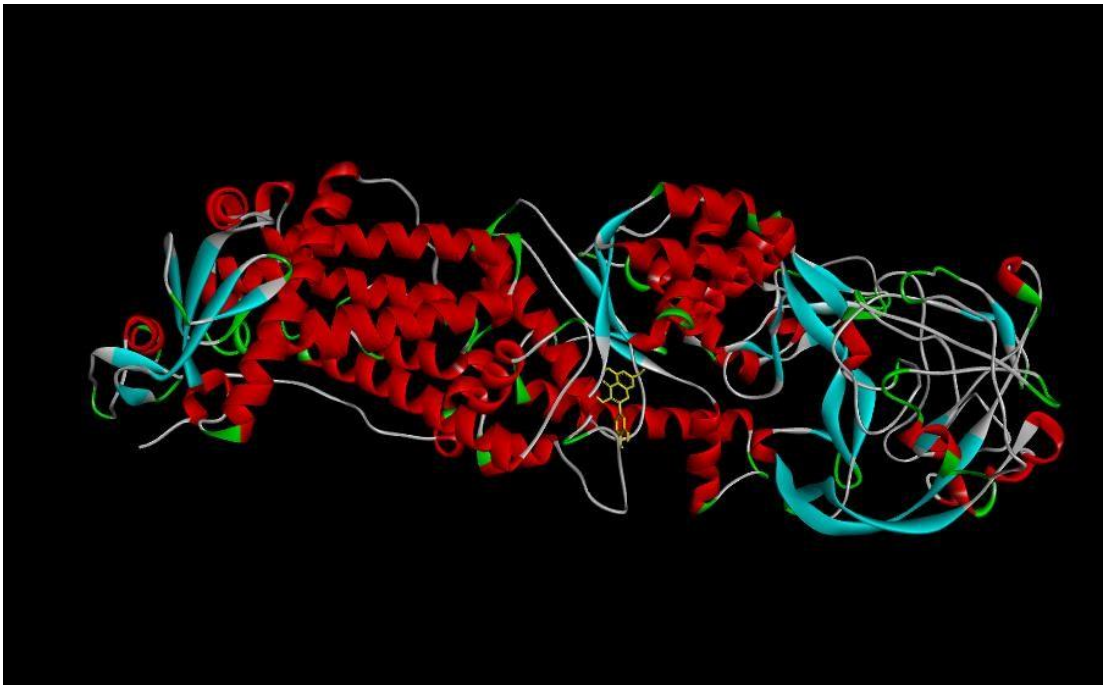


Figure 58: Binding mode of quercetin within the active site of IleRS receptor

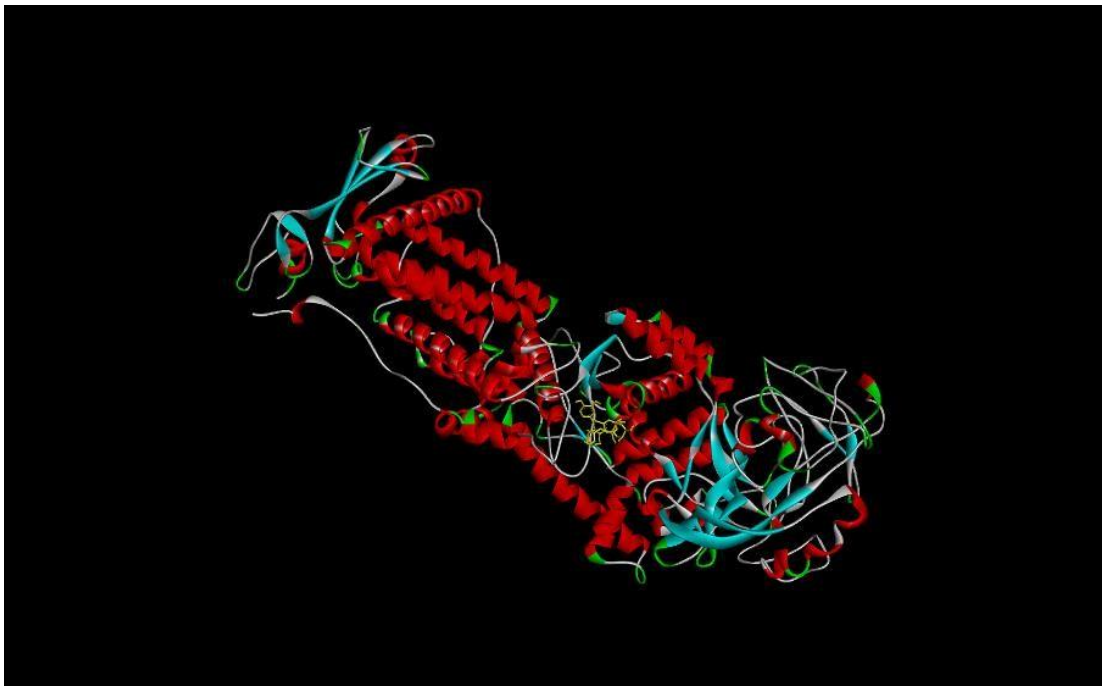


Figure 59: Binding mode of rutin within the active site of IleRS receptor

Figure 60: Binding mode of chlorogenic acid within the active site of IleRS receptor

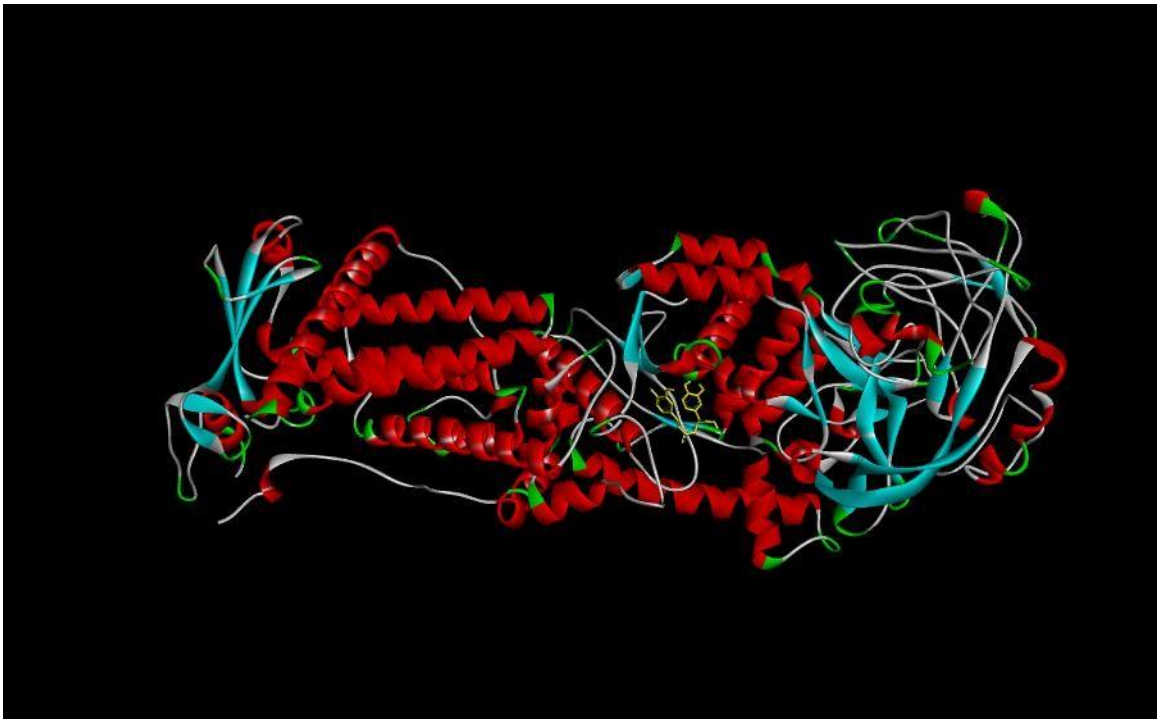
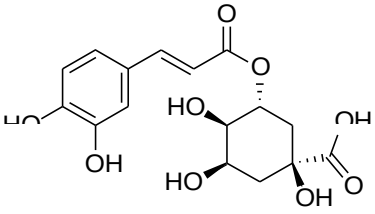
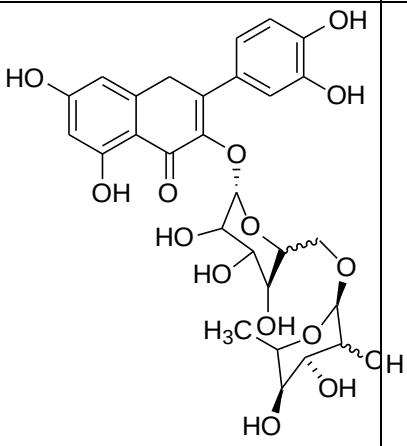
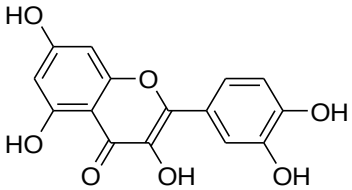
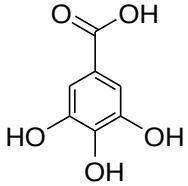


Figure 61: Binding mode of Gallic acid within the active site of IleRS receptor

Table 10: Result of docking of against GSK-3β enzyme.

S.No	Compound	Structure	B.E.	H-Bond	Residual Interaction	
					Pi-Interaction	van der Waals
1	Chlorogenic acid		-5.26	Val135, Asn186, Lys183, Cys199	Ala83, Leu188, Val70	Lys85, Phe67, Asp200, Gln185, Gly65, Tyr134, Thr138
2	Rutin		-5.55	Arg141, Gln185, Asn186, Lys85, Asp200, Asp133	Leu183, Val135, Ala83, Tyr134, Val70, Phe67, Cys199	Thr138, Gly63, Gly202, Leu132, Val110, Ile62, Leu189
3	Quercetin		-7.36	Asp133, Val135, Asp200,	Leu188, Cys199, Ala83, Phe67, Val70,	Tyr134, Val110, Leu132, Asn186, Glu97, Ile62
4	Gallic acid		-3.56	Arg144, Thr138, Gln185	Arg141, Tyr140	Asn186, Asn64, Gly63

Interactions

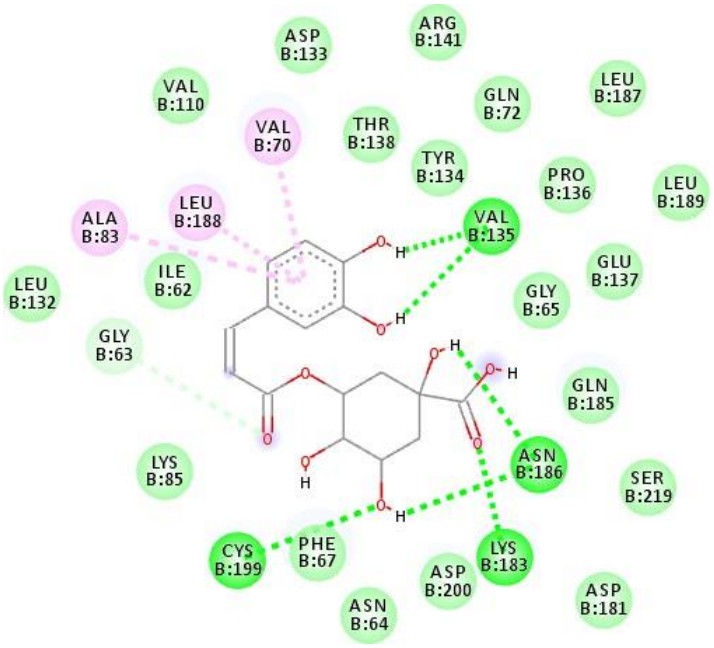


Figure 62: Binding interaction of chlorogenic acid with GSK-3β.

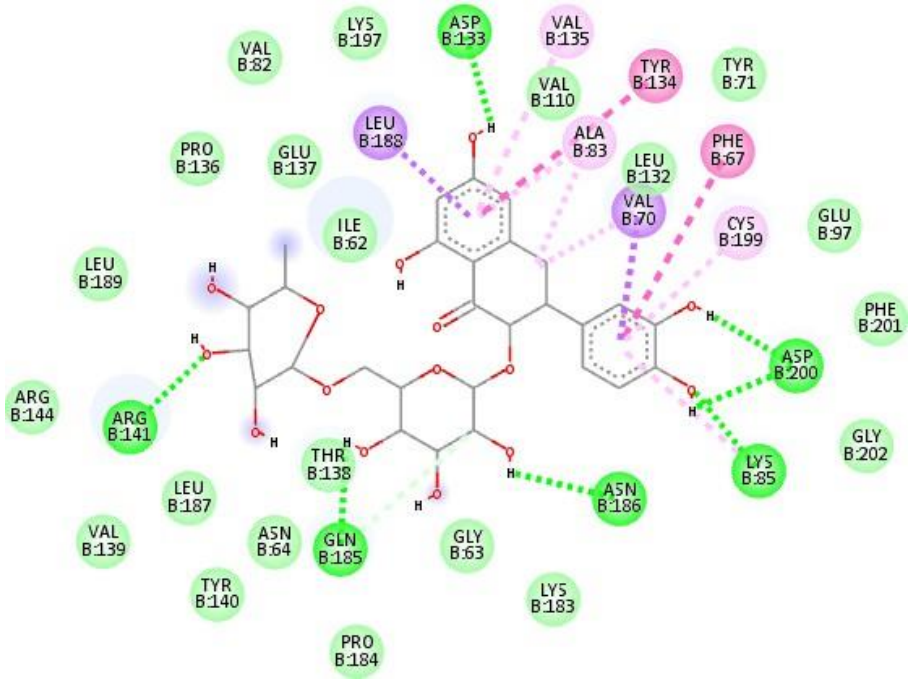


Figure 63: Binding interaction of rutin with GSK-3β.

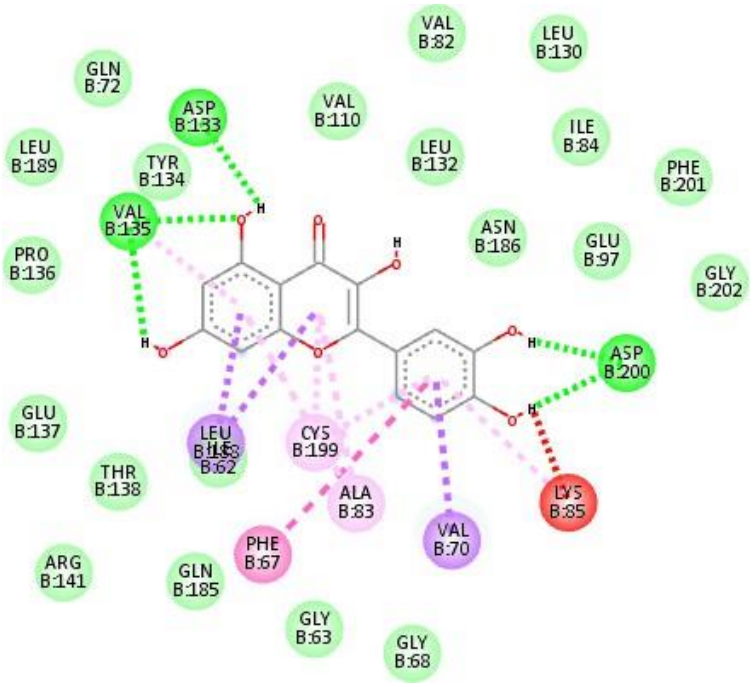


Figure 64: Binding interaction of quercetin acid with GSK-3β.

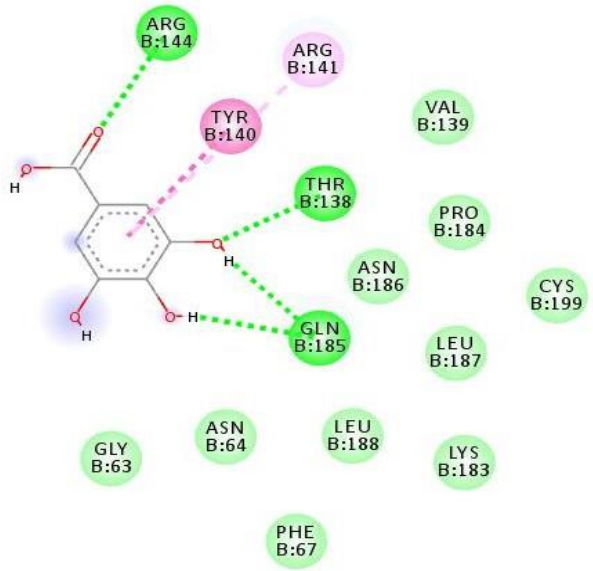


Figure 65: Binding interaction of gallic acid with GSK-3β.

Toxicity & ADME-T Studies

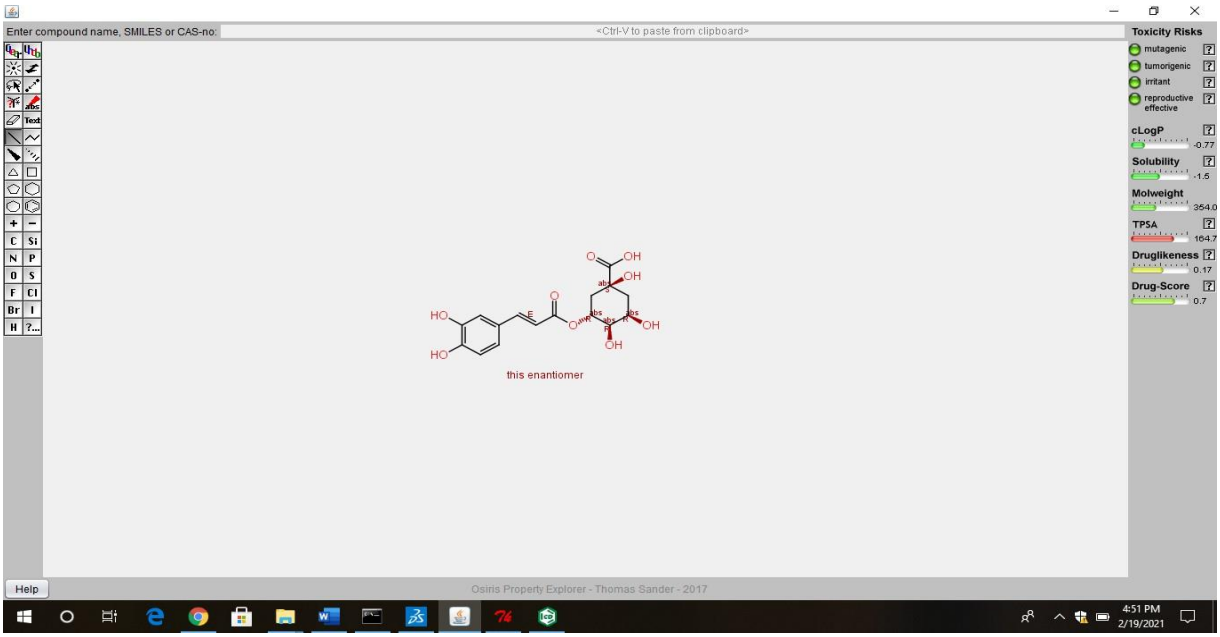


Figure 66: Pharmacokinetic and toxicity profiling of chlorogenic acid.

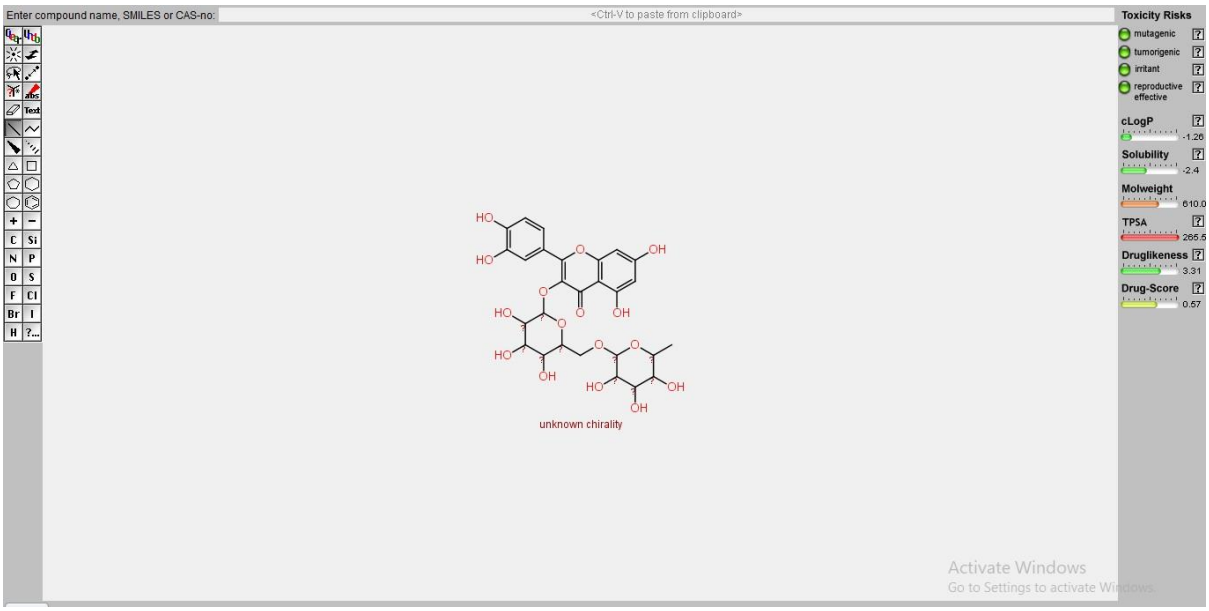


Figure 67: Pharmacokinetic and toxicity profiling of rutin.

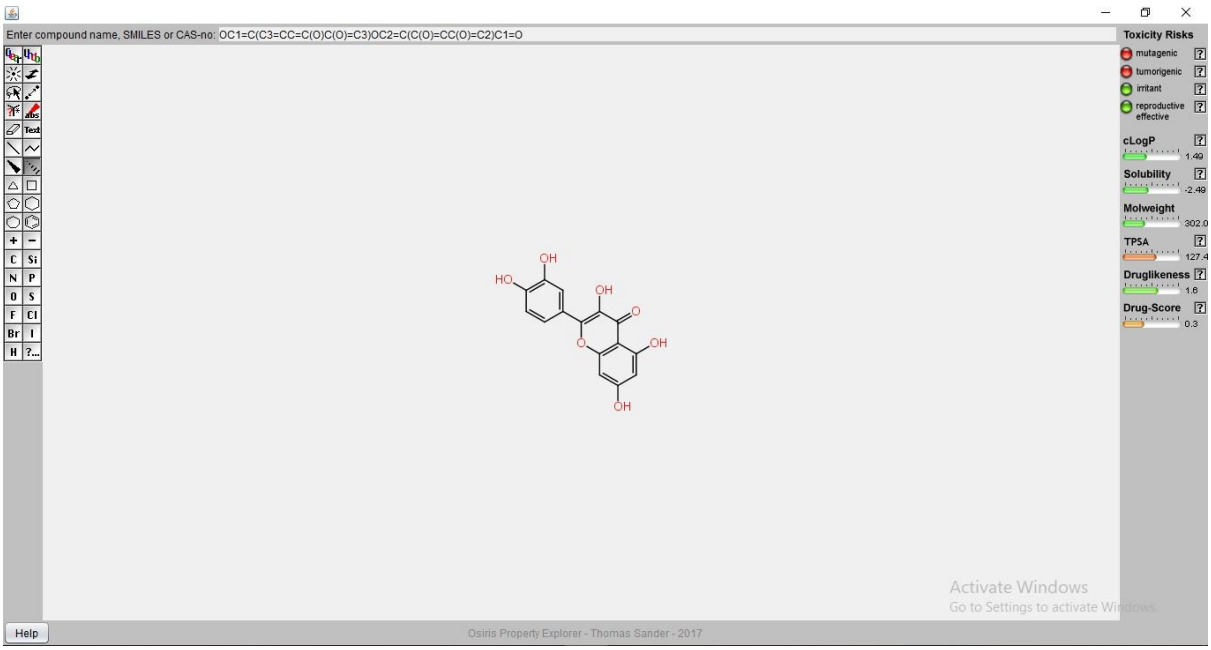


Figure 68: Pharmacokinetic and toxicity profiling of quercetin.

