ACKNOWLEDGMENT

This work has become successful because of the blessings of our parents, teachers, and almighty god. We are grateful thanks to one and all that have directly or indirectly helped and guided us in the successful completion of the work.

We have been constantly assimilate by the bountiful guidance of **Dr. B.N SUHAGIA.** We would thank him for his guidance, overwhelming enthusiasm, and untiring cooperativeness he conferred upon us.

A successful outcome in any research endeavour, therefore, attributes itself to the selfless guidance of the mentor. With a deep sense of gratitude, we take this opportunity to that our esteemed guide **Prof. DR. PUNIT BHATT** (Assistant professor Department of Pharmacology, Faculty of Pharmacy, DDU)

For his encouragement, critical evaluation, timely help so often sought and so generously given during the entire period of this research project. It is a great pleasure and privilege for us to acknowledge gratefully for his relentless countenance bestowed upon us.

We would also like to thank our friends and family who have inspired us to work hard and achieve new heights in our life. We would also like to express our gratitude to all the lab technicians and their coworkers for helping us.

INDEX

ABSTRACT

Wound healing is a complex biological process that involves the formulation and evaluation of a novel wound healing nanoemulgel containing extracts from various medicinal plants and oils. Specifically, methanol extract of Cinnamomum tamala, water extract of Punica granatum bark, and oil from Sesame were incorporated into the nanoemulgel formulation along with appropriate excipients such as carbopol, propylene glycol, and triethanolamine.

The nanoemulgel was subjected to a series of evaluations to assess its physicochemical properties, antimicrobial activity, skin compatibility, and wound healing potential. Organoleptic characteristics revealed a cinnamon-like colour and smell, indicative of the herbal extracts used. Physicochemical parameters including pH 6 for topical formulations. Moreover, the nanoemulgel demonstrated good washability upon application to the skin.

Antimicrobial susceptibility testing showed significant antibacterial activity against E. coli, particularly attributed to the gel and crude extract of Moringa oleifera. Skin irritation studies on human subjects confirmed the absence of irritation, further validating the safety profile of the nanoemulgel.

The nanoemulgel demonstrated non-irritating properties when applied to human hand skin. Additionally, its wound healing efficacy was evaluated using a rat model, aiming to substantiate its therapeutic potential through in vitro experimentation.

Keywords: Wound healing nanoemulgel, Polyherbal extracts, Physicochemical parameters, Antimicrobial activity, Safety and efficacy assessment, Animal study.

1. INTRODUCTION

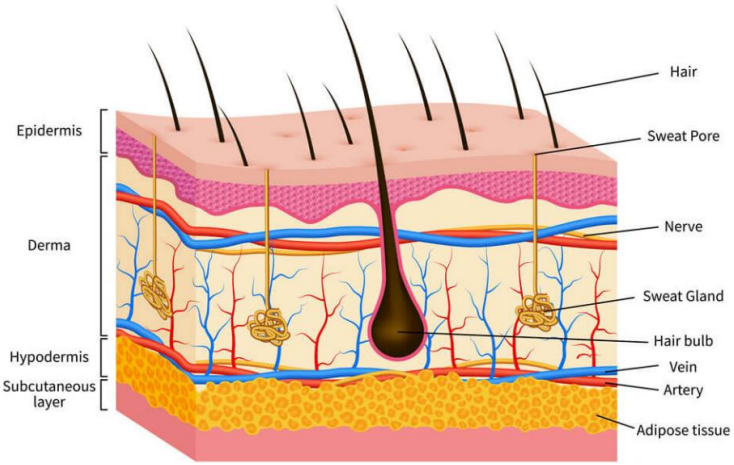


FIGURE-1

1.1 INTRODUCTION TO ANATOMY OF SKIN

Skin is the largest organ in the body and covers the body's entire external surface. It is made up of three layers, the epidermis, dermis, and the hypodermis, all three of which vary significantly in their anatomy and function. The skin's structure is made up of an intricate network which serves as the body's initial barrier against pathogens, UV light, and chemicals, and mechanical injury. It also regulates temperature and the amount of water released into the environment.

EPIDERMIS

The epidermis is made of stratified squamous keratinizing epithelial tissue and is thickest on the palms and soles. The cells that are most abundant are called keratinocytes, and there are no capillaries present between them. Although the epidermis may be further subdivided into four or five sublayers, two of these are of greatest importance: the innermost layer, the stratum germinativum, and the outermost layer, the stratum corneum.

Stratum basale, also known as stratum germinativum, is the deepest layer, separated from the dermis by the basement membrane (basal lamina) and attached to the basement membrane by hemidesmosomes. The cells found in this layer are cuboidal to columnar mitotically active stem cells that are constantly producing keratinocytes. This layer also contains melanocytes.