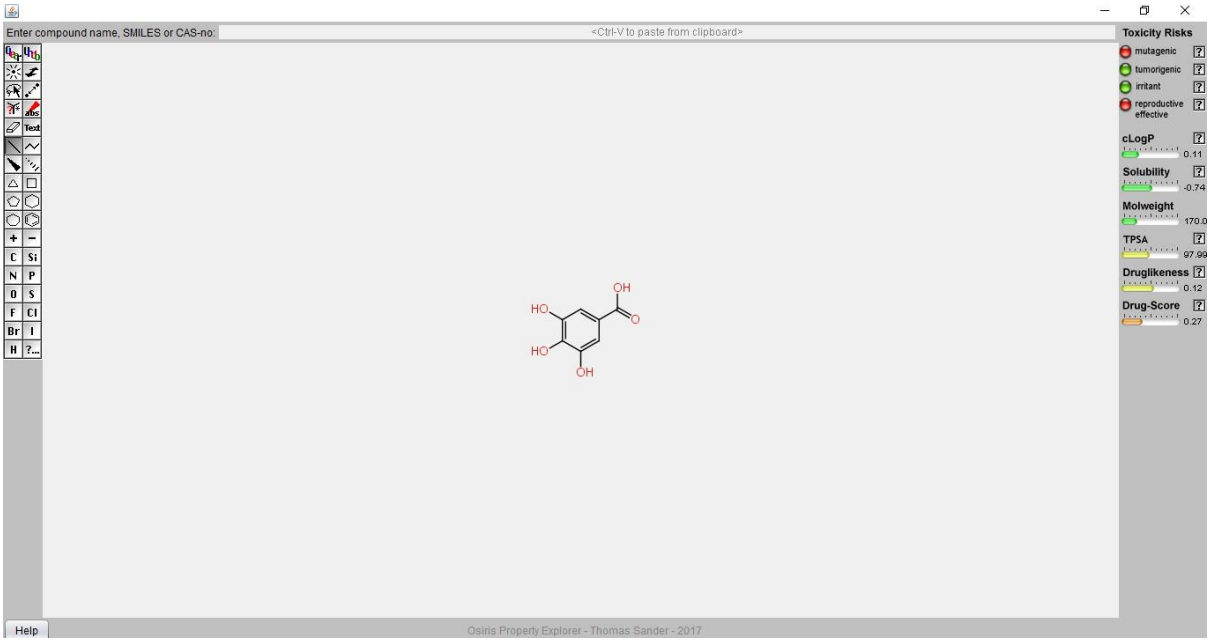


Figure 69: Pharmacokinetic and toxicity profiling of gallic acid.

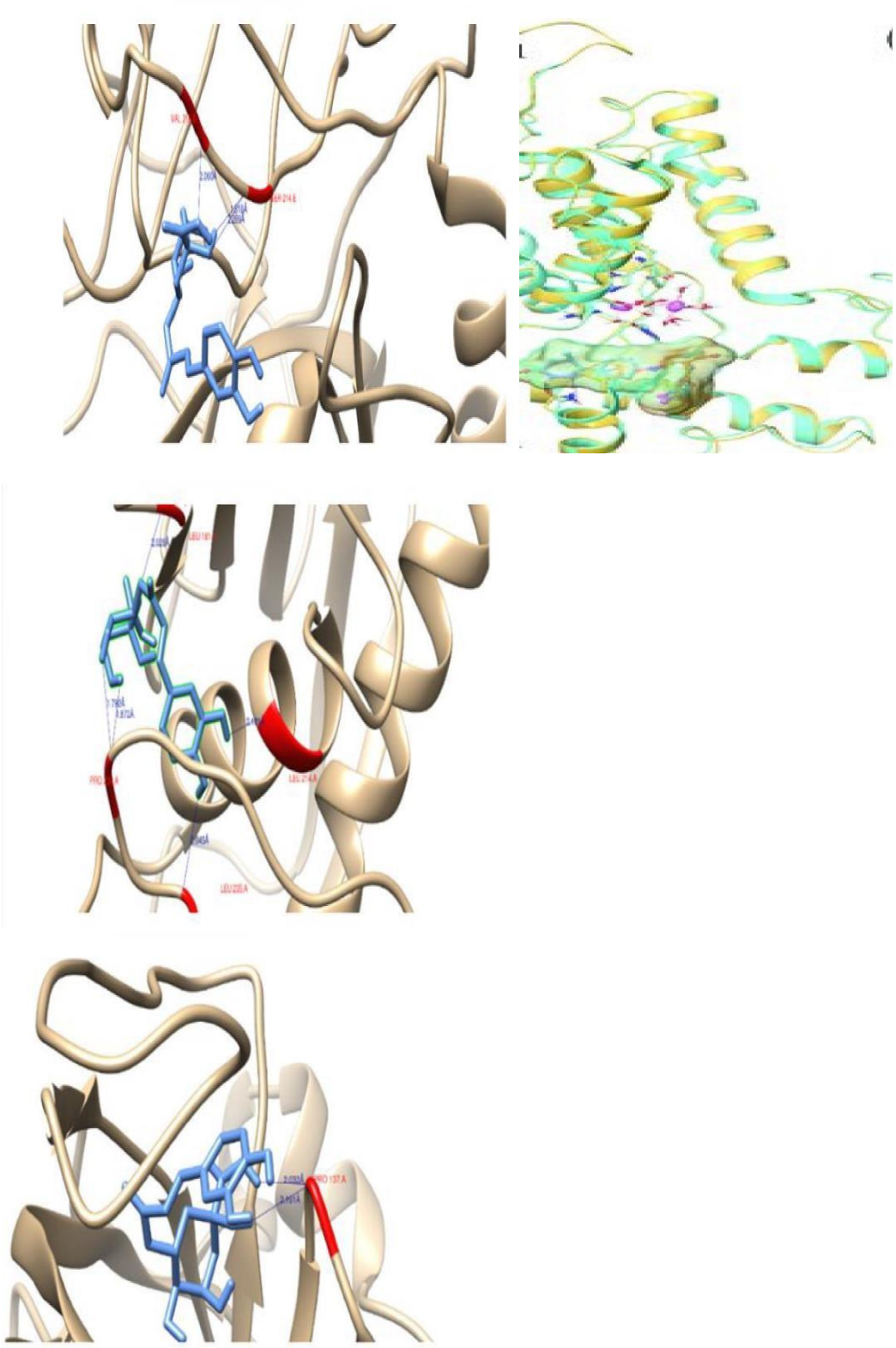


Figure 70: Binding Mode of Q,R, CA & GA.

6.8 Animal Care and Handling

The animals were transported for the study from the accredited animal house at P. K. University in Shivpuri, Madhya Pradesh. All of the rats were healthy and weighed between 150 and 200 grams. The temperature was maintained at 22°C (plus or minus 3°C), and the animals were housed in an air-conditioned environment. Every four days, the animal bedding was changed.

6.9 Preparation of polyherbal formulation (Gel)

Prepared three 200g gels. First, the individual weight of the medication extract was estimated, and an approximate amount was rounded about.

Shewaga- Approximately 20gm extract was extracted from 100gm of powdered crude medicate

Tulsi- Approximately 20gm extract was extracted from 80gm of powdered crude medication.

Neem- Approximately 20gm extract was extracted from 120gm of powdered crude medication.

Table.11: Formulation of Herbal gel

Ingredient	**F 1**	**F 2**	**F 3**
Shewaga Tulsi Neem	10gm 5gm 5gm	5gm 10gm 5gm	5gm 5gm 10gm
Carbopol 940 (Carbomer Homopolymer USP) Disodium Edetate IP Sodium Methylparaben IP Sodium Propylparaben IP Sodium Hydroxide IP Purified Water IP	1.25% w/w 0.05% w/w 0.1% w/w q.s. for pH adjustment	1.25% w/w 0.05% w/w 0.1% w/w q.s. for pH adjustment	1.25% w/w 0.05% w/w 0.1% w/w q.s. for pH adjustment

	0.05% w/w q.s. to 100% w/w	0.05% w/w q.s. to 100% w/w	0.05% w/w q.s. to 100% w/w

Methodology

20gm of drug extract & to its quantity 8 times of volume of water was added *i.e.* 160ml

↓

Whole extract was kept overnight with continuous stirring to get smooth gel

↓

Required quantity of methyl paraben was dissolved by heating on water bath

↓

Then volume was reduced to its 1/4th ie 40ml & finally, full mixed in gradients were mixed properly with the carbopol 940 gel with continuous stirring and NaOH was added drop wise to the formulation for adjusting the Required skin pH (pH: 6.8- 7.0) and to obtain required consistency.

6.10 Evaluation of formulation

Macroscopic Analysis of Formulation

The color, homogeneity, consistency, and spreadability of the created formulations (F1, F2, and F3) were evaluated visually. Natural light was used to examine the clarity, and all macroscopic measurements were taken in comparison to carbopol.

Spread ability

The spreadability of the resulting formulation was determined by applying 0.5 g of the gel to a circle with a premarket diameter of 2 cm on a glass plate, followed by a second glass

plate. For five minutes, a weight of 1/2 kg was permitted to lie on the top glass plate. The circumference of the circle was measured once the gel had spread (U. Shinde et al., 2012).

Post optimization of gel by Texture analyzer

Jones and his team's description of texture profile analysis to identify semisolid medication dosage forms was published in Jones et al. (1980). (Jone., et al;1980).

Table 12: Testing Criteria/Parameters

Test type	Compression	Probe type	Probe-38
Trigger point	5 g	Hold time	0 s
Target value for Gel Strength	5 mm	Testing Speed	30 mm/min

Release kinetics

The herbal gel data was changed to establish the active ingredient's release pattern. mathematical foundation (Martin, 1994). Kinetics of zero-order reactions. The first-order reaction kinetics are concentration independent. Drug release can occur in a dependent kinetics scenario. Constant expansion, erosion, or simple spreading. The Higuchi model is used to calculate the answer after data validation.

Accelerated Stability and Physicochemical Analysis of Gel

The resulting formulation (F1, F2, and F3) underwent accelerated stability testing. The topical herbal gel formulation (2%) was loaded in a humidity chamber (Technico, India) at 25°C ± 2°C/60 percent RH ± 5 percent RH, 32°C ± 2°C/60 percent RH ± 5 percent RH, and 40°C ± 2°C/75 percent RH ± 5 percent RH. The gel was kept at room temperature (20-40°C) so that its color, smell, pH, and viscosity could be determined. Each test was performed in triplicate using samples weighing 30 grams each. (S. A. Al-Suwayeh etal., 2014).

Extrudability

Approximately 20g of gel was inserted within a closed, foldable tube, which was then tightened to prevent rollback. The gel was extruded once the cap was removed. The amount of gel extruded was measured and weighed. Calculated was the proportion of extruded gel. (Nappinai, Pakalapati, Arimilli, 2006).

Skin irritation test

When the created herbal gels were tested for their ability to irritate the skin, none of the formulations showed any signs of erythema or edoema even after 10 days of testing, proving that they were safe. Studies on skin irritation are conducted to find irritation in the most stressful circumstances and when transdermal medication items are being evaluated. 14 days of research were conducted on 18 wistar rats. A study on skin irritation was conducted on three groups of six rats each.

Group 1 assigned as a control group apply with natural skin irritant, 0.9 % w/v saline,
Group 2 apply with placebo patch,
Group 3 apply with final optimized patch.

Gel was administered to the same skin location on the backs of hairless rats for 23 hours to 14 days. If irritation develops after 24 hours, apply a patch to a new area. Every day, the skin was inspected for both major and minor skin reactions using the 0-7 scale described below, which is identical to that used in an official book.

0 = no evidence of irritation

1 = minimal erythema, barely perceptible

2 = definite erythema, readily visible; minimal edema or minimal popular response

3 = erythema and papules

4 = definite edema

5 = erythema, edema, and papules

6 = vesicular eruption

7 = strong reaction spreading beyond test site

Individual daily results should be note down and mention in the table with each day which type of skin reaction occur.

6.11 pharmacological evaluations of polyherbal formulations

Antimicrobial Activity by Disc Diffusion Method

Chemicals and reagents

Peptone water, Muller-Hinton agar, nutrient broth (NB), nutrient agar (NA), and antibiotics. Gentamicin was sourced from Hi-Media Laboratories in Mumbai, India.

Test organisms

The test organisms, Staphylococcus aureus (MTCC 265), *Staphylococcus glurance* (MTCC 265), and *Eschericia coli* (MTCC 167), were obtained from the Microbial Tissue Culture Technology Centre in Chandigarh, India.

Preparation of inoculum

We employed strains of E. coli, S. glurance, and S. aureus. A 100 ml conical flask is filled with 50 ml of nutritional broth. Using a sterile loop and a multi-layer airflow from the retained slides, it was sterilised and then infected with inoculum. They were then placed in an incubator for long enough for the creature to grow at 37°C.

Preparation of media

Nutrient Agar Media(200ml) was prepared with maintained pH at 7 to 7.2.

Pour plate techenique

Prepared inoculums about 1ml was poured in sterile Petri dish & then poured 15ml of Nutrient Agar Media in it & allowed to solidify.

Disc diffusion method

After solidification, Whatman filter discs soaked in 20 µl of sample were carefully inserted by forceps in the centre of Petri dishes with varying doses and stored in an oven for 24 hours. **(Soni H *et al*., 2013).**

Standard

50 µg/disc Gentamicin was taken as the positive control.

About 1ml of the prepared inoculums were placed in a sterile Petri plate, followed by 15ml of Nutrient Agar Media, which was then let to set.

Measurment of zones

Zone of inhibition was measured by zone reader.

Anti-inflammatory activity

The animals were divided into eight groups of three, with the first three serving as controls: (a) no inflammation, saline injections; (b) inflammation, carrageenan injections; and (c) inflammation, therapy with the control product (sodium oleogel diclofenac). The remaining five groups were burned and given F1, F2, and F3 gel treatments. Carrageenan solution (1 percent w/v in normal saline) was used to induce inflammation. To minimize stress-related behavioral changes, the animals were kept apart in observation chambers for roughly 10 minutes. The mice were then returned to the observation chamber after receiving a 50 l injection of carrageenan solution into their left hind paw. Foot thickness (mm) was measured with an electronic digital calliper at 0, 1, 2, 3, and 4 hours following carrageenan administration (Soni H et al., 2013). The left hind foot's sub-plantar area was treated with Polyherbal gel and traditional formulations by gently rubbing 0.5 g of the solution 50 times with the index finger. Thirty minutes later, pleurisy was induced by injecting 50 l of a 1% w/v carrageenan solution under the skin of the rat's left feet. Non-inflammatory control animals' left feet were subcutaneously injected with 50 l of normal saline.

$$\% \text{ inhibition of edema} = (1 - Vt / Vc) * 100$$

Where,

Vt = Volume of edema in test
Vc = Volume of edema in control

6.12 *In-vivo* screening of Wound healing activity Excision wound model on albino rat-

a) Requirements-

- **Animal-** Albino rat 150gm-200gm
- **Drug-** Betadine ointment 25gm (Marketed Formulation)

b) Equipments- Cotton, Surgical blade, Measurement Scale, Black sketch, Surgical hand gloves, Chloroform **(mild)**

c) Procedure-

- For the excision wound study, 5 group was made & each group has 4 animals respectively.
- A circular wound of about 2.5 diameter made on the depilated dorsal thoracic region of the rats under light chloroform anesthesia in aseptic condition.
- The gel formulations was applied for 12 days the observation of percentage wound closure were made on 4 th,8 th,12 th post wounding days.
- Along with percentage wound closure epithelization period was also determined.
- Grouping of animal were done as follows
- The percentage wound contraction was determined using the following formula

$$\% \text{ Closure} = \frac{\text{Wound area on corresponding days - Wound area on day zero}}{\text{Wound area on day zero}} \times 100$$

- Group 1 **(Control)-** No treatment given
- Group 2 **(Standard)-** Treated with standard marketed formulation betadine
- Group 3 **(Test Formulation1)-** Treated with test formulation containing Sheawaga- 10gm

  Tulsi- 5gm, Neem- 5gm

- Group 4**(Test Formulation 2)-** Treated with test formulation containing

  Shewaga- 5gm

  Tulsi- 10gm

  Neem- 5gm

- Group 5**(Test Formulation 3)-** Treated with test formulation containing

  Shewaga- 5gm

  Tulsi- 5gm

  Neem-10gm

➢ On 12th day of post wounding days some parameters were evaluated that were percentage wound contraction & epithelization period.

Evaluation of parameters

The area that has contracted over a predetermined length of time is transformed into a % unit to calculate percentage wound contraction. The epithelization period is the amount of time until a scar forms and new skin grows in.

The area that has contracted over a defined period of time is converted into a percentage unit to calculate percentage wound contraction. The epithelization period is the time it takes to develop a scar and generate new skin.

***Incision wound model*: -**

Under light ether anaesthesia, 6 cm paravertebral incisions were performed on both sides of the spinal column with a sharp blade, cutting through the full thickness of the skin. The wounds were sutured with Ethicon 4-zero silk thread and a heterospherical body needle. Sutures will be removed on the eighth post-injury day, and tensile strength will be measured on the tenth post-injury day using a tensiometer.

Measurement of tensile strength:

Tensile strength is the capacity to withstand tearing when under tension. To do this, the scarred and freshly healed tissue were removed, and a tension gauge was used to assess the tensile strength. (2013) Gopalakrishnan and Rajangam.

Estimation of biochemical marker: Hydroxyproline

An approximately (500 mm2) circular wound was created, as depicted in the incision model. For 19 days, the ointment was given topically to the surgical wound. After 20 days, the scab was removed and dried in an oven at around 110 degrees Celsius. After being weighed, 10 mg was placed in a test tube with a glass stopper. Each tube containing 10 mg of dry scab had 1 mL of 6NHCl added. The tube was then heated for hydrolysis for 24 hours (12 hours each day over two days). The hydrolysis was then cooled, and excess acid was neutralized with 10NNaOH while employing phenolphthalein as an indicator. The neutral hydrolyzate was diluted with pure water to a concentration of 20 mg/ml. Using the final hydrolyzate to produce hydroxyproline. The hydroxyproline (HPR) level was determined. Each tube received 1 mL of hydrolyzate, 2.5 N NaOH, 0.01 M CuSO4, and 6% H 2O2. The tube was thoroughly shaken before being immediately immersed in an 80 °C water bath. The tube was removed after 15 minutes and immersed in cold water for 5 minutes. 41.2 mL of 3NH 2SO and 0.6 mL of freshly prepared para-dimethylaminobenzaldehyde in n-propanol solution were added. The test tube was heated in a water bath to 75 °C for 15 minutes before cooling for 5 minutes under running water. A spectrophotometer was used to analyze color intensity at 540 nm in comparison with a blank. An estimation of the tissue hydroxyproline content using a standard curve made with standard 4-hydroxyproline was 10-100 g/ml (S. Murthy etal.,2013).

Table.13: Evaluation of herbal gel

S.No.	Characterization	Inference
1.	Appearance	A brownish gel
2.	Colour	Sluggish brown
3.	Odor	Slight pungent
4.	pH	7.8
5.	Viscosity	135cps
6.	Spreadability	45.05(Good)
7.	Compatibility with container	Compatible
8.	Extrudability	Excellent

Table 14: Texture analyzer report

Formulation	Hardness(Kg)	Cohesiveness(Kg)	Strength(gm)	Adhesiveness(Kg)
F1	0.022	0.020	100	0.032
F2	0.023	0.019	100	0.030
F3	0.022	0.021	101	0.032

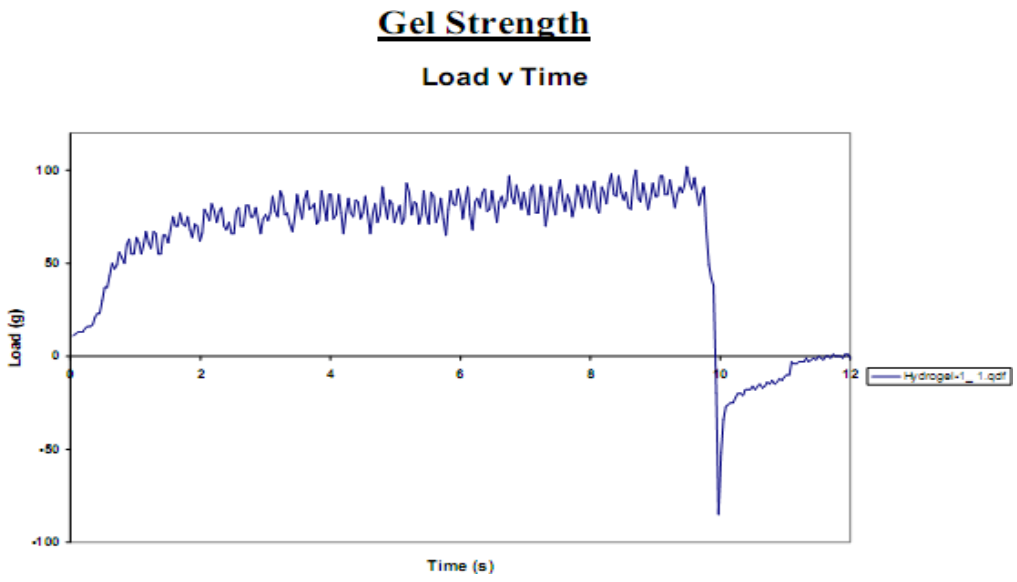


Fig 71: Texture analyzer graph

Table.15 *In-vitro* release kinetic of herbal gel formulation

Formulation	Zero order R^2	First order R^2	Higuchi model R^2	Best fitted model
F1	0.968	0.910	>1	Zero order
F2	0.955	0.946	0.926	Zero order
F3	0.982	0.914	>1	First order

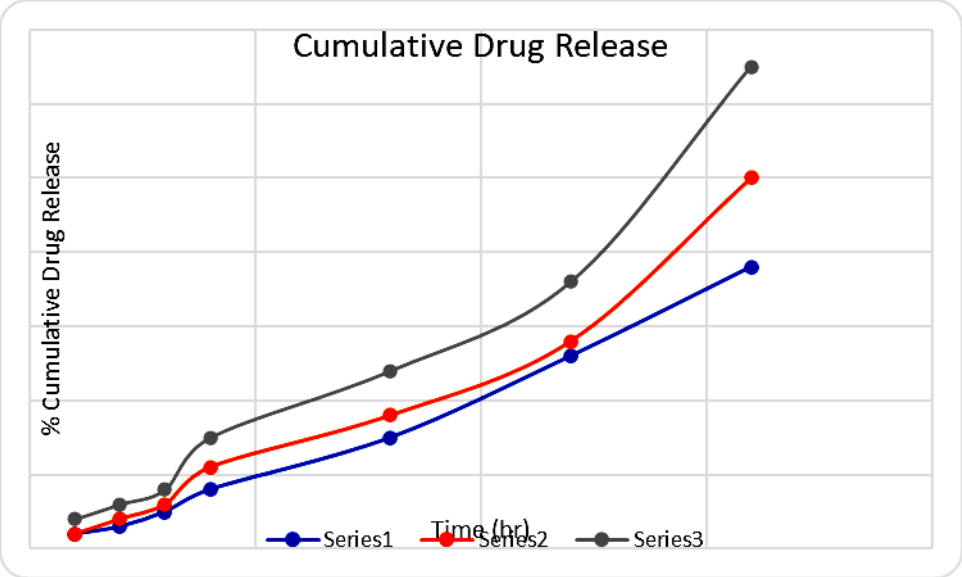


Fig 72: Graph Cumulative drug release

Table. 16: Stability studies

SNo.	Parameter	Storage condition		
		$25^0C\pm2^0C/60\%RH\pm5$	**$32^0C\pm2^0C/60\%RH\pm5$**	**$40^0C\pm2^0C/60\%RH\pm5$**
		0 1 2 3 4 5 6 months	0 1 2 3 4 5 6 months	0 1 2 3 4 5 6 months
1.	Color	No change	No change	No change
2.	Odor	No change	No change	No change
3.	Homogeneity	Smooth	Smooth	Smooth
4.	Viscosity(poise)	7.8-7.2	7.8-7.1	7.8-6.90
5.	pH	0.385-0.378	0.385-0.372	0.385-0.348
6.	Sterility	No microbial growth was observed at 24,48&72 hrs	No microbial growth was observed at 24,48&72 hrs	No microbial growth was observed at 24,48&72 hrs

Table.17 : Skin Irritation study of Group (Group with0.9%w/v Saline)

Sr. No.	Skin Irri-tation Sympt om	D 1	D 2	D 3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	0	-	-	-	-	-	-	p	p	p	p	P	P	p	p
2	1	-	-	-	-	-	-	-	-	-	p	P	P	p	p
3	2	-	-	-	-	-	-	-	-	-	-	-	P	p	p
4	3	-	-	-	-	-	-	-	-	-	-	-	-	p	p
5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	p
6	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table.18 : Skin Irritation study of Group (Applied with Placebo gel of F 1)

Sr. No.	Skin Irritation Symptom	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	0	-	-	-	-	-	-	-	-	-	-	P	p	p	p
2	1	-	-	-	-	-	-	-	-	-	-	-	P	p	p
3	2	-	-	-	-	-	-	-	-	-	-	-	-	P	p
4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table.19: Skin Irritation Study of Group (Applied with Placebo gel of F 2)

Sr. No.	Skin Irritation Symptom	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	0	-	-	-	-	-	-	-	-	P	P	P	p	p	p
2	1	-	-	-	-	-	-	-	-	-	-	P	P	p	p
3	2	-	-	-	-	-	-	-	-	-	-	-	P	P	p
4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	P
5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table.20: Skin Irritation Study of Group (Applied with Placebo gel of F3)

Sr. No.	Skin Irritation Symptom	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14
1	0	-	-	-	-	-	-	-	-	-	-	-	P	P	P
2	1	-	-	-	-	-	-	-	-	-	-	-	-	P	P
3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	P
4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table.21: Primary irritation study of Batches (gel)

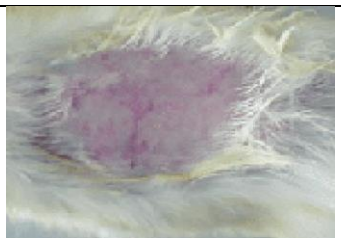
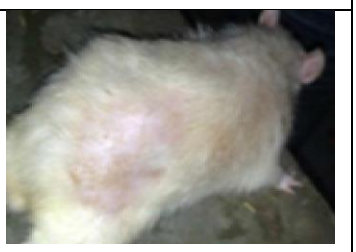
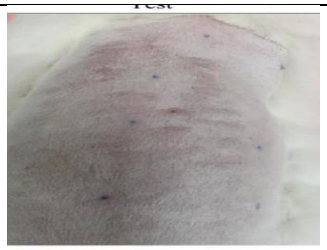
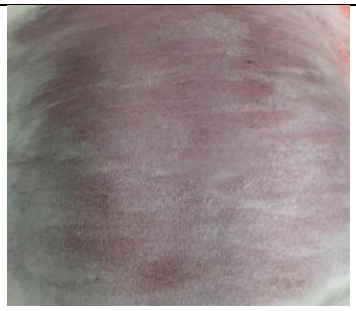
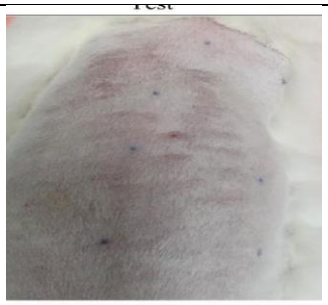
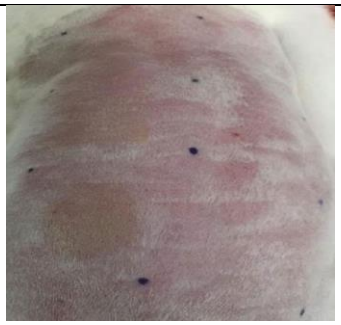
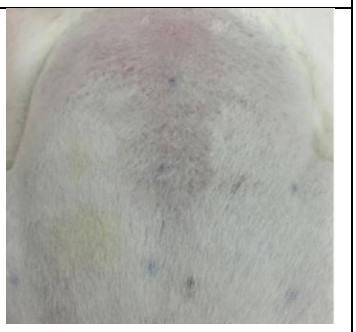
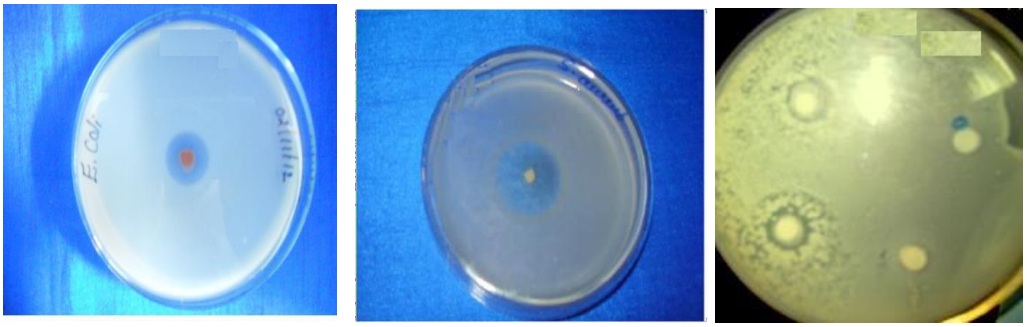
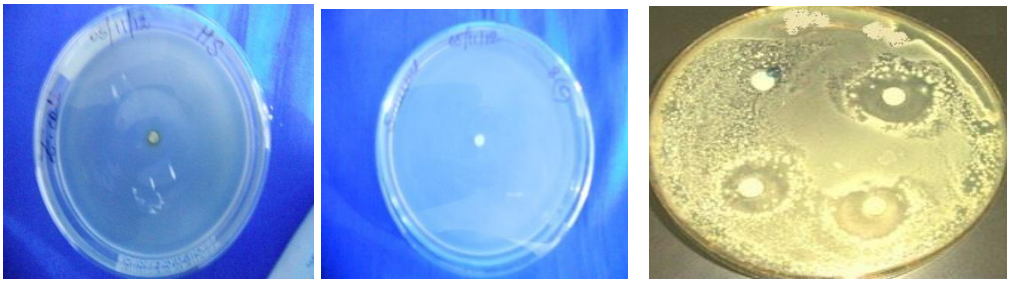
Batches	D1	D14
Group 0.9% w/v Saline		
F1(Plecebo)		
F2		
F3		

Table 22: Anti-microbial effect of polyherbal formulation by cup plate method

S. No.	Bacterial Strains	Zone of inhibition (Diameter in mm)				
		Ctl.	Std.	Formulation		
				F1	F2	F3
1.	*E. coli*	-	22	07	02	05
2.	*S.aureus*	-	30	06	01	03
3.	*S. glurence*	-	17	14	Nil	10



Standard for *E.coil* Standard for *S.aureus* Standard for *S. glurance*



H2 for *E.coli* H2 for *S.aureus* H2 for *S.glurance*

Fig 73: Observation of zone of inhibition of F1,F2 & F3 formulation

Table 23:Anti-inflammatory effect in term of % inhibition of edema

Time in hrs	F1	F2	F3	Oleogel (standard)
1	71.67	73.78	70.04	81.11
2	72.05	72.35	73.97	88.54
3	39.87	37.49	34.01	49.37
4	29.56	26.07	24.63	42.44
5	27.91	19.96	17.04	29.34

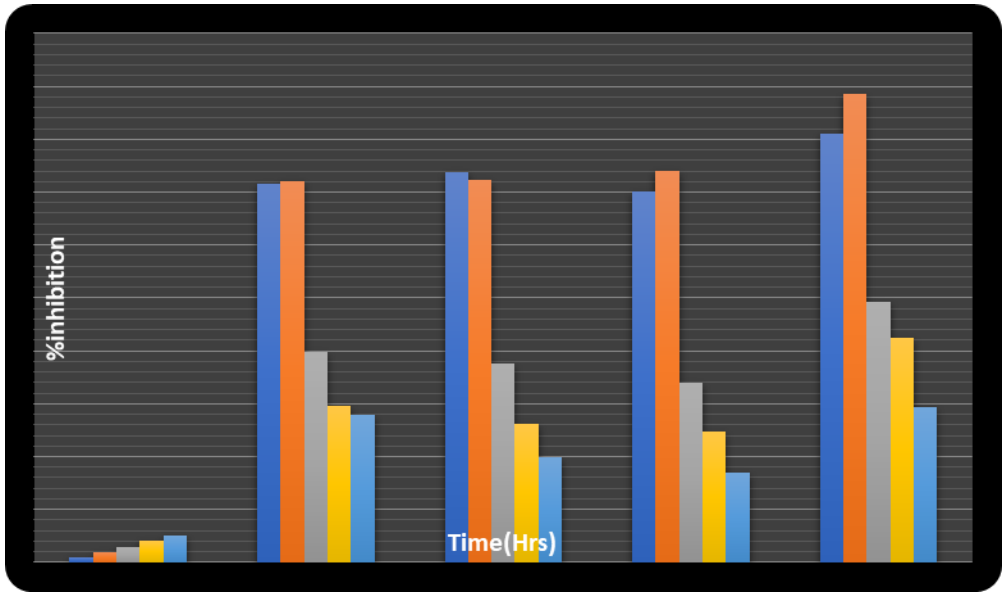


Fig 74: Graph % inhibition of edema

Table .24: Effect of topical application of drug on Excision wound

DAYS	CONTROL	STANDARD	TEST F-1	TEST F-2	TEST F-3
4th Day	43.3875±0.9446	65.13±1.335 a***	53.33±1.244 a*** b***	40.225±2.264 a** b***	54.947±3.003 a*** b**
8th Day	61.71±1.914	93.98±2.493 a***	56.44±1.643 a*** b***	39.27±2.961 a*** b***	55.447±0.415 a*** b***
12th Day	62.237±1.837	96.31±1.408 a***	9.802±1.34 ns b***	68.61±3.277 a*** b***	86.582±0.610 a*** b***

*P<0.05, **P<0.01, ***P<0.001, P>0.05 ns-Non-Significant, n=4

a- significance difference as compared to control

b- significance difference as compared to Standard

One-way Analysis of Variance (ANOVA)

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Tukey-Kramer Multiple Comparisons Test

If the value of q is greater than 5.080 then the P value is less than 0.05.

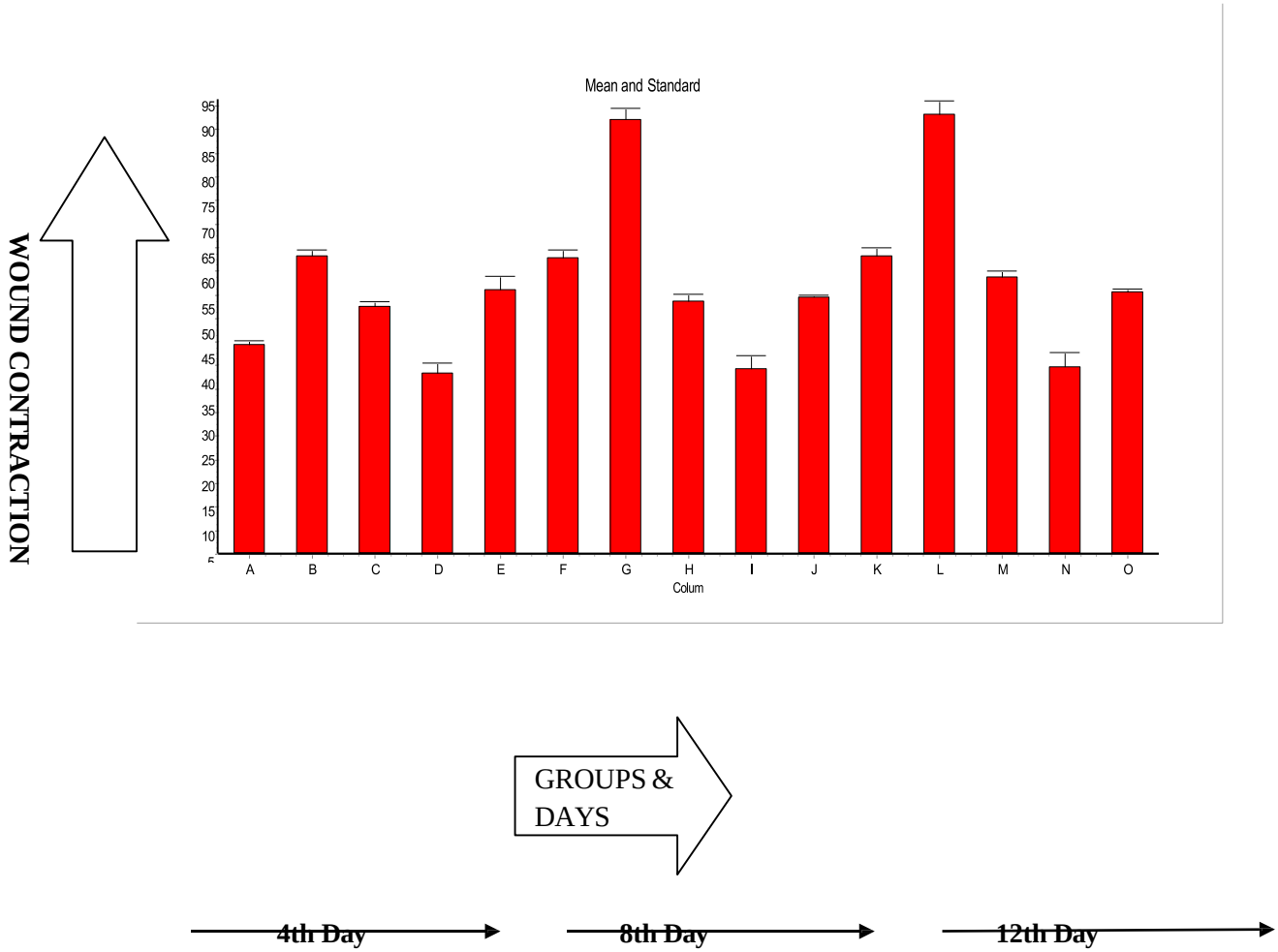
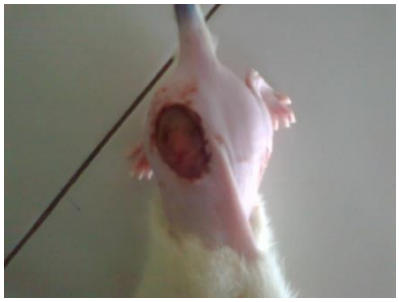
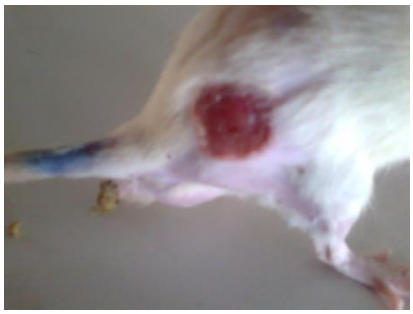


Fig 75:Graph showing mean & standard deviation of wound contraction

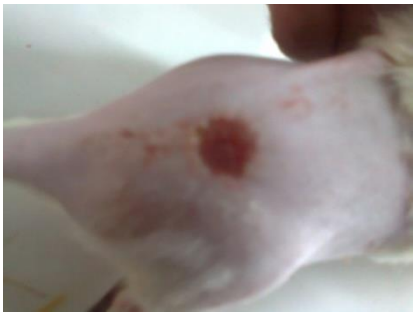


0th day

4th day

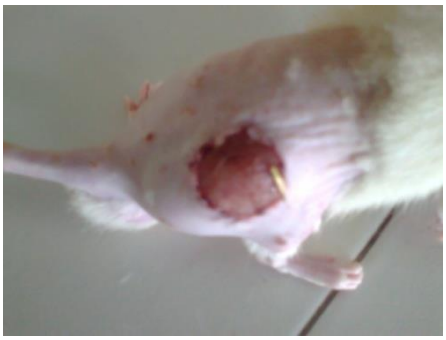


8th day



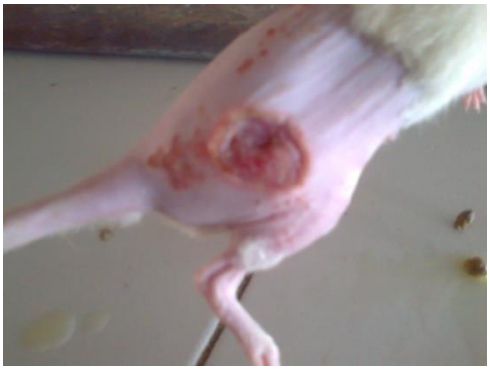
12th day

CONTROL- Fig 76(a)

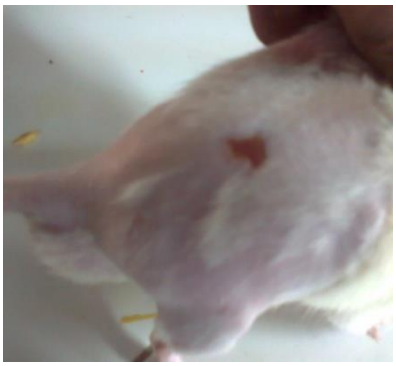


0th day

4th day

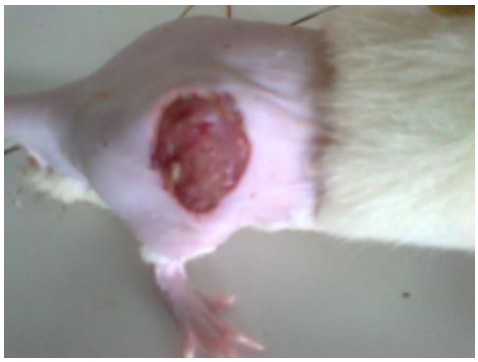


8th day

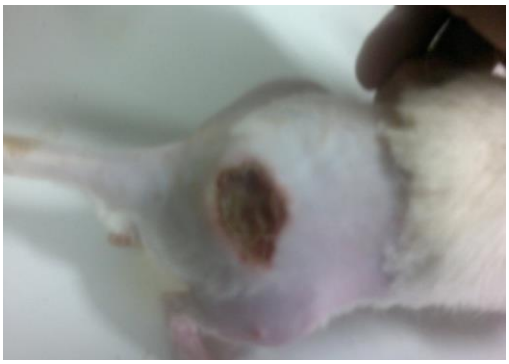


12th day

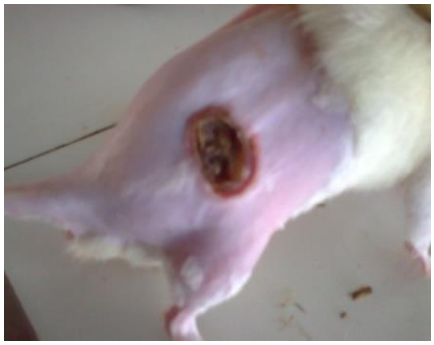
STANDARD- Fig 76(b)



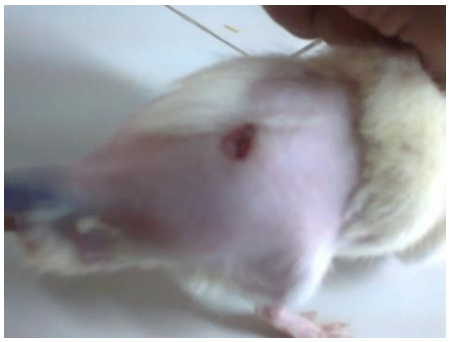
0th day



4th day

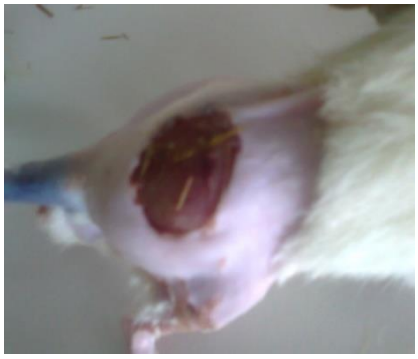


8th day

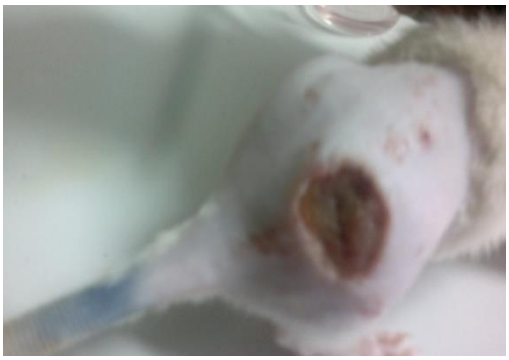


12th day

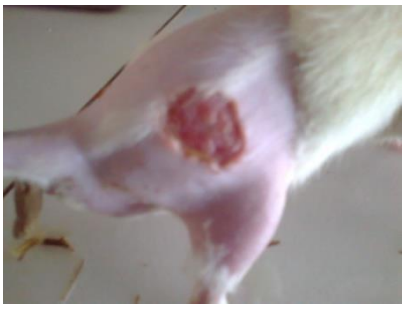
TEST F-1- Fig 76(c)



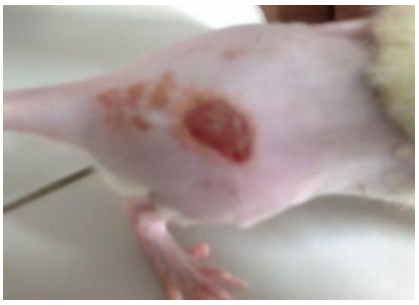
0th day



4th day

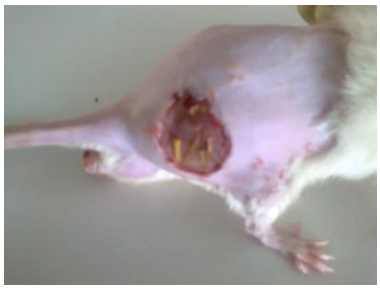


8th day

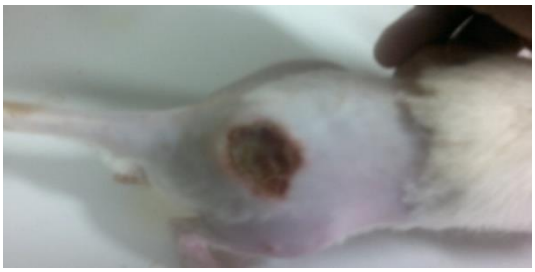


12th day

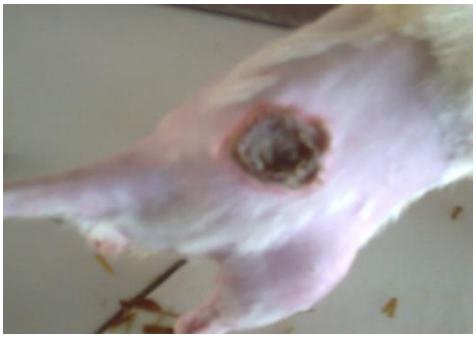
TEST F-2- Fig 76(d)



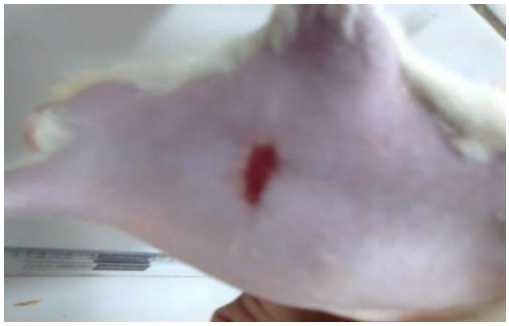
0th day



4th day



8th day



12th day

TEST F 4- Fig: 76(e)

Table.25: Effect of topical application of drug on epithelization period

Group	**Control**	**Standard**	**Test F-1**	**Test F-2**	**Test F-3**
Epithelization Period	21.500±1.915	16±1.633 a**	17±2.582 a*	20±1.63 Ns	17.75±1.708 Ns

*P<0.05, **P<0.01, P>0.05 ns-Non-Significant,n=4

a- significance difference as compared to control

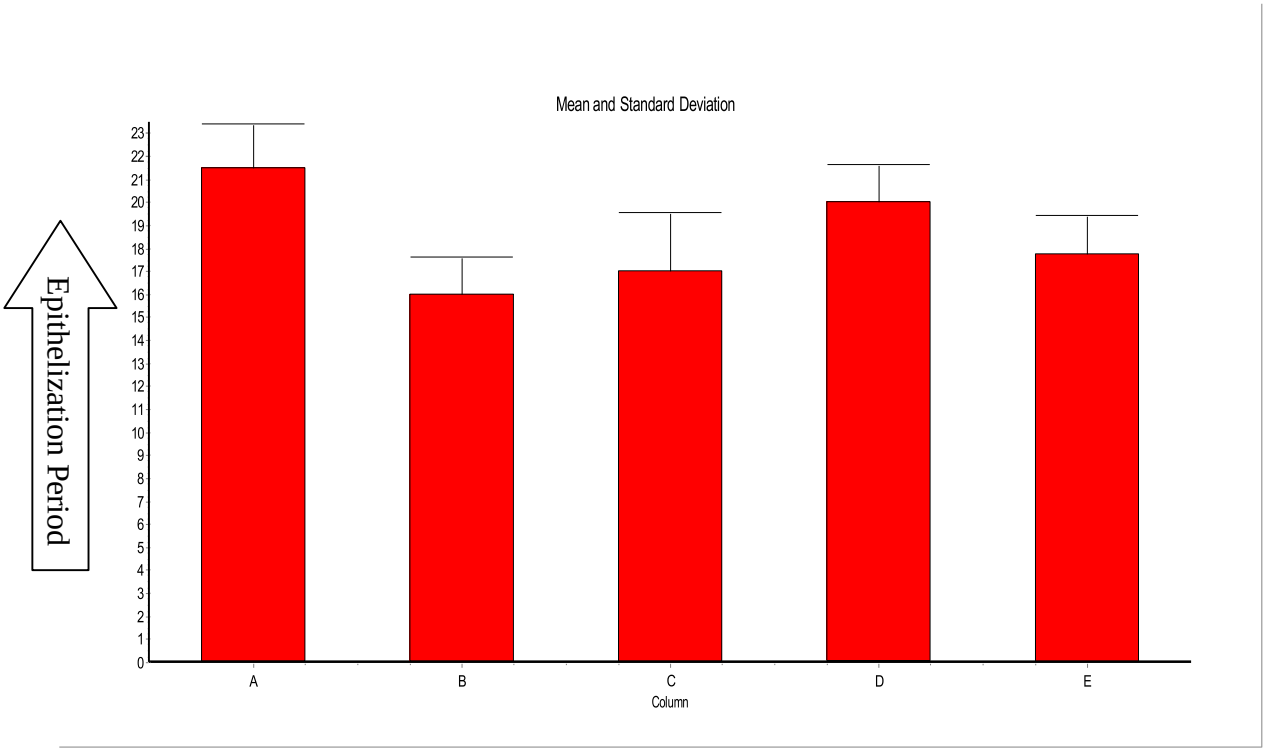
b- significance difference as compared to Standard

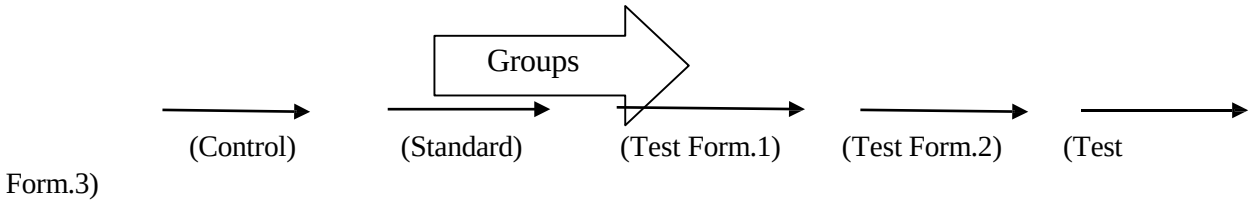
One-way Analysis of Variance (ANOVA)

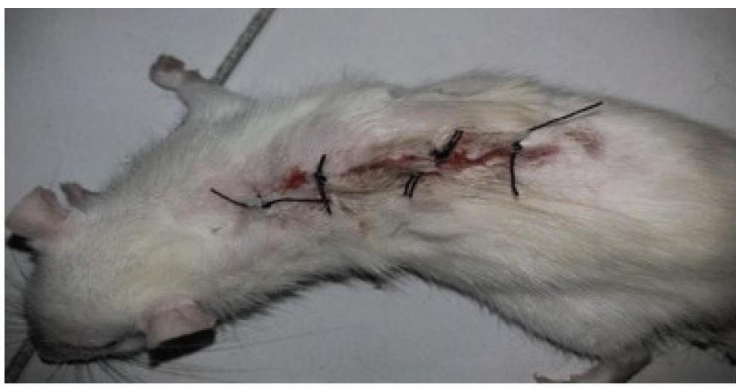
The P value is 0.0064, considered very significant. Variation among column means is significantly greater than expected by chance.

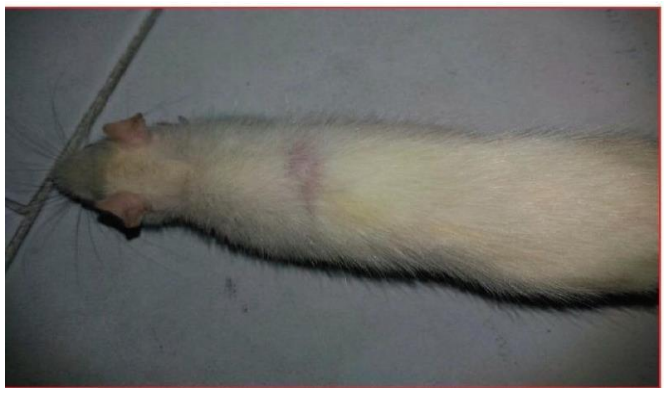
Tukey-Kramer Multiple Comparisons Test

If the value of q is greater than 4.367 then the P value is less than 0.05.









Graph.14: Graph showing mean & standard deviation of epithelization period

Fig:81 Incision wound model

Fig.82 : Healed excision wound on 17thday

Fig.83: Tensiometer: for the measurement of tensile strength

Table 26: Mean Tensile strength of resutured incision wound on 10th Post Wounding Day

S.No.	Groups	Breaking strength (gm)
1.	**Control**	244.86 ± 1.47
2.	**F1**	411.42±0.79*
3.	**F2**	392.2±0.36
4.	**F3**	409.96±01.29
5.	**Standard**	419.2±3.8

Value are expressed as the Mean ± SEM, n = 6 in each group P< 0.001 significance Vs control

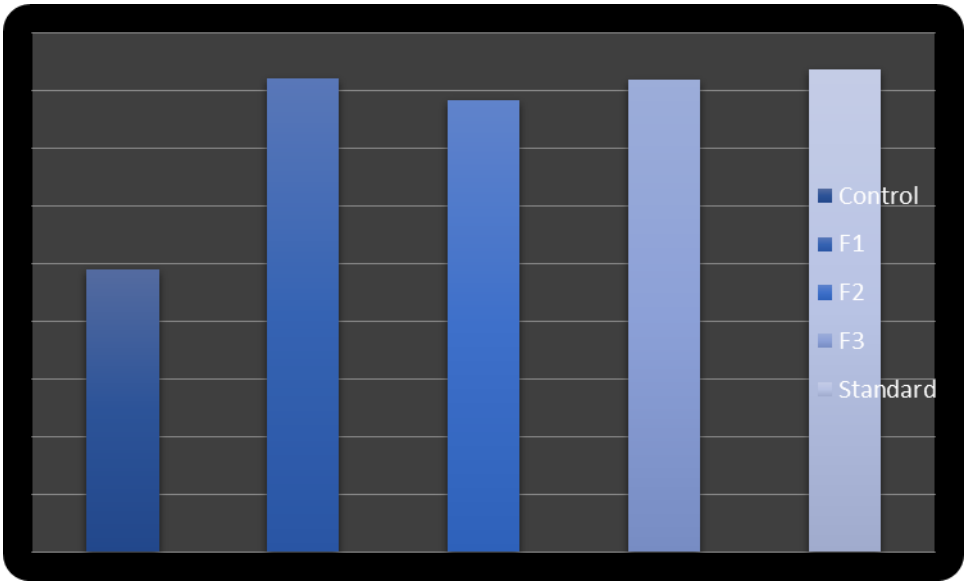


Fig 84:Graph Mean Tensile strength of resutured Incision wound on 10th Post Wounding Day

Table .27: Effect of applying different Polyherbal formulation on content of hydroxyproline in the eschar of excision wound

Group (N= 6)	Hydroxyproline (µg/ 500 mg)
Gr I(Control)	29.22 ±0.67
Gr II(Standard)	43.02 ±0.70
F1	41.58 ±0.45***
F2	38.45±0.15**
F3	40.86±0.90**

SO, simple gel base; n = 6 animals in each group.

The treated groups are compared by Student t-test with the control group.

** P < 0.01, *** P < 0.001

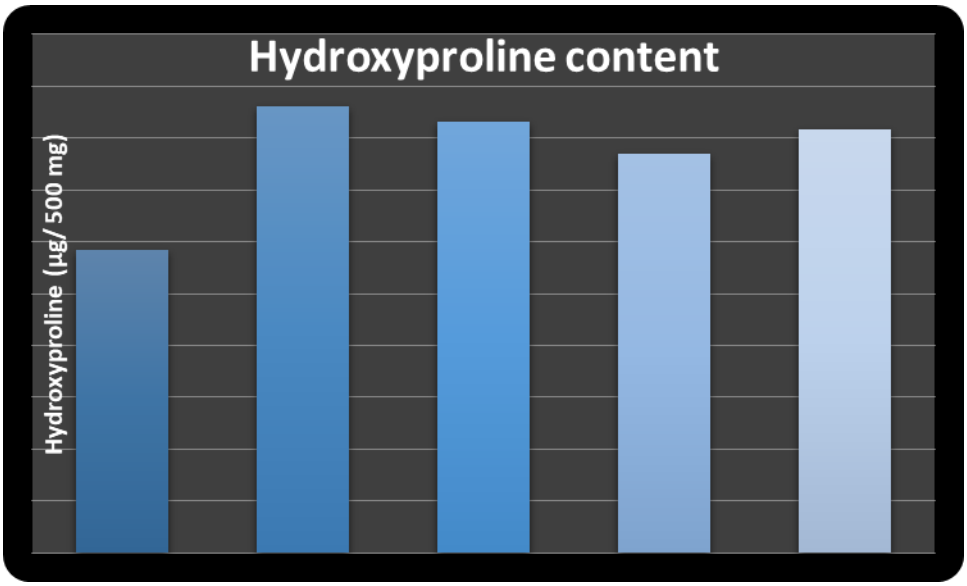


Fig 85: Graph Hydroxyproline content

CHAPTER - 7
RESULT AND DISCUSSION

Wounds are the outcome of injuries to the skin that cause disruption to the other surrounding soft tissue. In response to an injury, the process of tissue remodelling and repair is what comprises the healing of a wound. This process is both complicated and drawn out. In order to treat wounds, numerous compounds derived from plants have been utilized over the course of time. Blood clotting, infection management, and wound healing are all areas that can benefit from the use of herbal extracts that speed up the healing process. Discovering phytoconstituents that are derived from plants and determining whether or not they possess antibacterial properties is necessary for the treatment of wounds.

The Moringa oleifera Lam. plant, more commonly referred to as munga, is widely farmed in India and is considered to be one of the most significant botanical species. The family Moringaceae is the one that it belongs to. There is a plant known as Moringa oleifera Lam that is utilized extensively as a food supplement. Anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-cancer, antimicrobial, anti-oxidant, cardiovascular, anti-ulcer, central nervous system activity, anti-allergic, wound healing, analgesic, and antipyretic effect are some of the valuable pharmacological features that it possesses. The plant is also known as the Drumstick Tree and the Horse-radish Tree. Both of these names are used interchangeably. This herb possesses remarkable therapeutic properties in every aspect of human health. Vitamin A, vitamin C, and milk protein are all found in high concentrations in this food. A number of active phytoconstituents are present, some of which include alkaloids, proteins, quinine, saponins, flavonoids, tannin, steroids, glycosides, fixed oil, and lipids. These are just some of the available phytoconstituents.

The herb tulsi, whose scientific name is as *Ocimum sanctum* Linn, is considered to be the most significant plant used in Ayurveda, and recent research is proving that it has numerous positive effects on health. Increasing evidence suggests that the distinctive combination of pharmacological properties that tulsi possesses can alleviate stress on multiple levels,

including the physiological, metabolic, and psychological levels. It has been demonstrated that tulsi can protect organs and tissues from both chemical and physical stress. This includes the stress that is brought on by prolonged physical activity, ischemia, physical restraint, exposure to cold, and excessive noise. Pollutants from industrial processes and heavy metals are the root causes of chemical stress. Tulsi has also been discovered to combat psychological stress by enhancing memory and cognitive performance. Additionally, it has been found to combat metabolic stress by normalizing blood glucose, blood pressure, and lipid levels. Additionally, it has been found to counteract metabolic stress through its anxiolytic and antidepressant properties. The broad-spectrum antimicrobial activity of tulsi, which includes activity against a variety of human and animal pathogens, suggests that it can be used as a hand sanitizer, mouthwash, and water purifier. Additionally, it can be utilized in animal husbandry, wound healing, the preservation of food and herbal raw materials, and the health of travelers. In addition, it can be utilized for the purpose of preserving food and herbal raw materials, treating wounds, elevating animals, and raising animals.

Neem, also known *as Azadirachta indica*, is a plant that is often found in nations such as India, Pakistan, Bangladesh, and Nepal. It belongs to the Meliaceae family and is addressed in this article in terms of its potential therapeutic use for the treatment of a wide range of illnesses. There are several distinct components that may be found in *Azadirachta indica*, such as limonoids, nimbin, nimbidin, and nimbolide along with other components. Altering a variety of genetic pathways and other processes is one of the ways that these kinds of substances can be helpful in the disorder management process. Quercetin and ß-sitosterol were the first polyphenolic flavonoids to be extracted from neem leaves. These flavonoids were also recognized to possess antibacterial and antifungal activities because of their flavonoid composition. Numerous biological and pharmacological effects, including antibacterial, antifungal, and anti-inflammatory actions, have been reported. These effects will be discussed more below. Previous researchers have demonstrated that they possess anti-inflammatory, anti-arthritic, antipyretic, hypoglycemic, anti-gastric ulcer, antifungal, antibacterial, and antitumor properties. Additionally, they have been shown to have antitumor.

Throughout the process, the crude medication underwent a first physiochemical evaluation. Table 1 and figures 1–3 show that the results revealed that the ethanol extract of each plant had a high percentage of extractive value when compared to other extracts. A standard approach was employed to determine the percentage of moisture content, and the LOD values for M. oleifera, O. sanctum, and A. indica were determined to be 4.1, 0.7, and 15.2 degrees, respectively (table 2 and graph 4). We will be able to use drugs as a home treatment with the help of these simple yet reliable criteria. They also help businesses determine and select raw materials for pharmaceutical production. Because quality standards are based on the proper selection of raw materials, standardization is important in the production of high-quality pesticides. The examination of crude medicines is extremely important in the pharmaceutical industry because authorized monographs only cover a restricted number of specified standards. Soxhlation, which uses ethanol as a solvent, was used to extract the leaves of each plant.

The objective of this preliminary phytochemical study of ethanolic leaf extracts was to determine the numerous active components present. The results revealed that each extract included tannin and flavonoid, but the ethanolic leaf extracts of O. sanctum and A. indica contained alkaloid and glycoside. Each extract contained tannins and flavonoids.

Phytochemicals, particularly flavonoids, are widely known for their ability to prevent and treat diseases. Flavonoids, the most common and widely distributed form of plant phenol, can be found in nearly every plant component, especially photosynthesis-producing cells. Flavonoids are prevalent in plants and belong to a broad class of polyphenol chemicals that are structurally related to benzopyrone. Flavonoids exhibit a wide variety of pharmacological actions. Flavonoids have the ability to activate enzyme systems that protect humans. A growing amount of evidence suggests that flavonoids may protect against a variety of infectious (bacterial and viral) and degenerative diseases, such as cardiovascular disease, cancer, and other age-related issues. Flavonoids in plant tissues subjected to various biotic and abiotic stressors function as a secondary antioxidant defense system. Flavonoids can be found in both the nucleus of mesenchymal cells and in ROS generation sites. Flavonoids are known to have a variety of therapeutic qualities, including antibacterial, hepatoprotective, anti-inflammatory, anticancer, and antiviral effects. The

$Alcl_3$ colorimetric method was used to calculate the total flavonoid concentration. M. oleifera and O. sanctum contained 33.2 and 25.38 mg/gm of flavonoid, respectively, while A. indica had a high flavonoid concentration (52.9 mg/gm). Table 4 and Graph 5 summarized the results for with them.

Spectrophotometric analysis was used to determine the spectrum of the Shimadzu 1700 UV spectrophotometer. Figure 13 illustrates that ethanolic leaf extracts of M. oleifera, O. sanctum, and A. indica had λmax values of 334, 210, and 366 nm, respectively. Furthermore, the spectrum data aid in the chromatographic fingering of the extract by HPLC.

The term "herb pattern fingerprints" refers to a set of chromatographs or spectral signals that are unique to a specific plant and help identify a single sample. Numerous chromatographic techniques, such as high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography (GC), and high speed counter current chromatography (HSCCC), were used to identify the fingerprinted structures. Chromatographic fingerprint technology has lately emerged as an important quality control tool in traditional Chinese medicine. Simultaneously, a rising number of people are focusing on integer characterisation and identification, as well as quantitatively evaluating sample composition. The system's stability chromatographic fingerprint is a sort of approach that displays chemical information from pharmaceuticals using chromatograms, spectrograms, and other figures obtained through analytical methods. Until now, chromatography technologies have included fingerprint TLC, gas chromatography, high-performance liquid chromatography, and others. The Cyber Lab C-18 column (250 x 4.0 mm, 5μ) separated ethanolic leaf extracts of M. oleifera, O. sanctum, and A. indica using high-performance liquid chromatography (HPLC). According to the HPLC chromatogram, the ethanolic leaf extracts of M. oleifera, O. sanctum, and A. indica contained rutin and quercetin (M. oleifera), chlorogenic and gallic acid (O. sanctum), and rutin and quercetin (A. indica). These flavonoids and phenolic chemicals are present in plants. According to the study's findings, the RT values for rutin and quercetin (M. oleifera) were 5.70, 7.70 minutes, chlorogenic and gallic acid (O. sanctum) were 5.48, 7.9 minutes, and rutin and

quercetin (A. indica) were 7.70, 8.80 minutes. Table 5 and pictures 14-18 summarized the study's findings.

Several methodologies were used to evaluate the antioxidant activity of different plant extracts. Solid absorbance at 517 nm is demonstrated by DPPH, a free radical with a consistent nitrogen-focused concentration that can be successfully removed by antioxidants. The example induced the extinction of DPPH-radical absorbance due to an interaction between the antioxidant molecule and the sample. This reaction resulted in the removal of radicals via the creation of H atoms. It may appear as a discoloration ranging from purple to yellow. To determine the amount to which DPPH radicals were removed, the decline in purple intensity was compared to the IC50 value. The ethanolic leaf extracts of O. sanctum, A. indica, and M. oleifera demonstrated radical scavenging effectiveness of 30.92, 82.2, and 86.1 µg/ml, respectively. The IC50 for ascorbic acid (standard) was 3.17 µg/mL. Table.6 and Graph.6 presented the results in tabular format.

It has been proven that the potency of some antioxidants is related to the reducing power of their atoms, which is linked to the presence of reducing agents in general. According to the reduction potential test, the presence of a reducing agent, also known as an antioxidant, in the tested samples causes the Fe3+/ferricyanide complex to be reduced to the iron form, represented by the symbol Fe2+. It is so possible to monitor Fe2+ by measuring the generation of Perl's Prussian blue at 700 nm. The ethanolic leaves extract of A. indica, O. sanctum, and M. orlifera had a reduction power of 2.89, 1.16, and 3.1, respectively. In contrast, the concentration of the reference component (BHT) increased and reached 0.769 at 500 µg/mL (see to table 7 and graph 7-10).

High-performance liquid chromatography (HPLC) analysis of the ethanolic extracts of O. sanctum, A. indica, and M. oleifera revealed the presence of active flavonoid/phenolic compounds such as rutin, quercetin, chlorogenic acid, and gallic acid. These compounds were chosen as lead molecules for additional validation efforts to establish their efficacy in terms of anti-inflammatory, antimicrobial, and wound healing capabilities.

Phosphodiesterase 4 (PDE4) and phosphodiesterase 7 (PDE7), both members of the PDE superfamily, are in charge of breaking down cyclic adenosine monophosphate, which

causes an increase in inflammatory processes in immunomodulatory and pro-inflammatory cells. Dual inhibitors of PDE4 and PDE7 are a novel class of pharmacological candidates capable of regulating both pro-inflammatory and T-cell function. These therapeutic candidates have the potential to be notably effective in the treatment of a wide range of immune system disorders and inflammatory diseases, with fewer undesired side effects. **(Grewal AS etal.,2017)**.

The binding energies of chlorogenic acid, rutin, quercetin, and gallic acid against the PDE4 enzyme are -4.06, -5.74, -6.76, and -3.55 kcalmol-1, respectively. Table 8 presents the results. Figures 25–29 show the 2D and 3D interactions of selected compounds.

The results demonstrated that the minimal binding energies were Quercetin > Rutin > Chlorogenic acid > Gallic acid. The aforesaid findings led to the conclusion that plant phenolic and flavonoid compounds could serve as starting points for the development of effective, powerful, and selective PDE4 inhibitors for the promising treatment of inflammatory illnesses.

The current phenomenal increase in antibiotic resistance in microorganisms poses a serious threat to global public health. Contamination with MRSA, penicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci, and mycobacteria is especially concerning. Tuberculosis, and many of these animals are resistant to certain types of antibacterial drugs. This condition makes it easier to find novel antimicrobial drugs that block critical sites for bacteria that are immune to current chemotherapeutic resistance mechanisms. In this regard, the enzyme aminoacyl-tRNA synthetase (AaRS) has been the subject of contemporary study into the identification of antibacterial drugs. These enzymes are important in protein biosynthesis because they catalyse the synthesis of aminoacyl-RNA (aarRNA). When these enzymes are inhibited, protein production stops, limiting bacterial growth in both in vitro and infectious settings. Antibacterial medicines could potentially target these enzymes (Julian Gregston Hurdle et al. 2020).

With this endeavour, natural plants Phenolics and flavonoids present in plant extracts, such as gallic acid, chlorogenic acid, rutin, and quercetin, have been discovered as ligands, and their inhibitory action against aminoacyl-tRNA synthetase (AaRS) enzymes has been tested

in silico using a docking technique. The binding energies of chlorogenic acid, rutin, quercetin, and gallic acid are -4.20, -5.06, -7.42, and -4.56 kcalmol-1, respectively. Table 9 presents the results. Figures 30–37 show the 2D and 3D interactions of selected compounds.

The molecular docking results revealed that chlorogenic acid, gallic acid, quercetin, and rutin had favourable docking scores. As a result of the preceding findings, it is possible to conclude that phenolics and flavonoids detected in plant extracts were effective inhibitors of the IleRS enzyme.

A computational molecular docking study of selected plant lead compounds against the GSk-3β enzyme validated the current findings.

According to Soni H et al. (2012), the ordered and coordinated process of wound healing includes inflammation, matrix deposition, cell proliferation, tissue modeling, collagenation, and epithelialization. The Wnt/b-catenin pathway promotes wound healing by inhibiting the glycogen synthase kinase-3 (GSK-3) protein, which is a key regulatory enzyme. A variety of medicinal plants have been studied to see if they may be used to make wound-treatment drugs. We tested the phytoconstituents rutin, quercetin, chlorogenic acid, and gallic acid on the GSk-3β enzyme.

Plant phenolics and flavonoids found in selected plants have been shown to efficiently treat wounds. The lead molecules bind to the target protein GSk-3β enzyme with binding energies of -5.26, -5.55, -7.36, and -3.56 kcalmol-1 for chlorogenic acid, rutin, quercetin, and gallic acid, respectively. Table 10 presents the results. Figure 46 shows the binding mode of chosen lead compounds. Figures 38–41 show the 2D and 3D interactions of selected compounds. In molecular docking and dynamics experiments, the ethanolic extract of A. indica, O.sanctum, and M.oleifera gallic inhibited the GSK-3β protein the most effectively. The minimal binding energies were Quercetin > chlorogenic acid > Rutin > Gallic acid. Gallic acid has a low binding energy (-3.56 kJ/mol) but an appropriate affinity for the active pocket. All active drugs effectively inhibit GSK-3β.

The pharmacokinetic profile shows that it has a decent pharmacokinetic profile but no significant hazardous effects such as mutagenicity, tumorogenicity, or reproductive

impacts. Figures 43–45 illustrate the pharmacokinetic and toxicity profiling data of ligands such as chlorogenic acid, rutin, quercetin, and gallic acid. Theoretically, all of the ligand molecules have an encouraging docking score.

Table 11 shows the formulation of polyherbal gel with various extract concentrations utilizing carbopol 940 (Carbomer Homopolymer USP), Disodium Edetate IP, Sodium Methylparaben IP, Sodium Propylparaben IP, and Sodium Hydroxide IP as excipients. A created formulation appears brownish in color and has a pungent odor, with a pH of 7.8 and a viscosity of 135 cps. The pH values of the F1, F2, and F3 were found to be within the range, as expected given that the carbopol was formulated with a pH of 5 to 7.5, which is sufficient to achieve a good viscosity and clarity of the gel. The spread of gels determines their therapeutic potential. The gel spreading aids in the uniform application of the gel to the skin, so the manufactured gels must have good spreadability and meet the optimal quality for topical use. The spreadability (45.05) was good, and the extrudability was superb. Table 13 presents the results. The texture analyser was used to further optimize the gel (F1-F3).The results demonstrated that the formulated polyherbal gels had good hardness, cohesion, strength, and adhesiveness. Table 15 and graph 11 show the in-vitro diffusion profiles of the F1 to F3 formulations. Because the pH of the membrane utilized ranged from 5 to 7.8, the gel formulations' in vitro release tests were conducted using phosphate buffer saline pH 7.4. The in vitro release patterns of all three formulations including carbopol 934 resulted in approximately 100% release within 5 hours. Based on our kinetic release investigation, we discovered that F1 and F2 formulations had zero order kinetics, whereas F3 had first order kinetics. Because zero order kinetics is preferable for sustained release, gel composition. To ensure quality, safety, and efficacy throughout the shelf life, a stability study was conducted in accordance with ICH requirements for the F1-F3 formulation (made with carbopol 934), which displayed higher quality features. The color, odor, homogeneity, pH, viscosity, and net content of the topical herbal gel formulation remained unchanged after 0, 1, 2, 3, and 6 months of stability testing. The study's results clearly showed that the prepared topical gel was stable (Table 16).

The skin irritation test was conducted on 18 wistar rats for 14 days. The results showed that because saline solution is a skin irritant, it caused irritation with minor erythema after 10

days and obvious erythema with easily apparent edema after 12 days. Compared to this, neither the placebo nor the optimized formulation caused irritation for up to 10 days, after which there was minor erythema and light redness at the application site. These results of the in-vivo skin irritation study suggested that optimized formulation F1-F3 does not cause any major irritation on rat skin for 14 days and can be safely used for 24 hours. Photographs of optimized formulation F1-F3 before and after the in-vivo skin irritation study are shown in table 17-21.

Polyherbal gels (F1-F3) were tested for antibacterial and anti-inflammatory properties. Antimicrobial activity was assessed using the disc diffusion method. The zone of inhibition for three different strains of bacteria, S. aureus, S. glurence, and E. coli, for F1 and F2 formulations was found to be the most significant when compared to standard (table 22 and figure 48), while formulation F3 also demonstrated considerable inhibition for bacterial strain.

The anti-inflammatory efficacy of several polyherbal gel formulations was assessed using the standard Oleogel. Within 5 hours of delivery, the prepared Polyherbal gels F1, F2, and F3 reduced paw oedema volume by 27.91%, 19.96%, and 17.04%, respectively, whereas the standard medicine reduced paw oedema volume by 29.34% (Table 23 & graph 12).

Various metrics such as wound contraction, tensile strength, and hydroxyproline as a biomarker were used to assess the wound healing capability of various polyherbal gel formulations. Table 14 and graph 3 show the reduction in wound area of the different groups over the 4th, 8th, 12th, and 16th days (fig 49 a-e). Animals treated with F1 and F3 formulations demonstrated the fastest wound healing. The period of epithelization for F1 and F3 was found to be similar to the standard (Table 24-25 & graph 13).

The incision wound models demonstrated that the mean tensile strength of resutered incision wound on the tenth post-wounding day showed F1 & F formulation to be the most significant when compared to standard (table 26, fig. 50-52, and graph 15).

The generated collagen was applied to the wound site and cross-linked to create fibers. Collagen not only provides strength and stability to the tissue matrix, but it also plays a vital role in late homeostasis and epithelialization during the healing process. Collagen is

the most abundant extracellular protein in the scab of healing wounds, and its synthesis in the wound area following injury is quickly rising. Collagen degrades, releasing free hydroxyproline and associated peptides. The measurement of hydroxyproline acts as a marker of collagen turnover. The F1 treated animal had a higher hydroxyproline level (41.58±0.45) than the standard. Although the F2 treated group revealed hydroxyproline content 40.86±0.90. Table 27 and Graph 16 show the results.

CHAPTER - 8
SUMMARY AND CONCLUSION

Botanicals have long been used to heal chronic and infected wounds, despite modern science's inadequate understanding of the molecular basis of these treatments. Further research is needed to completely understand the molecular basis of herbal wound healing therapy. The most important finding is that research into the growing conditions, postharvest handling, and pharmacological preparation of these botanicals has confirmed their importance in terms of the chemical composition and pharmacological activity of the finished product used in pre-clinical and clinical trials.

Wound healing is a multiphase process that involves stages including hemostasis, inflammation, proliferation, and remodelling. Poor blood flow to the wound bed and microbial infection are two additional reasons that could hinder wound healing.

A polyherbal formulation containing plant extracts (*M. oleifera, O. sanctum, and A. indica*) accelerates wound healing by increasing angiogenesis at the site of damage, as well as fibroblast and keratinocyte proliferation and mobilization.

This study looks at the potential efficacy, safety, and molecular modeling of three traditional medicinal plants used to treat wounds: M. oleifera, O. sanctum, and A. indica. The main ingredient in the ethanolic extracts was tested for its ability to promote wound healing utilizing in silico molecular docking against the GSK-3 enzyme. In addition, in-vivo and in-silico testing were performed to examine the potential anti-inflammatory and antibacterial efficacy of ethanolic extracts of *M. oleifera, O. sanctum,* and *A. indica* as a wound healing support mechanism.

A preliminary phytochemical investigation found that the ethanolic extracts of *M. oleifera, O. sanctum,* and *A. indica* contained flavonoids and phenolic content and yielded more extract than other extracts. Ethanolic leaf extracts were chosen to evaluate their anti-inflammatory, antibacterial, and wound healing properties. In an in-vivo wound healing study, the polyherbal formulation significantly enhanced the healing power of an excision wound model compared to standard.

Many parts of wound healing rely on redox control to maintain a precise balance of oxidative stress and antioxidants. While minimal levels of reactive oxygen species and oxidative stress are required for optimal wound healing, excessive oxidative stress inhibits wound healing. Antioxidants are hypothesized to accelerate wound healing by lowering the oxidative stress induced by wounds. In the current investigation, ethanolic leaf extracts *from M. oleifera, O. sanctum,* and *A. indica* shown considerable antioxidant activity when compared to standard ascorbic acid.

The ethanolic leaf extracts contained rutin, quercetin, chlorogenic acid, and gallic acid, according to an HPLC analysis. These active components were then chosen as the lead compounds for the in-silico validation research.

The FGF/TGF- and Wnt/-catenin pathways are two essential cell signaling pathways that promote wound healing and regeneration. A disruption in the release of these substances may affect fibroblast cell activity and wound healing. One of them, the Wnt/-catenin pathway, is required for cell proliferation during wound healing. The most critical signalling stage in this route is the GSK3- enzyme, which phosphorylates and degrades the -catenin protein. When GSK3- is blocked, activated-catenin travels to the nucleus and regulates gene expression. Previous research utilizing a variety of animal models suggests that tiny compounds such as glycogen synthase kinase 3 (GSK3-) inhibitors could be useful medicines for enhancing wound healing.

A computational docking investigation demonstrated that the lead compounds have strong GSK3 inhibitory characteristics. The results revealed a positive docking score and a pattern of strong covalent contact between the lead chemical and the target protein's active region. The synergistic interplay of plant phenolic and flavonoid chemicals is responsible for the polyherbal formulation's wound-healing properties.

Carbopol 940 (Carbomer Homopolymer USP), Disodium Edetate IP, Sodium Methylparaben IP, Sodium Propylparaben IP, and Sodium Hydroxide IP were utilized to create the Polyherbal Gel, which was then tested for anti-inflammatory, antibacterial, and wound healing properties.

The disc diffusion method was employed to determine antibacterial activity. Formulations F1 and F2 had the greatest zone of inhibition for three separate bacterial strains, *S. aureus, S. glurence,* and *E. coli,* as compared to the standard (table 22 and figure 48), while formulation F3 also showed some bacterial strain suppression.

The anti-inflammatory effectiveness of several Polyherbal gel compositions was evaluated using the widely used Oleo gel. The prepared Polyherbal gels F1, F2, and F3 reduced paw oedema volume by 27.91 percent, 19.96 percent, and 17.04 percent, respectively, within 5 hours of treatment (Table 23 and graph 12).

Animals administered the F1 and F3 formulations demonstrated the quickest wound healing. It was revealed that the epithelization time of F1 and F3 was nearly identical. The incision wound models suggested that, as compared to the standard, the mean tensile strength of the recovered incision wound on the tenth post-injury day was most significant for the F1 and F3 formulation.

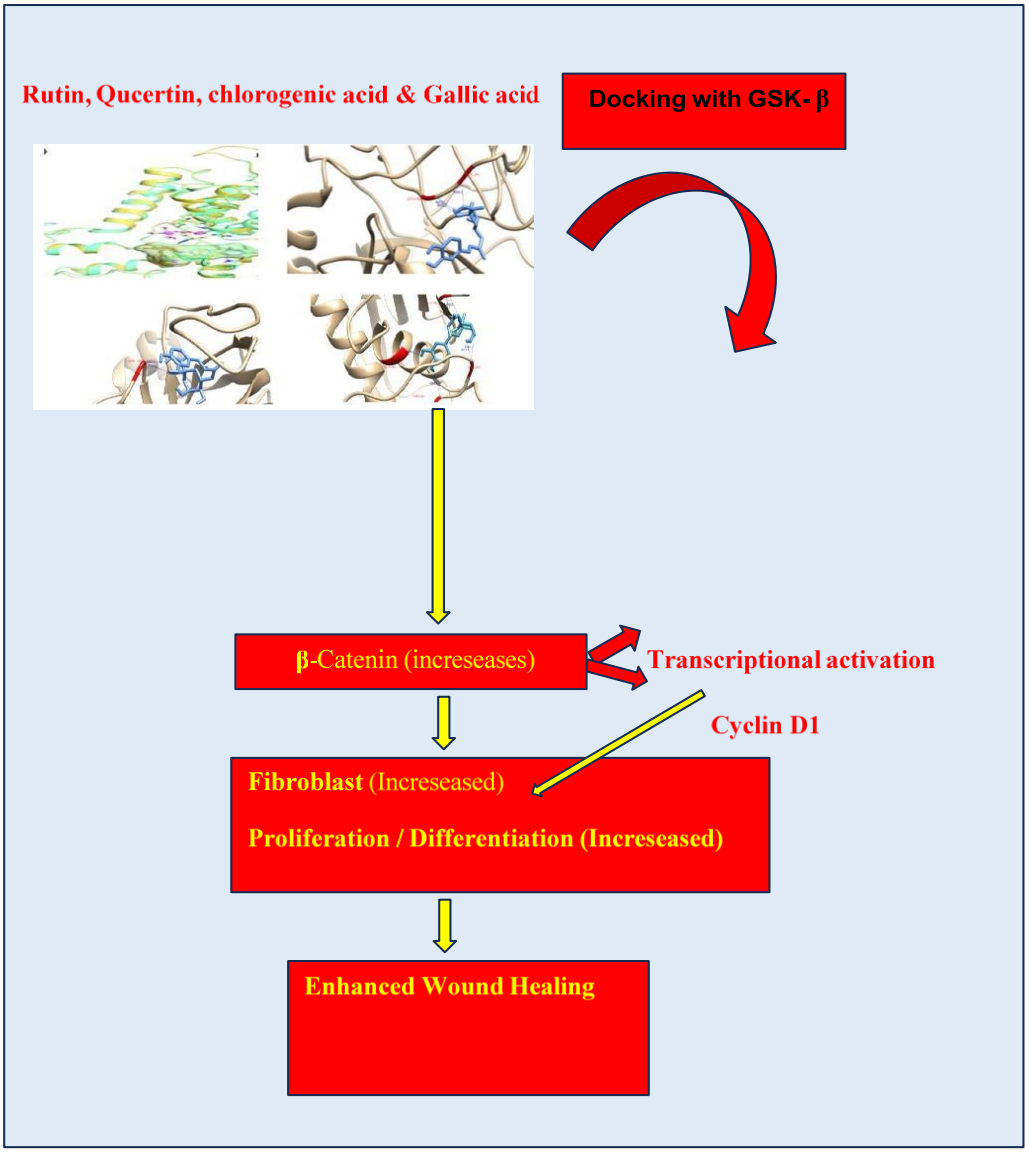
Hydroxyproline levels indicate collagen turnover. The results showed that the F1-treated animal had considerably more hydroxyproline than the control. Despite this, the F2 treated group had hydroxyproline levels that were relatively close to those of F1.

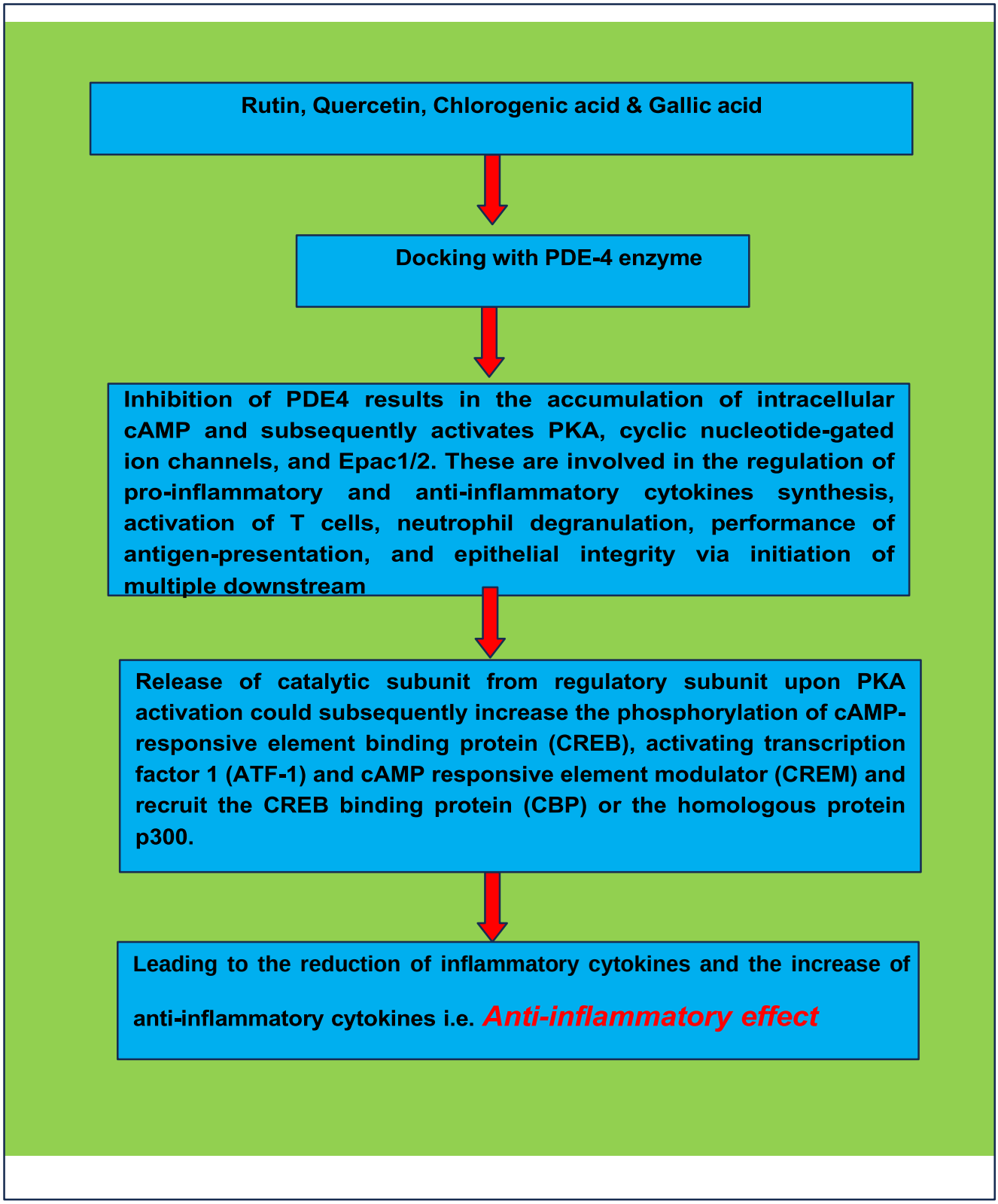
Several therapeutically useful natural bioactive compounds discovered in medicinal plants are synthesized into different drug formulations. Numerous studies have focused on the many benefits of polyherbal formulations, but in order to properly utilize them, a systematic approach is required. However, the main findings of the present study show that polyherbal formulations are quite effective at accelerating wound healing. They have the ability to initiate a variety of physiological processes that promote wound healing. Because of the synergistic effects of flavonoid and plant phenolic extracts, the current study found that polyherbal formulations containing ethanolic leaf extracts of *M. oleifera, O. sanctum,* and *A. indica* had potent antimicrobial, anti-inflammatory, and wound healing efficacy.

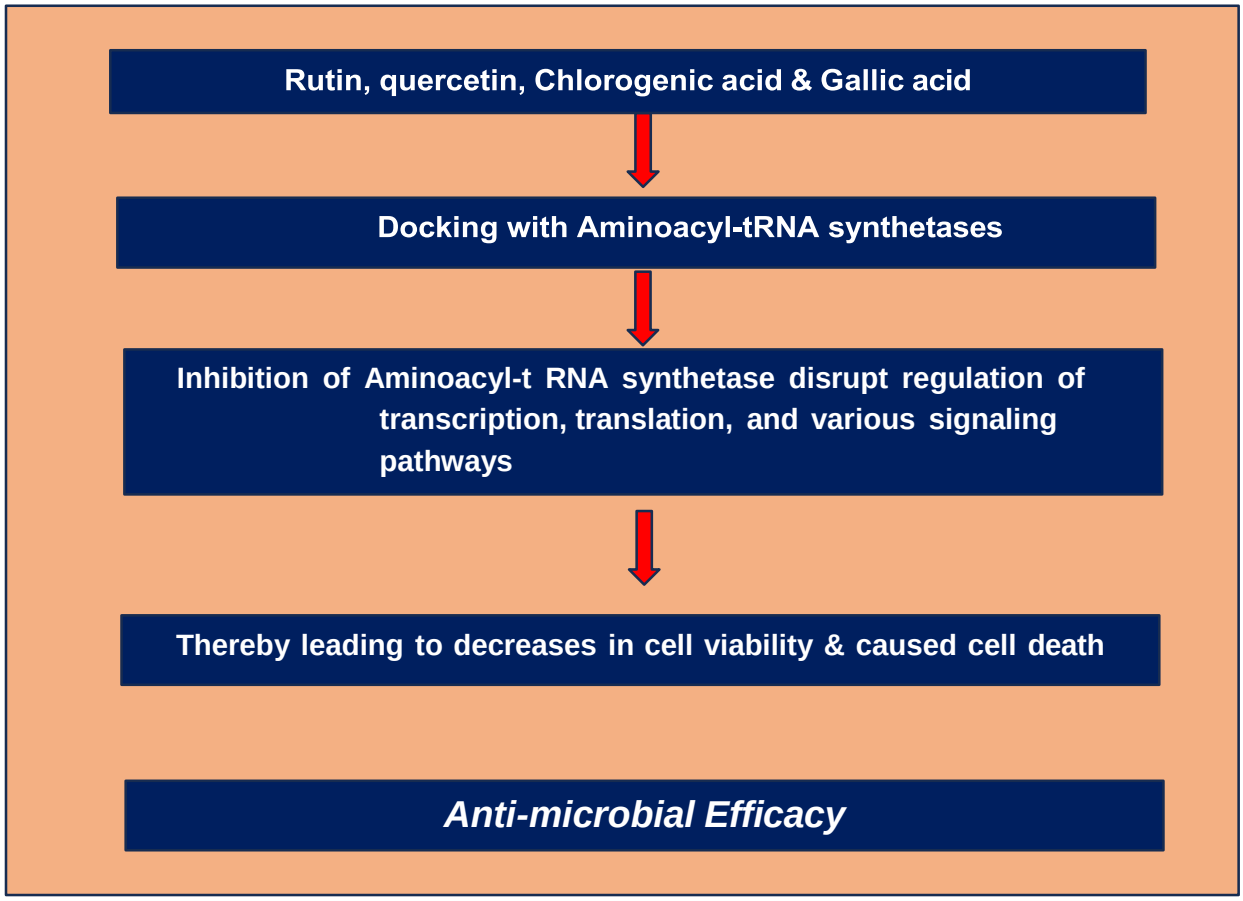
Divulgence of Investigation

The results of the study revealed that ethanolic leaf extracts of M. oleifera, O. sanctum, and A. indica, in addition to rutin, quercetin, chlorogenic acid, and gallic acid, have

considerable anti-microbial, anti-inflammatory, and wound healing action. Contrary to rutin, quercetin accelerates wound healing by reducing inflammation, oxidative stress, and increasing neovascularization. By raising hydroxyproline content, flavonoid content stimulates collagen formation, which improves wound contraction and reepithelialization in wound healing by increasing collagen and fibronectin synthesis. Gallic acid is a powerful antioxidant that directly stimulates the expression of antioxidant genes and accelerates keratinocyte and fibroblast cell migration. Chlorogenic acid's antioxidant activity may have an impact on wound healing effectiveness. The following illustrates the hypothesized anti-inflammatory, antimicrobial, and wound-healing processes of lead compounds:




Rutin, Quercetin, Chlorogenic acid & Gallic acid
Docking with PDE-4 enzyme
Inhibition of PDE4 results in the accumulation of intracellular cAMP and subsequently activates PKA, cyclic nucleotide-gated ion channels, and Epac1/2. These are involved in the regulation of pro-inflammatory and anti-inflammatory cytokines synthesis, activation of T cells, neutrophil degranulation, performance of antigen-presentation, and epithelial integrity via initiation of multiple downstream
Release of catalytic subunit from regulatory subunit upon PKA activation could subsequently increase the phosphorylation of cAMP-responsive element binding protein (CREB), activating transcription factor 1 (ATF-1) and cAMP responsive element modulator (CREM) and recruit the CREB binding protein (CBP) or the homologous protein p300.
Leading to the reduction of inflammatory cytokines and the increase of anti-inflammatory cytokines i.e. Anti-inflammatory effect


Rutin, quercetin, Chlorogenic acid & Gallic acid
Docking with Aminoacyl-tRNA synthetases
Inhibition of Aminoacyl-t RNA synthetase disrupt regulation of transcription, translation, and various signaling pathways
Thereby leading to decreases in cell viability & caused cell death
Anti-microbial Efficacy

CHAPTER - 9

SIGNIFICANCE OF INVESTIGATION

Polyherbal formulations containing phytopharmaceuticals for treating skin wounds via anti-inflammatory and antibacterial action were developed using ethanolic leaf extracts from *M. oleifera, O. sanctum,* and *A. indica.* Furthermore, it has been shown that plants contain a high concentration of plant phenolics and flavonoids. The polymer Carbopol 940 shows acceptable formulation compatibility. This study shows that polyherbal formulations with varying proportions of plant active components are possible. However, in comparison to other formulas, F1 and F3 had the best results. The antioxidant activity of ethanolic leaf extracts was assessed using the ferric-reducing antioxidant power test and the DPPH technique. The results of these approaches are consistent with antioxidant action. The anti-inflammatory activity of several formulations was measured using the traditional Oleo gel paw oedema method, and many properties such as wound contraction, tensile strength, and hydroxyproline content were utilized to assess their potential for wound healing. Furthermore, in-silico molecular docking was used to clarify the proposed mechanism of action, demonstrating the scientific validity of the previously reported activity.

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Review Article

Recent Progression in Wound healing Technologies

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Abstract: Optimizing patient local and systemic circumstances, as well as creating an optimum wound healing environment, are essential components of successful wound care. Many products have been developed to impact the wound environment in order to offer a pathogen-free, protected, and moist environment in which to heal. In the wound healing cascade, newer items are being employed to replace or supplement various substrates. The latest applications of silver in microbial prophylaxis and treatment, including issues involving resistance and side effects, the latest uses of negative pressure wound devices, advanced dressings and skin substitutes, biologic wound products, including growth factor applications, and hyperbaric oxygen as an adjunct in wound healing are all covered in this review of the current state of the art in wound-healing products. With so many options, it's easy to get overwhelmed.

Keywords: Wound healing, dressing, Hydrogel & Technologies.

INTRODUCTION

Wound is defined as the disruption of the cellular and anatomic discontinuity of a tissue [1] .Wound may be produced by chemical, physical, thermal, microbial or immunological insult to the tissue. Wound cause discomfort and are more prone to infection and other troublesome complications [2]. Some diseases like diabetes, Immune compromised conditions, ischaemia and conditions like malnourishment, ageing, local infection, local tissue damage due to burn or gunshot often leads to delay in wound healing. Infection is the major complications of burn injury and is responsible for 50-75% of hospital deaths [3]. Wound healing consists of an orderly progression of events that reestablish the integrity of the damaged tissue. Many of the synthetic drugs currently used for the treatment of wounds are not only expensive but also pose problems such as allergy, drug resistance etc and this situation has forced the scientists to seek alternative drugs[4]. More than 80% of the world population still depends upon traditional medicines for their ailments [5]. Especially for wound management [6] as they provide a moist environment to encourage the establishment of the suitable environment. Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine though their mechanism of action and efficacy have not been evaluated scientifically. Wound in a normal state of body get healed by various processes which is fundamentally a connective tissue response, initial stage of this process involves an acute inflammatory phase followed by the synthesis of collagen and other extra cellular macromolecules which are later remodeled to form a scar [7]. Wound is a physical trauma where the skin is torn, cut, burn or punctured [8]. Normally on wound site various mechanisms of body participate in wound healing i.e. white blood cell [9] fibroblasts, keratinocytes etc. While carbohydrates [10], lipids [11], and proteins [12] metabolism increases with the increase in the resting energy expenditure (RES). Wound healing is also affected by the other diseases such as diabetes etc. antineoplastic drug and antibiotics may also interfere with the wound healing. Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions.[13]Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin [14]. In other words wound is a break in the epithelial integrity of the

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skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, haematoma, laceration or an abrasion [15]. Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase, and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue [16]. 70% of the wound healing Ayurvedic drugs are of plant origin, 20% of mineral origin, and the remaining 10% consisting of animal products and these drugs are stated to be effective in different conditions such as *Vrana* (wounds or ulcers), *Nadivrana* (sinuses), *Vidradhi* (abscess),*Visarpa* (erysipelas), *Upadamsha* (syphilitic ulcers), *Vranajakrimi* (maggots in wounds), *Dustavrana* (septic wounds), *Vranashotha* (inflammatory changes of wounds), *Vranavisha* (cellulitis), *Ugravrana* (purulative ulcer), *Netravrana* (hordeolum or stye sepsis), *Pramehapidaka* (diabetic carbuncle), and *Bhagandara* (fistula-inano) [17]. Some very common plants like *Aloevera, Azadirachta indica, Carica papaya, Celosia argentea, Centella asiatica, Cinnamomum zeylanicum , Curcuma longa, Nelumbo nucifera, Ocimum sanctum, Phyllanthus emblica, Plumbago zeylanica, Pterocarpus santalinus, Terminalia arjuna ana Terminalia chebula* have been extensively reported in ayurveda, siddha and unani systems of medicines for their wound healing potentials [17]. Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China have valuable information of many lesser-known hitherto unknown wild plants for treatingwounds and burn [18]. Traditional forms of medicine practiced for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of wounds related disorders [19, 20]. According to various traditional medicinal practices throughout the world, wounds have been treated mostly topically with different medicinal herbs or with their extracts solely or in combination with some other plant parts [20].

Ancient history of wound healing plants

In ancient times men tried out different plants to see which ones helped cure certain diseases. They probably watched to see what plants the animals ate, especially when they are sick. By trial and error, over the ages, men came to use thousands of plants as remedies for their ills. For example, many American Indian tribes used willow bark to treat rheumatism. How they selected it is not known, but scientists have found that the willow bark contains a pain killing chemical related to one used in aspirin. Many medicinal plants discovered by primitive people are still in use today. The leaves of 'Foxglove' furnish digitalis for the treatment of heart ailments. Quinine, from the bark of the South American Cinchova tree was long used to combat malaria. Curarae, a powerful poison applied by South American Indians to the tips of their arrows, is valuable in the treatment of disease that causes muscular spasms and anesthesia. Rauwolfa, used in the treatment of high BP is derived from the root of a plant that grows in Southeast Asia. It has long been used to treat fevers, insomnia and nervousness. Belladonna and atropine, obtained from the deadly night shade are important in the treatment of eye diseases. Painful spasmodic conditions and other ailments. Ephedrine, used for hay fever and in nose drugs is one of the few drugs that are derived from conifers. Spagnum moss is used for surgical dressings. Antibiotics produced by molds are the most important medicinal discoveries of 20th century. Many plants are rich source of vitamins. Some plant drugs are violent poisons and habit-forming narcotics. About 4500 years ago, when the great civilizations arose in ancient China, India, Babylon and Egypt, men put their knowledge of plant remedies in writing. These written accounts were called 'herbals'. The earliest herbal known was probably written by the Chinese emperor, Shen Nung, about 2700 BC. It contains the accounts of the healing value of about 250 plants. In India, the references to the curative properties of some herbs in the Rigveda seem to be the earliest records of use of plants in medicine. But references to plants in the Rigveda are very brief. More detailed account is available in the Atharva-veda. The period of Rigveda is estimated to be between 3500 and 1800 BC. After the Vedas, there is no information on the development of this science in India for a period of about 1000 years. Charak-Samhila (1000 BC), one of the earliest treatises on Indian Medicine, records the use of over 340 drugs of plant origin; some of these drugs were not indigenous to India. In Egypt carvings on tomb and temple walls show that people used plants for medicine as early as 3000 BC. A long document written about 1500 BC describes more than 800 remedies for all sorts of ailments, from headaches to heart trouble and from sore throats to insect bites. India recognizes more than 2500 plant species as having medicinal value, Sri Lanka about 1400 and Nepal around 700. In Ayurveda about 2000 plant species are considered to have medicinal value. The Indian Pharmacopoiea (1966) recognized 85 drug plants whose ingredients are used in pharmaceutical preparations. The Chinese Pharmacopoiea lists over 5700 traditional medicines, most of which are of plant origin. It has been estimated that out of about 2000 drugs that have been used in curing human ailments in India, only about 200 are of animal origin and a similar number are of mineral origin. The rest, i.e., about 1500 are of plant origin [21].

Phases of Wound Healing

Wound healing involves continuous cell–cell and cell–matrix interactions that allow the process to proceed in three overlapping phase's viz. inflammation cellular proliferation and remodeling.

Phase 1

It is a coagulation and inflammatory phase (0–3 days) and this involves migration of neutrophils at margin of incision, moving towards the fibrin clot.

Phase 2

It is a proliferative phase (3–12 days) in which the neutrophils are largely replaced by the macrophages. Granulation tissue progressively invades the incision space and the incissional space is filled with granulation tissue. Collagen fibrils become more abundant and begin to bridge the incision.

Phase 3

It is a remodeling phase (3–6 months), involving continuous accumulation of collagen and proliferation of fibroblasts. There is marked reduction in leukocyte infiltration and edema. The phase involves synthesis of collagen fibers, leading to increase in tensile strength of the skin. Healing requires the collaborative efforts of many different tissues and cell lineages. It involves platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, alteration in the ground substances, angiogenesis and re-epithelization. Healing is not complete until the disrupted surfaces are firmly knit by collagen. Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. three different phases constitute the physiologic process of wound healing (i***) substrate phases (ii) proliferative phase (iii) remodeling phase.*** All these steps are orchestrated in controlled manner by a variety of cytokines including growth factors. Some of this growth factor like platelet derived growth factor, transforming growth factor B, fibroblast growth factor and epidermal growth factor etc. has been identified in self healing wounds. In chronic wound the application of some growth promoting agents or some compounds which can enhance the in situ generation of these growth factors is required to augment the healing process [22].

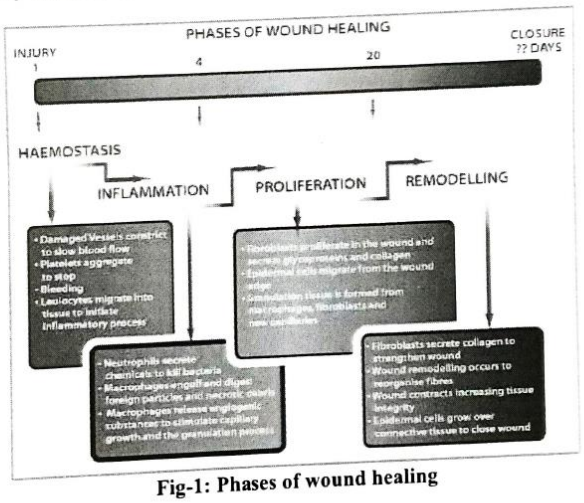


Fig-1: Phases of wound healing

Role of Cell Signaling in Tissue injury

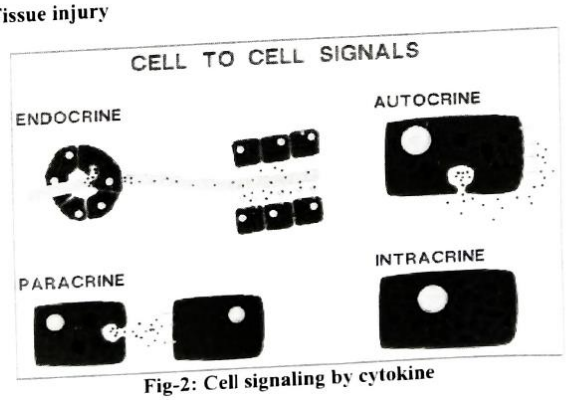


Fig-2: Cell signaling by cytokine

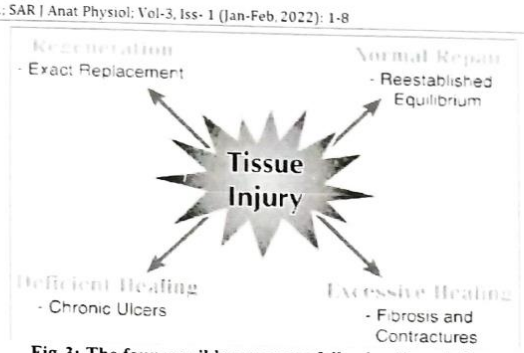


Fig-3: The four possible responses following tissue injury

The Healing Cascade

The healing cascade begins immediately after injury when platelets come into contact with exposed collagen. When platelets aggregate, clotting factors are released, resulting in the deposition of fibrin clots at the site of damage. Fibrin clots serve as temporary substrates and form the basis for subsequent healing processes. In addition to releasing clotting factors needed to control bleeding and loss of fluid and electrolytes, platelets provide a set of chemical signals known as cytokines or growth factors that initiate a healing response. The two most important signals are platelet-derived growth factor (PDGF) and transforming growth factor beta (TGFß). PDGF starts Homotacia neutrophils, macrophages, smooth muscle cells and fiber acetopes. It also stimulates the mitogenesis of fiber aceto and smooth muscle cells. TGFβ initiates cascade healing and stimulates cascade healing to start cascade healing and to emphasize additional cine, including FGF (fiber-aceto growth factor), PDGF, TNFA (alpo necrosis), PDGF, TNFA (Alpha necrotic tumor) and IL1 (interleukin1) . In addition, TGFβ also improves fiber aceta and smooth muscular cell chemale and adjust collagen expression and collage. The net result of this excessive signal is the energetic response of the matrix production cells to ensure rapid deposition of new coupling tissues at the impairment site during the following proliferation during the following proliferation. Neutrophils are the next predominant cellular marker in wounds up to 24 hours post-injury. The main function of neutrophils is to remove foreign substances, bacteria and non-functional host cells, as well as damaged matrix components that may be present at the wound site. Bacteria provide a chemical signal by attracting neutrophils, which engulf the neutrophils through a phagocytosis process. During bacterial protein synthesis, a waste product represented by f-MetLeuPhe tripeptide is released to attract inflammatory cells. The neutrophils swell until they are full of bacteria and form "pus" in the wound 48 hours after injury, fixed tissue monocytes are activated to become wound macrophages. These specialized wound macrophages are perhaps the most important inflammatory cells involved in the normal healing response. Inhibition of macrophage function delays the healing response. When activated, these wound macrophages release PDGF and TGFß to further recruit fibroblasts and smooth muscle cells to the wound site. These highly phagocytic macrophages are also responsible for clearing non-functional host cells, bacteria-filled neutrophils, damaged matrix, foreign debris, and any remaining bacteria from the wound site.

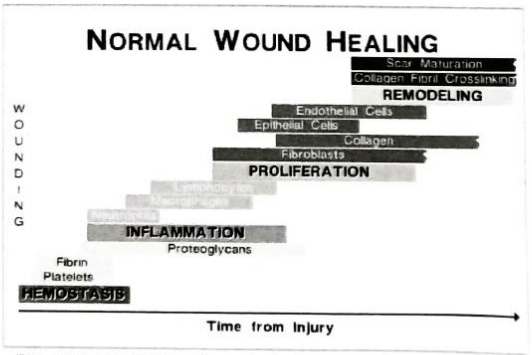


Fig-4: The sequence of events during normal wound healing

Phases of wound healing

PHASES OF HEALING	*DAYS POST INJURY*	*CELLS INVOLVED IN PHASES*
Hemostasis	Immediate	Platlets
Inflammation	Days 1-4	Neutophils
Proliferation	Days 4-21	Macrophages
Granulation		Lymphocyte
		Angiocytes
		Neurocytes
Contracture		Fibroblasts
		Keratinocytes
Remodelling	Days 21-2 yrs	Fibrocytes

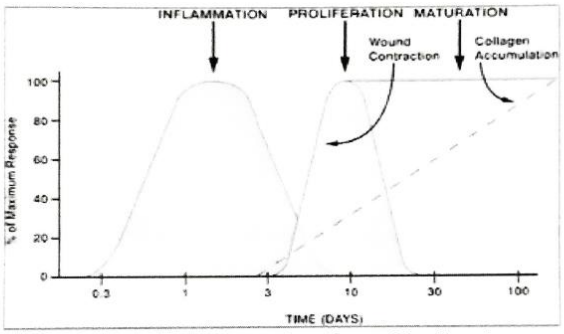
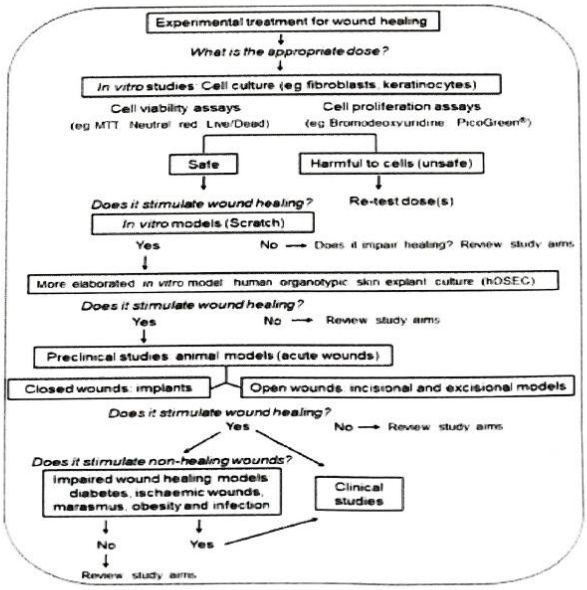
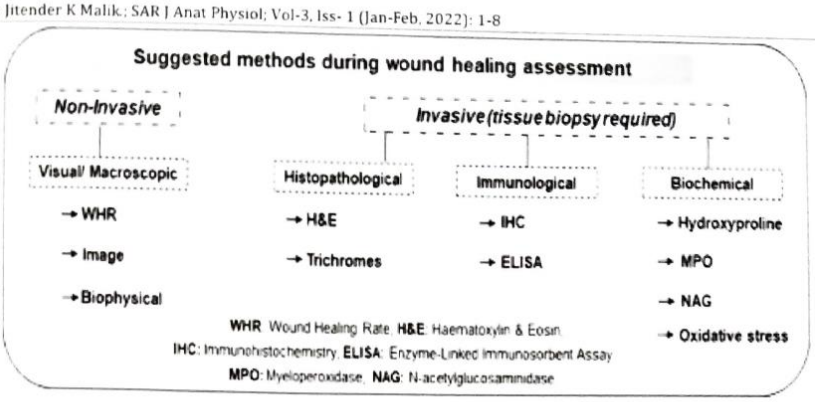


Fig-5: Graphical representation of wound healing phases

Advancement in Screening of Wound healing Potential





Biophysical Model of wound Healing Activity

Optical coherence tomography (OCT) is a new technique for diagnosing and monitoring inflammatory dermatological disorders. It produces high-resolution real-time images of the cutaneous architecture.

Greaves *et al.* evaluated OCT and histological assessments of in vivo acute wound healing to see how well they agreed on inflammation, proliferation, and remodelling. The authors proposed that OCT may be used as a diagnostic option to punch biopsies because the results were equivalent [23].

Tsai *et al.* used optical coherence tomography (OCT) to study in vivo wound healing following non-ablative fractional laser or ablative fractional laser treatments. The treated areas were scanned at several time periods to monitor the wound healing process, and an algorithm was devised to quantitatively quantify the morphological changes at different tissue depths during recovery [24].

Recent advancement in dressings for wound

Hydrogel dressings for wound

Hydrogels are applied to the wound as gels; they required a second cover such as gauze. Besides, if they are applied as films to the wound area, they can be used both as a primary and secondary dressing.

Hydrogels suitable for wound dressing as they

- Aid to the rehydration of dead tissues and elevated the healing of debridement
- Suitable for cleansing of dry or necrotic wounds
- Act as inert with biological reactants
- Penetrable to metabolites

Hydrogel is a cross-linked polymer matrix which has the potential to absorb and hold water in its network structure. Hydrogels act as a moist wound dressing medium and have the ability to absorb and retain the wound exudates along with the foreign bodies, such as bacteria, within its network structure. In addition to this, hydrogels have been found to encourage fibroblast proliferation by minimized the fluid loss from the wound surface and protect the wound from external harm necessary for rapid wound healing. Hydrogels help to support a micro-climate for biosynthetic reactions on the wound surface necessary for cellular activities. Fibroblast proliferation is requisite for complete epithelialisation of the wound, which starts from the edge of the wound. Since hydrogels help to keep the wound moist, keratinocytes can voyage on the surface. Hydrogels may be transparent, depending on the nature of the polymers, and provide soften and cooling/ soothing effects to the wound surface. The main advantage of the transparent hydrogels includes examined the wound healing without removing the wound dressing. The process of angiogenesis can be begins by using semi-occlusive hydrogel dressings, which is initiated due to temporary hypoxia. Angiogenesis of the wound ensures the growth of granulation tissue by maintaining appropriate supply of oxygen and nutrients to the wound surface [25].

Skin substitutes

Bioengineered skin replacements, including biosynthetic skin substitutes and cultured autologous engineered skin, are available in large quantities and pose no danger of infection or immunologic difficulties, making them ideal for temporary or permanent coverage. The cost of these items is their biggest drawback. We will briefly review currently available products and then go through some of them that may have an advantage over autologous tissue in terms of wound healing potential in chronic wounds. Bio-membrane is a temporary dressing made up of a knitted nylon mesh that is attached to a thin silicone membrane and covered with porcine polypeptides. It's used to cover donor areas in split-thickness skin grafting and clean superficial and mid-dermal depth burns It's used to cover donor areas in split-thickness skin grafting and clean superficial and middermal depth burns. It has been found in studies to be just as effective as silver sulfadiazine in wound healing without the need for frequent dressing changes. TransCyte is a biosynthetic dressing that combines a semi-permeable silicone membrane with a nylon mesh coated with porcine

collagen and newborn human fibroblast cells to create a biosynthetic dressing. It's used as a temporary cover for excised burns before grafting or as a dressing for superficial burns that don't require skin grafting. In terms of healing time, infections, and scar formation, it has been demonstrated to be superior to antibiotic creams or silver sulfadiazine in several investigations, particularly on facial burns [26].

Hyperbaric oxygen

For the past 40 years, hyperbaric oxygen has been used as an adjuvant in wound healing. It entails putting the patient in a sealed chamber with 100 percent oxygen at 1.5 to 3 atmospheres absolute (ATA) for 60 to 120 minutes over the course of several sessions. It has indications for use in carbon monoxide poisoning, crush injuries, compartment syndrome, acute traumatic ischemia, ischemia-reperfusion injury, radiation injury, compromised skin grafts, infections with anaerobic organisms, and refractory osteomyelitis. It was originally designed for use in decompression illness in deep sea divers. In addition, HBO treatment has particular special indications in chronic wounds [27].

CONCLUSION

The field of wound healing is constantly expanding with technological advances. There is still no good alternative to reconstruction using the patient's own tissue and carefully designed reconstruction procedures. The new product provides preventative measures against healing barriers, increases wound healing factors, delays and a bridge time to final repair, and optimize the outcome of final wound reconstruction and helps to promote good healing. Current wound healing products and modality expand the surgeon's arsenal to consider all aspects of wound healing.

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In-Silico Consideration of Anti-Microbial Prospective of Plant Phenolic and Flavonoids

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ABSTRACT: ***Background:*** Pathogenic microorganism infections pose a serious threat to human health. The need for innovative, safe, and efficient antimicrobial medicines has been driven by rising drug resistance cases, unfavorable antibiotic side effects, and the reemergence of previously identified illnesses. Virtual screening techniques used in drug development, such as drug-likeness and ADMET analysis, use computation to quickly and cheaply identify compounds that are likely to demonstrate physiological activity. ***Methods*:** In this regard, the enzyme aminoacyl-tRNA synthetase (AaRS) has been the focus of recent research in the discovery of antibacterial agents. Docking studies were performed Molecular docking of aminoacyl-tRNA synthetase (AaRS) with chlorogenic acid, rutin, quercetin and gallic acid was carried out by AutoDock. ***Results*:** The molecular docking result revealed that chlorogenic acid, gallic acid, quercetin and rutin showed encouraging docking score. Hence from above finding it can be predicted that phenolics and flavonoids found in the plants extract exhibited good inhibitor of IleRS enzyme.

Keywords: Chlorogenic acid, rutin, quercetin, gallic acid and *in-silico* molecular docking.

RESEARCH PAPER

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INTRODUCTION

The rise, dissemination, and persistence of multidrug-resistant (MDR) bacteria, colloquially known as "superbugs," which cause infections that do not respond to traditional therapies, has led to antibiotic resistance being one of the most important public health challenges of this century [1-2]. One of the main factors contributing to the emergence and spread of antimicrobial resistance is the rising use and abuse of antibiotics in both humans and animals, as well as the lack of innovation in antibiotic research (reduction in the number of new antibiotic classes). There is an urgent need for new chemical entities to be developed as antibacterial agents, as well as policies to restrict the improper and irrational use of antibiotics. A group of MDR micro organism together acknowledged as "ESKAPE", which incorporates Gram-effective and Gram-terrible species (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.*), are regularly remoted in sanatorium environments, in which they're accountable for the bulk of nosocomial infections [3]. In precise, Gram-effective micro organism have predominantly advanced resistance to all of the to be had antibiotics and pose a critical trouble now no longer simplest in hospitals however additionally for the overall population [4, 5]. Infections of methicillin-resistant Staphylococcus aureus (MRSA) are of precise concern. In plants, Flavonoids are widely distributed as a naturally occurring polyphenols. Being both dietary and biologically active compounds, flavonoids have attracted much attention of investigators as potent species capable of affecting various biological processes in living organisms. They are able to modulate various enzymes present in biological system. Flavonoids and the other phenolic compounds are generally referred to as plant secondary metabolites that maintain an aromatic ring bearing as a minimum one hydroxyl groups. More than 8000 phenolic compounds as obviously going on materials from vegetation were pronounced [6]. It may be very thrilling to word that 1/2 of of those phenolic compounds are flavonoids offering as aglycone, glycosides and methylated derivatives [7]. These phytochemical materials are provided in vitamins and natural medicines, each flavonoids and lots of different phenolic additives were pronounced on their powerful antioxidants, anticancer, antibacterial, cardioprotective agents, anti-inflammation, immune

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gadget promoting, pores and skin safety from UV radiation, and thrilling candidate for pharmaceutical and scientific application [8-9]. Since some many years ago, the studies research specializing in flavonoids and the other phenolics compounds from medicinal plant species have improved considerably, due to their flexible advantages for human health. In this consideration, the enzyme aminoacyl-tRNA synthetase (AaRS) has been the focus of recent research in the discovery of antibacterial agents. With this endeavor chlorogenic acid, rutin, quercetin, and gallic acid are taken as active compound for elucidation of antimicrobial potential *via* molecular docking.

Experimental works

Ligand Preparation

2D Structure of ligand like chlorogenic acid, rutin, quercetin, and gallic acid was drawn using ChemDraw [10]. The two-dimensional structures of ligands were converted into 3-D structures with optimized 3D geometry by using Chem3D software. The optimized structure was saved in PDB format for AutoDock compatibility [11].

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.392 Å and No. of points considered are 40, 40 and 40 points in the x, y, and z dimensions are67.561, 31.934 and 19.359 as x, y, z centers [12].

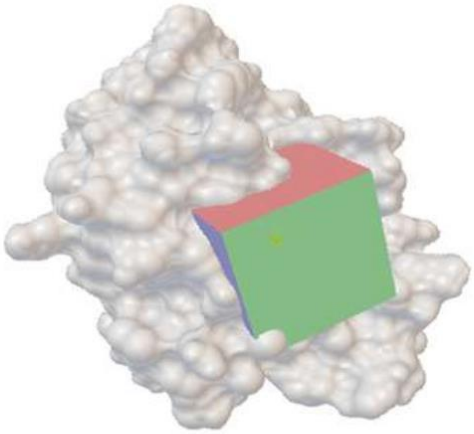


Figure 1: Grid box covering all active sites in receptor

Preparation of the docking file

All the calculations were carried out by using Autodock4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [13].

Docking of Isoleucyl-transfer RNA (tRNA) synthetase (IleRS)

Crystal structure

The crystal structure of the protein consisting of receptor associated with bound ligand is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (1WNZ.pdb) registered in the Protein data bank was used. The bound ligand 2'-(L-valyl) amino-2'-deoxyadenosine (2VA) was found within the receptor [14].

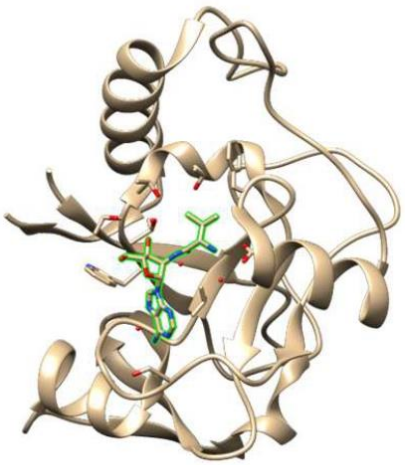


Figure 2: Crystal structure of IleRS enzyme with bound ligand 2VA(PDB ID-1WNZ)

Processing of Protein

The downloaded receptor protein is having a single chain A, which has been selected for the experimental purpose. The bound ligand 2VA was separated from the macromolecular complex by using software Chimera [15].

Molecular Docking Simulation Studies

Docking of ligand like chlorogenic acid, rutin, quercetin, and gallic acid against IleRS enzyme was performed by Autodock. All the bonds of ligand were kept flexible, while no residues in receptor were made flexible [16].

Toxicity & ADME-T Studies

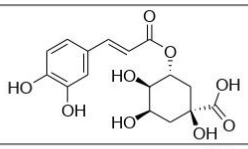
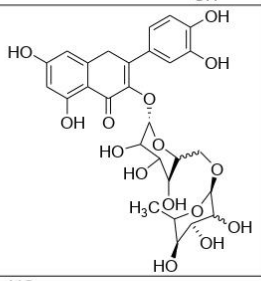
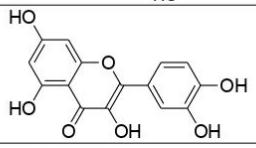
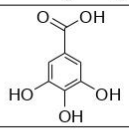
The modified lead molecules are studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [17].

RESULTS AND DISCUSSION

The current sensational increase within the unfold of antimicrobial resistance in microorganisms poses a true threat to the well-being of the general public round the world. Of explicit concern are contamination with methicillin-resistant staphylococci aureus (MRSA), antibiotic drug *staphylococci aureus*, vancomycin-resistant enterococci and mycobacteria. Tuberculosis, several of those creatures are impervious to some categories of medication agents. This scenario facilitates the explored for novel antimicrobial agents that block essential targets for microorganism that don't seem to be littered with current therapy resistance mechanisms. During this regard, the protein aminoacyl-tRNA synthetase (AaRS) has been the focus of recent analysis within the discovery of medication agents. These enzymes play a very important role in super molecule synthesis by catalyzing the synthesis of aminoacyl-RNA (aarRNA). Once these enzymes are suppressed, protein biosynthesis ceases, leading to restricted bacterial growth underneath each *in-vitro* and infectious conditions. These enzymes are attention-grabbing targets for antibacterial drugs. The result of *in-Silico* molecular docking revealed that binding energy ($Kcalmol^{-1}$) of chlorogenic acid, rutin, quercetin, and gallic acid against IleRS enzyme were found to be -4.20,-5.06,-7.42 & -4.56 respectively (Table 1). Molecular stimulation interaction showed in following pattern Rutin> Chlorgenic acid > Gallic acid > Quercetin (Figure 3-10).

The pharmacokinetic profiling of the ligand has revealed that chlorogenic acid and rutin are having good pharmacokinetic profile without presence of any major toxic effects, while quercetin is associated with some mutagenic and tumorogenic properties. The Gallic acid is also associate with mutagenic and reproduction effects. The pharmacokinetic and toxicity profiling results were shown in Figure 11-14.

Table 1: Result of docking of against IleRS enzyme.

S. No	Compound	Structure	B.E.	H-Bond	Residual Interaction	
					Pi-Interaction	van der Waals
1	Chlorogenic acid		-4.20	Gly316, Ser310, Thr229, His319, Asp328, Thr230	Val318	Asp313, Val309, Trp227, Thr228, Tyr308, Thr233
2	Rutin		-5.06	Thr229, Trp227, Thr315, Ile317, Ser310, Tyr308, Asp328	Trp227, Val318	Gly314, Val309, Thr230, Thr228, Glu327, Gln320, Ala321
3	Quercetin		-7.42	Thr315, Ser310, Ile317	Val318, Asp313	Gly316, Gly314, Thr228, Thr229, Thr230, Tyr308
4	Gallic acid		-4.56	Asp313, Ser310, Trp227, Ile317, Gly316	Val318, Trp227	Thr315, Thr228, Val309, Ile226

Interactions

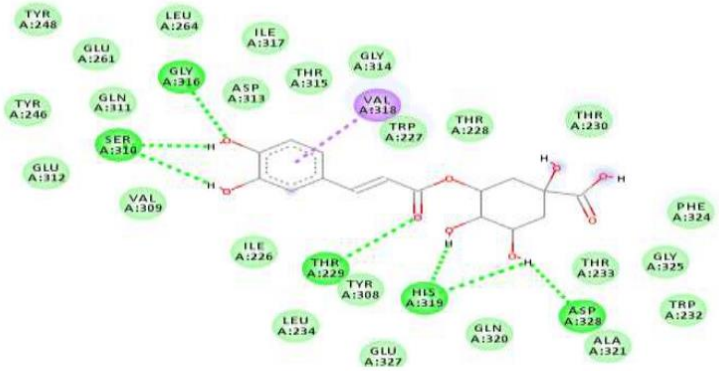


Figure 3: Binding interaction of chlorogenic acid with IleRS

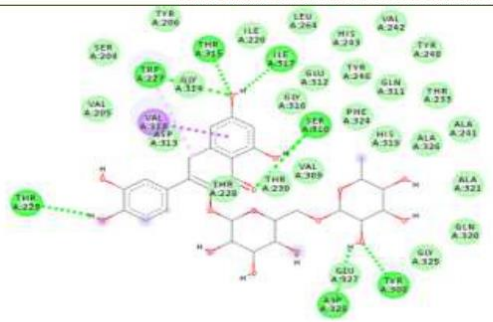


Figure 4: Binding interaction of rutin with IleRS

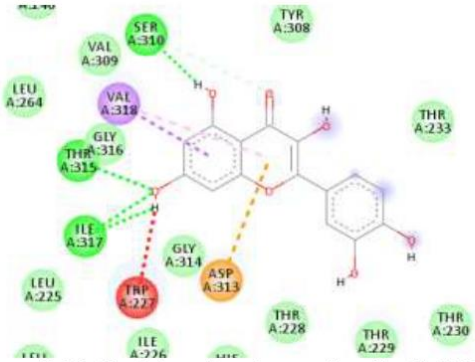


Figure 5: Binding interaction of quercetin acid with IleRS

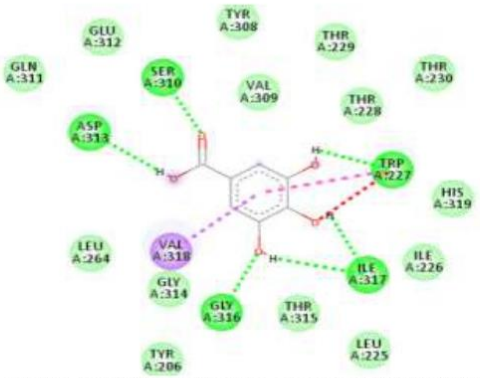


Figure 6: Binding interaction of gallic acid with IleRS

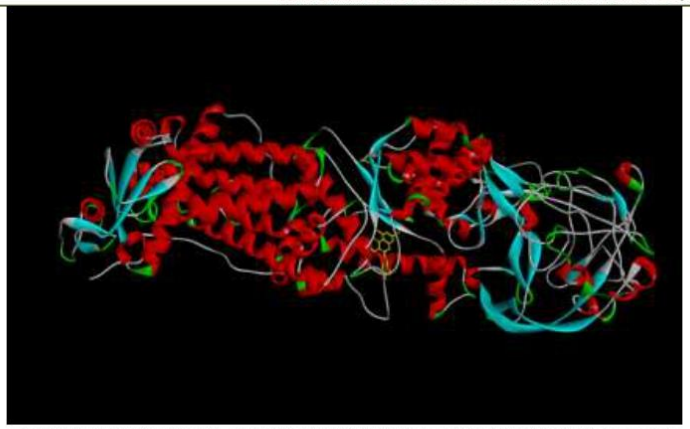


Figure 7: Binding mode of quercetin within the active site of IleRS receptor

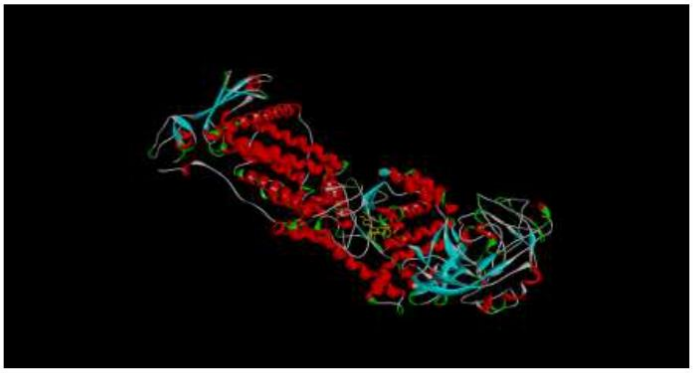


Figure 8: Binding mode of rutin within the active site of IleRS receptor

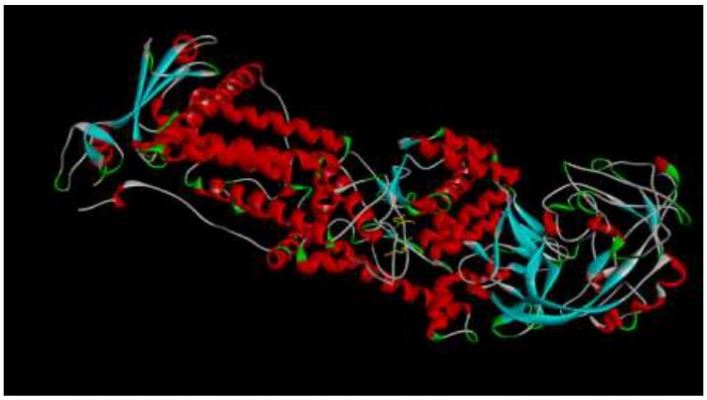


Figure 9: Binding mode of chlorogenic acid within the active site of IleRS receptor

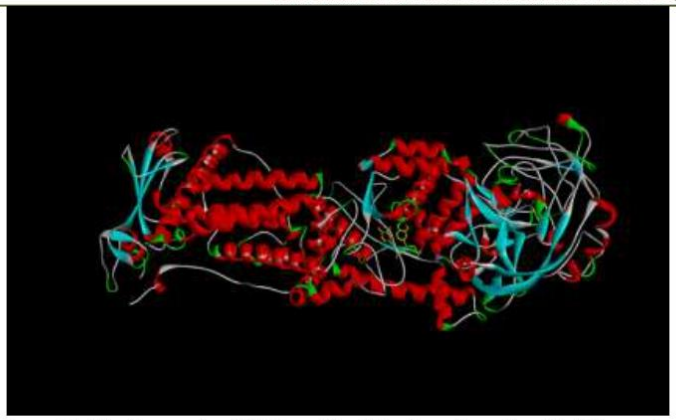


Figure 10: Binding mode of Gallic acid within the active site of IleRS receptor

Figure 11: Pharmacokinetic and toxicity profiling of chlorogenic acid

Figure 12: Pharmacokinetic and toxicity profiling of rutin

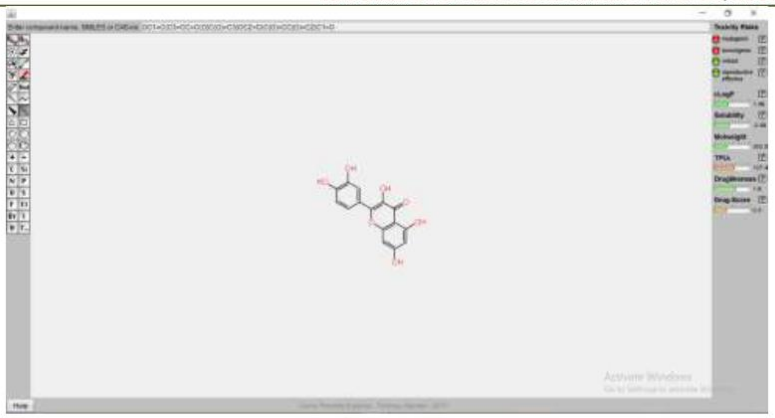


Figure 13: Pharmacokinetic and toxicity profiling of quercetin

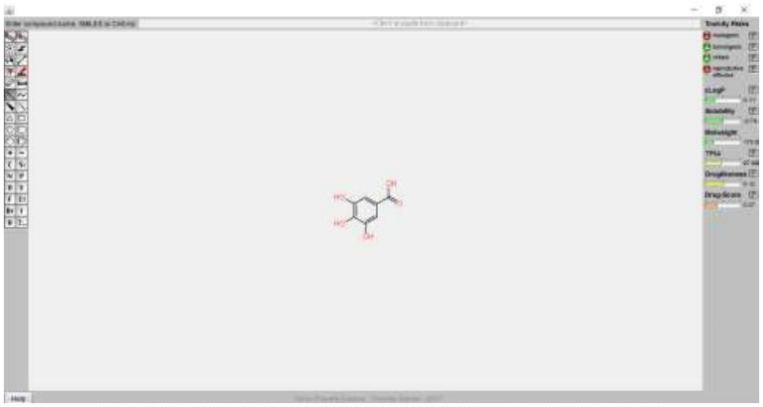


Figure 14: Pharmacokinetic and toxicity profiling of gallic acid

CONCLUSION

With this attempt, natural plant Phenolics & flavonoids found in extracts of the plants known as, gallic acid, chlorogenic acid rutin and quercetin has been identified as ligand and their aminoacyl-tRNA synthetase (AaRS) enzymes inhibitory activity has been checked *in-silico* with the facilitated of docking approach. The molecular docking result revealed that chlorogenic acid, gallic acid, quercetin and rutin showed encouraging docking score. Hence from above finding it can be predicted that phenolics and flavonoids found in the plants exhibited good inhibitor of IleRS enzyme.

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In-Silico assessment of Wound healing Potential of Plant Phenolic and Flavonoids

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Abstract

Background: The destruction of a tissue's cellular and anatomic discontinuity is referred to as a wound. A chemical, physical, thermal, microbiological, or immunological insult to the tissue might result in a wound. Uncomfortable wounds are more likely to become infected and develop other problematic problems. Chlorogenic acid, rutin, quercetin, and gallic acid are well known plant phenolic and flavonoids with numerous pharmacological efficacy. In the present work an attempt had been made to target this phenolic and flavonoid compound as lead molecule for *in-silico* molecular docking with GSK-3β and thereby assess the wound healing potential.

Methods

In this regard, current studies in the quest for therapeutic drugs have focused on the enzyme GSK-3. Docking investigations were undertaken AutoDock was used to molecularly attach GSK-3 with chlorogenic acid, rutin, quercetin, and gallic acid.

Results

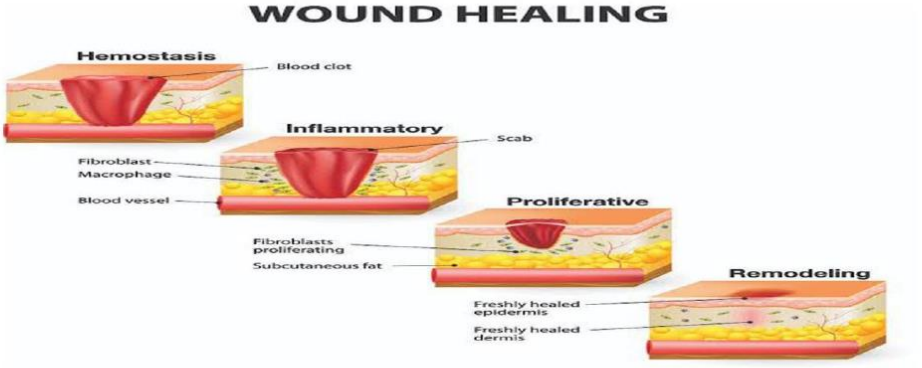
Chlorogenic acid, gallic acid, quercetin, and rutin all had favourable docking scores, according to the molecular docking results. So, based on the results discussed above, it may be assumed that the phenolics and flavonoids present in the plant extract were effective GSK-3 enzyme inhibitors.

Key words: Chlorogenic acid, rutin, quercetin, gallic acid and *in-silico* molecular docking.

Introduction

The destruction of a tissue's cellular and anatomic discontinuity is referred to as a wound [1]. A chemical, physical, thermal, microbiological, or immunological insult to the tissue might result in a wound. Uncomfortable wounds are more likely to become infected and develop other problematic problems [2]. Delays in wound healing can be caused by illnesses including diabetes, immune system disorders, ischaemia, malnutrition, ageing, local

infections, and local tissue damage from burns or gunshots. The primary consequences of burn injuries are infections, which cause 50–75% of hospital fatalities [3].



Phase of Wound healing

Both phenolic compounds and flavonoids are well-known as antioxidants and other significant bioactive substances that have attracted study for a long time due to their advantages for human health and their potential to treat and prevent a wide range of disorders [4].

Active Compound	**Therapeutic Potential**
Chlorogenic acid	Antioxidant activity, antibacterial, hepatoprotective ,cardioprotective,anti-inflammatory,antipyreticneuroprotective, anti-obesity, antiviral, anti-microbial, anti-hypertension, free radicals scavenger and a central nervous system (CNS) stimulator [5].
Rutin	Antioxidants, anti-inflammatory, anti-allergic, antiviral as well as an anticancer activity. They have also been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases [6].
Quercetin	Anti-carcinogenic, anti-inflammatory and antiviral activities; as well as attenuating lipid peroxidation, platelet aggregation and capillary permeability [7].
Gallic acid	Antioxidants, anticancer, antimicrobial, chondro-protective effect, carbonic anhydrase inhibitors, antidiabetic activity, anti-ulcerogenic, cathepsin D inhibitor[8].

Experimental Work

Ligand Preparation:

ChemDraw was used to create a 2D structure of a ligand such as chlorogenic acid, rutin, quercetin, and gallic acid [8]. Chem3D software was used to transform the two-dimensional ligand structures into three-dimensional structures with optimum 3D geometry. For AutoDock compatibility, the optimised structure was saved in PDB format.[9].

Preparation of the grid file

By creating a grid box around the active sites, Autodock's regions of interest were identified by taking grid area into account. Grid boxes are essential to the docking process because they are designed to cover all amino acids other than those found in receptors that are present in active sites and required for binding. Three thumbwheel widgets on the grid box allow us to adjust the number of points in the x, y, and z dimensions. The study's chosen value for the spacing between grid points is 0.392, and the number of points studied are 40, 40, and 40 points in the x, y, and z dimensions are 23.936, -17.104, and 9.189 as x, y, and z centres [10].

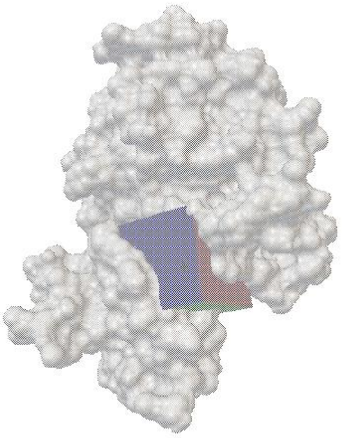


Figure 1: Grid box covering all active sites in receptor

Preparation of the docking file

The docking tool used for all the computations was Autodock4.2. The visualisation and other programmes necessary for docking experiments were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus[11].

Docking of Glycogen Synthase Kinase-3β (GSK-3β)

Crystal structure

Download the protein's crystal structure from the Protein Data Bank website. The protein has a receptor linked to a binding ligand. The Protein Data Bank's 7OY5.pdb database's primary information on receptor and structure was used[12].

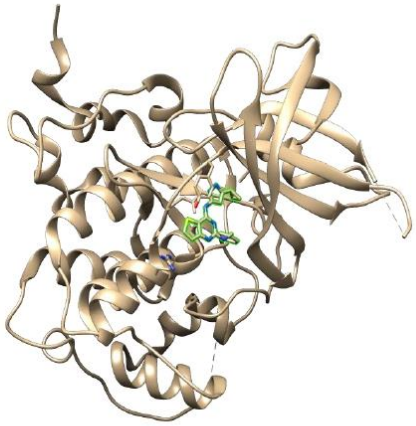


Figure 2: Crystal structure of GSK-3beta enzyme with bound ligand

Processing of Protein

The downloaded receptor protein is having two chains A and B, out of which chain B has been selected for the experimental purpose. The bound ligand 39I was separated from the macromolecular complex by using software Chimera [13].

Molecular Docking Simulation Studies

Docking of ligandslike chlorogenic acid, rutin, quercetin, and gallic acid againstGSK-3β enzyme was performed by Autodock. All the bonds of ligand were kept flexible, while no residues in receptor were made flexible [14].

Toxicity & ADME-T Studies

The modified lead molecules are studied by online program OSIRIS, for prediction of presence of any toxicgroup as well as presence of any toxic group and ADME- T properties [15].

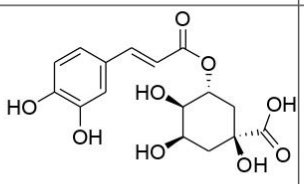
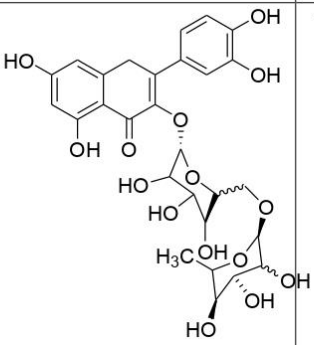
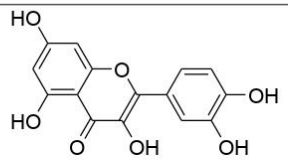
RESULTS AND DISCUSSION

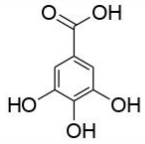
Wound-healing is an ordered and coordinated process that includes inflammation, matrix deposition, cell proliferation, tissue modelling, collagenation and epithelialization **Soni H *etal* 2012**. It has been revealed that the Wnt/b-catenin pathway can enhance wound-healing by inhibiting glycogen synthase kinase-3β (GSK-3β) protein, a significant regulatory

enzyme. A variety of medicinal plants have been studied to establish whether they are possible sources of wound-healing drugs. We screened the phyto-constituents Rutin, Quercetin, Chlorgenic acid and gallic acid *in silico* on GSK-3β.The docking result revealed that binding energy of chlorogenic acid, rutin, quercetin and gallic acid with GSK-3β energy showed -5.26,-5.55,-7.36 &-3.56 respectively. The result was tabulated in table 1. The binding interaction of lead molecule with GSK-3β was showed in fig 3-6.The pharmacokinetic profiling of the ligand has revealed that chlorogenic acid and rutin are having good pharmacokinetic profile without presence of any major toxic effects, while quercetin is associated with some mutagenic and tumorogenic properties. The Gallic acid is also associate with mutagenic and reproduction effects. The pharmacokinetic and toxicity profiling results were shown in figure 7-10.

Following result were observed in docking studies of GSK-3β enzyme with ligands like chlorogenic acid, rutin, quercetin, and gallic acid.

Table 1: Result of docking of against GSK-3βenzyme.

S.No	Compound	Structure	B.E.	H-Bond	Residual Interaction	
					Pi-Interaction	van der Waals
1	Chlorogenic acid		-5.26	Val135, Asn186, Lys183, Cys199	Ala83, Leu188, Val70	Lys85, Phe67, Asp200, Gln185, Gly65, Tyr134, Thr138
2	Rutin		-5.55	Arg141, Gln185, Asn186, Lys85, Asp200, Asp133	Leu183, Val135, Ala83, Tyr134, Val70, Phe67, Cys199	Thr138, Gly63, Gly202, Leu132, Val110, Ile62, Leu189
3	Quercetin		-7.36	Asp133, Val135, Asp200,	Leu188, Cys199, Ala83, Phe67, Val70,	Tyr134, Val110, Leu132, Asn186, Glu97,

						Ile62
4	Gallic acid		-3.56	Arg144, Thr138, Gln185	Arg141, Tyr140	Asn186, Asn64, Gly63

Interactions

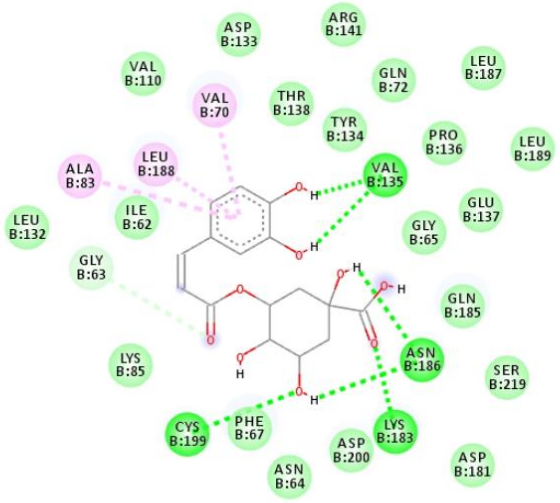


Figure 3: Binding interaction of chlorogenic acid with GSK-3β.

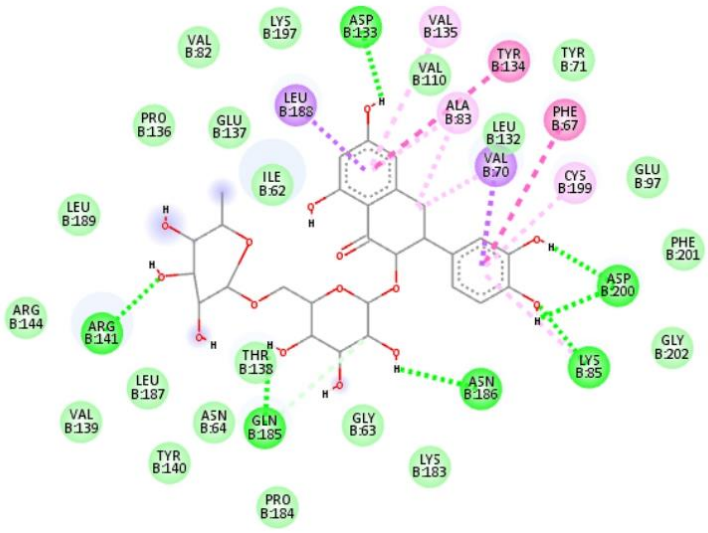


Figure 4: Binding interaction of rutin with GSK-3β.

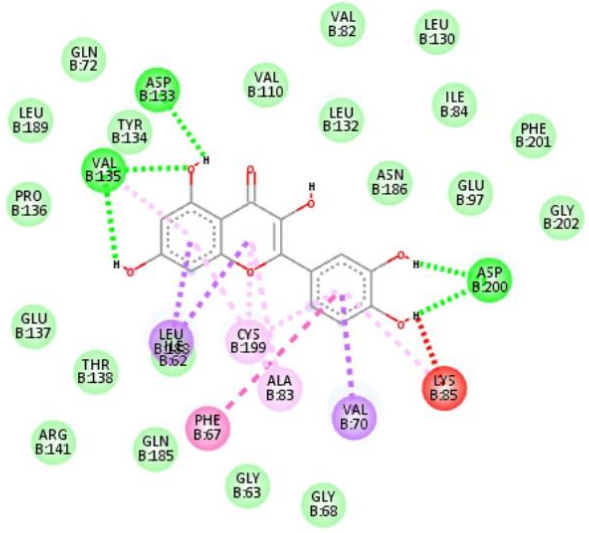


Figure 5: Binding interaction of quercetin acid with GSK-3β.

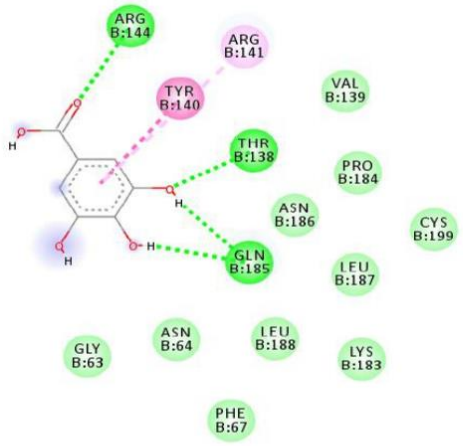


Figure 6: Binding interaction of gallic acid with GSK-3β.

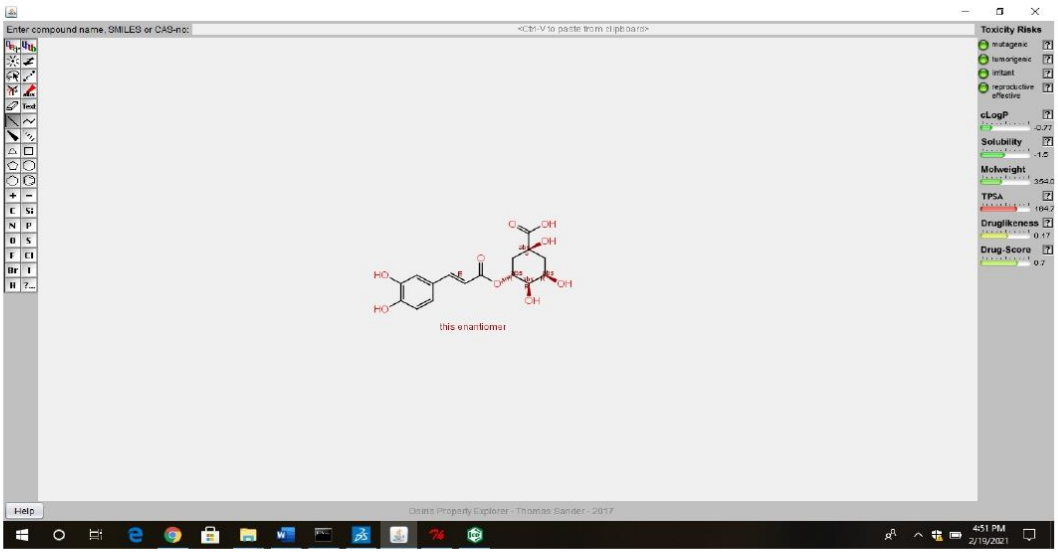


Figure 7: Pharmacokinetic and toxicity profiling of chlorogenic acid.

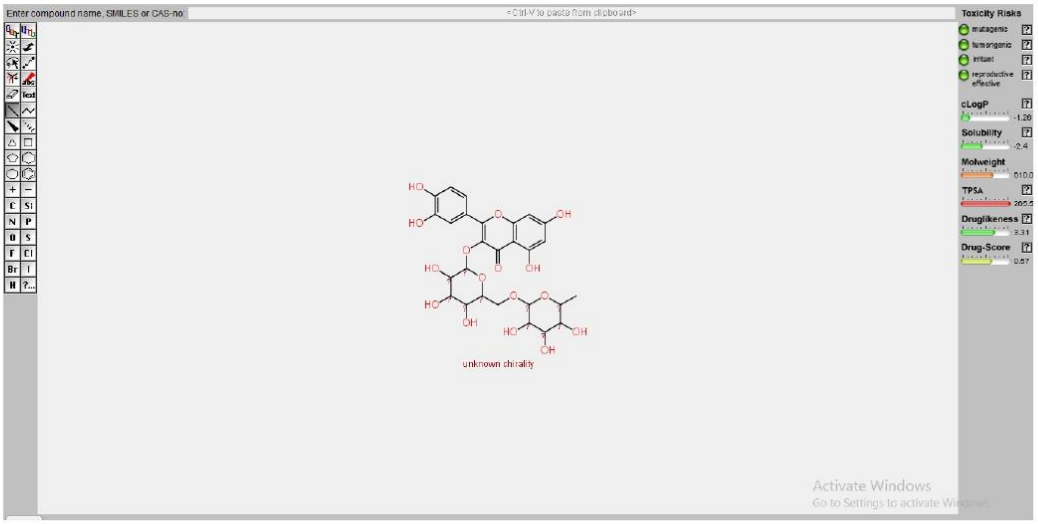


Figure 8: Pharmacokinetic and toxicity profiling of rutin.

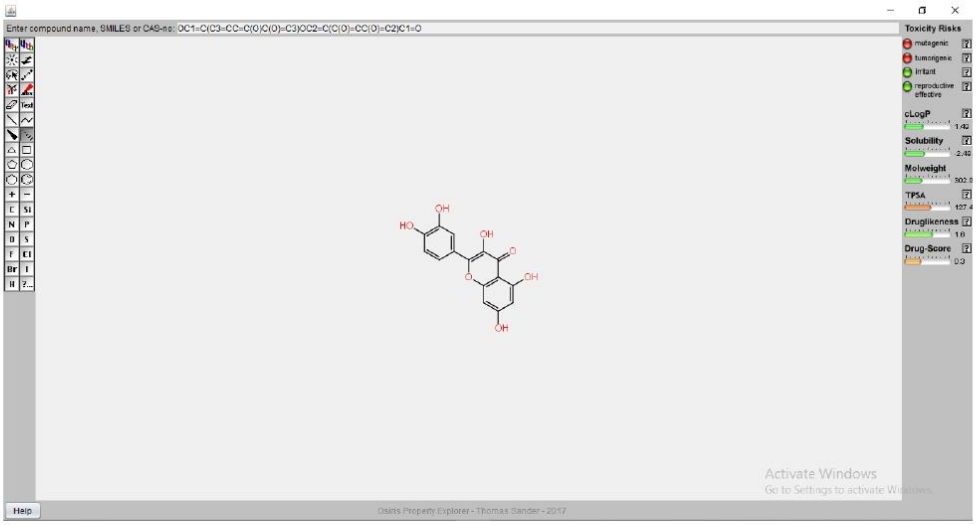


Figure 9: Pharmacokinetic and toxicity profiling of quercetin.

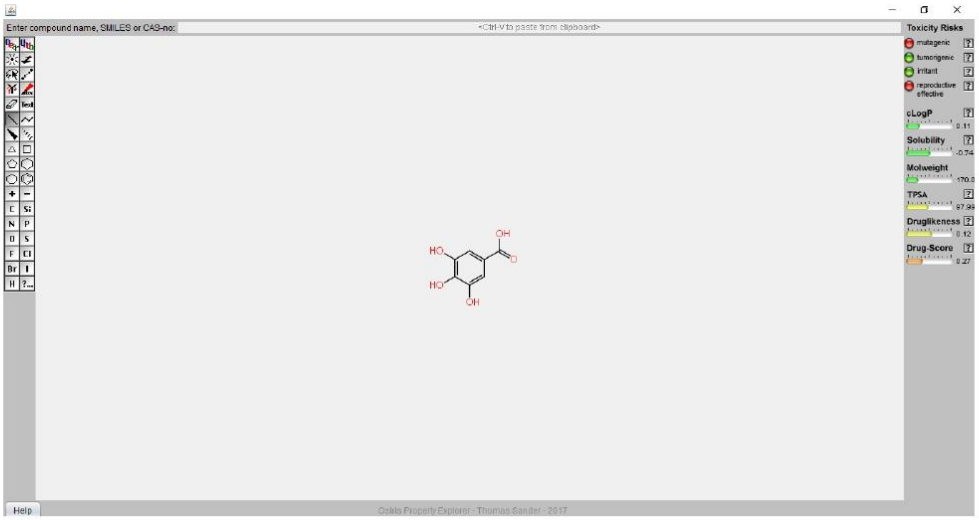
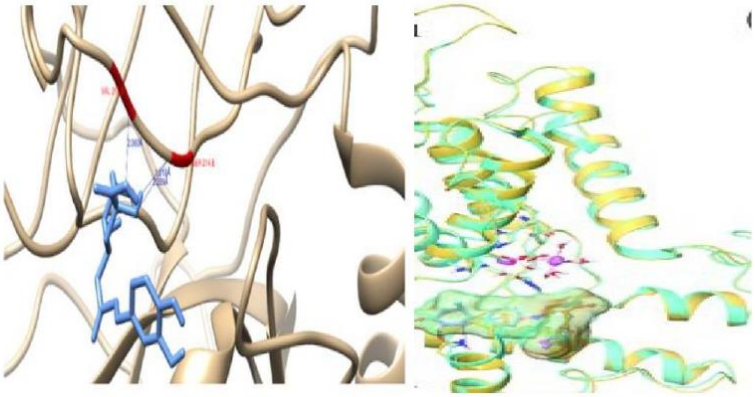


Figure 10: Pharmacokinetic and toxicity profiling of gallic acid.



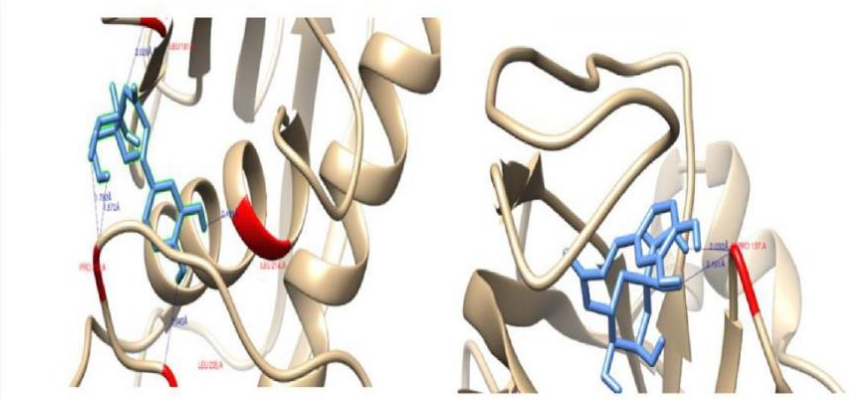


Figure 11: Binding Mode of Q,R, CA & GA.

Conclusion

Of the four compounds present in the ethanolic extract of *A. indica*,*O.sanctum*and *M.oleifera* gallic showed greatest inhibition of GSK-3β protein in molecular docking and dynamics studies. The minimum binding energies showed Quercetin>chlorgenic acid>Rutin> Gallic acid. Gallic acid showed minimum binding (-3.56 kJ mol^{-1}) with acceptable affinity towards the active pocket. It can be considered that all active compounds have good inhibitor of GSK-3β.

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SHIVPURI (M.P.)

University Established Under section 2f of UGC ACT 1956 Vide MP Government Act No 17 of 2015

Ref no PhD/PKU/2024/PhD Cell/Invitation/361

Date. 01/10/2024

An Intimation Letter

To
Mr.Amit Kumar
Research Scholar
Faculty of Pharmacy
P.K.University.

Greetings from P.K.University. Mr.Amit Kumar, (2021-22) your **Phd. Pre-Submission Viva voce (PSDC)** is scheduled on **09/11/2024 at 12:00 pm** in P.K.University ,Dr.A.P.J.Abdul Kalam Conference Hall., Guru Vashista Administrative Block. Make sure to attend and present in ICT mode of presentation.Equip you to accompany ICT gadgets / Laptop / Pendrive etc.

01/10/2024

All the best
Dr Nalla Bhaskar
Dean Research & I/c Ph.D Cell
P.K.University
Thanra village Karera Tehsil
Shivpuri - M.P

ADDRESS: VILL: THANRA, TEHSIL: KARERA, NH-27, DIST: SHIVPURI, M.P. 473665,
MOB: 7241115088, Email:registrar.pkuniversity@gmail.com

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

भारत सरकार / GOVERNMENT OF INDIA
MINISTRY OF ENVIRONMENT, FORESTS & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण / BOTANICAL SURVEY OF INDIA
पर्यावरण , वन एंव जलवायु परिवर्तन मंत्रालय
मध्य क्षेत्रीय केंद्र / CENTRAL REGIONAL CENTRAL
10. चैतम लाइन्स, इलाहबाद -211002 / 10, CHATAM LINES, ALLAHABAD-211002

सा०भा.व.स.म.झे.के./ प्रशा./2022-23/01801 दिनांक/ Date: 18/11/23

Mr. Amit Kumar

C/O Faculty of Pharmacy, P K University, Shivpuri, M P

विषय / subject: Identification and authentication of plant specimen /पादप नमूना पहचान एवं प्रमाणीकरण के सन्दर्भ में।

महोदया,

आपके पत्र,दिनांक- (भारत कोश भुगतानसं. 2406230006346, दिनांक 05/10/2023) जो की पादप नमूनों के पहचान एंव सत्यापन के सन्दर्भ में है, आपके द्वारा भेजे गए नमूनों को निमन्लिखित नाम से प्रमाणित किया जाता है।

With reference to your latter, vide dated nil (Bharat Kosh transaction ref. no. 2406230006346, dated 05/10/2023) regarding authentication and identification of the plant specimen, the specimen is identified and authenticated as under:

S. No.	Name of plant species	Family
01	*Azadirachta indica* Herb.	Meliaceae
02	*Ocimum sanctum* Herb.	Lamiaceae
03	*Moringa oleifera* Herb.	Moringaceae

साधनयवाद/ Thanking you,

(आरती गर्ग/Arti Garg)
वैज्ञानिक -एवं प्रधान कार्यालय/
Scientist- E & Head of Office