Stratum spinosum, 8-10 cell layers, also known as the prickle cell layer contains irregular, polyhedral cells with cytoplasmic processes, sometimes called "spines", that

extend outward and contact neighboring cells by desmosomes. Dendritic cells can be found in this layer.

Stratum granulosum, 3-5 cell layers, contains diamond shaped cells with keratohyalin granules and lamellar granules. Keratohyalin granules contain keratin precursors that eventually aggregate, crosslink, and form bundles. The lamellar granules contain the glycolipids that get secreted to the surface of the cells and function as a glue, keeping the cells stuck together.

Stratum lucidum, 2-3 cell layers, present in thicker skin found in the palms and soles, is a thin clear layer consisting of eleidin which is a transformation product of keratohyalin.

Stratum corneum, 20-30 cell layers, is the uppermost layer, made up of keratin and horny scales made up of dead keratinocytes, known as anucleate squamous cells. This is the layer which varies most in thickness, especially in callused skin. Within this layer, the dead keratinocytes secrete defensins which are part of our first immune defence.

Cells of the Epidermis

1. Keratinocytes
2. Melanocytes
3. Langerhans' cells
4. Merkel's cell
5. Keratinocytes

TABLE-1

Part	Function
Stratum corneum (keratin)	● Prevents loss or entry of water ● If unbroken, it prevents entry of pathogens and most chemicals.
Stratum germinativum (stratum basale)	● Continuous mitosis produces new cells to replace worn-off surface cells ● Produces antimicrobial defensins.
Langerhans cells	● Phagocytize foreign material and stimulate an immune response by lymphocytes.
Merkel cells	● Receptors for sense of touch.
Melanocytes	● Produce melanin on exposure to UV rays.
Melanin	● Protects living skin layers from further exposure to UV rays.

BASEMENT MEMBRANE

Basement membranes are a dense, sheet-like form of extracellular matrix (ECM) that underlie epithelial and endothelial, and surround muscle, fat and Schwann cells. Basement membranes separate tissues and protect them from mechanical stress. Epidermal-Dermal anchoring fibrils (made from collagen VII) physically connect the lamina densa to the papillary dermis.

DERMIS

The dermis is made of an irregular type of fibrous connective tissue, irregular meaning that the fibers are not parallel, but run in all directions. Fibroblasts produce both collagen and elastin fibers. Recall that collagen fibers are strong, and elastin fibers are able to recoil after being stretched. Strength and elasticity are two characteristics of the dermis. With increasing age, however, the deterioration of the elastin fibers causes the skin to lose its elasticity. We can all look forward to at least a few wrinkles as we get older.

The uneven junction of the dermis with the epider- mis is called the papillary layer. Capillaries are abundant here to nourish not only the dermis but also the stratum germinativum. The epidermis has no capillaries of its own, and the lower, living cells depend on the blood supply in the dermis for oxygen and nutrients.

Within the dermis are the accessory skin structures: hair and nail follicles, sensory receptors, and several types of glands. Some of these projects through the epidermis to the skin surface, but their active portions are in the dermis.

SUBCUTANEOUS TISSUE

The subcutaneous tissue may also be called the superficial fascia, one of the connective tissue mem- branes. Made of areolar connective tissue and adipose tissue, the superficial fascia connects the dermis to the underlying muscles.

Areolar connective tissue, or loose connective tissue, contains collagen and elastin fibers and many white blood cells that have left capillaries to wander around in the tissue fluid between skin and muscles. These migrating white blood cells destroy pathogens that enter the body through breaks in the skin. Mast cells are specialized connective tissue cells found in areolar tissue; they produce histamine, leukotrienes, and other chemicals that help bring about inflammation, the body response to damage.

1.2 INTRODUCTION TO WOUND

FIGURE-2

A wound is any disruption of or damage to living tissue, such as skin, mucous membranes, or organs. Wounds can either be the sudden result of direct trauma (mechanical, thermal, chemical), or can develop slowly over time due to underlying disease processes such as diabetes mellitus, venous/arterial insufficiency, or immunologic disease. Wounds can vary greatly in their appearance depending on wound location, injury mechanism, depth of injury, timing of onset (acute vs chronic), and wound sterility, among other factors. Treatment strategies for wounds will vary based on the classification of the wound, therefore it is essential that wounds be thoroughly evaluated by a healthcare professional for proper management.

CLASSIFICATION OF WOUNDS

Class 1 wounds are categorized as clean wounds. These types of wounds are not infected, do not exhibit any signs of inflammation, and are typically closed. If drainage is required, a closed draining approach is recommended. It is worth noting that Class 1 wounds do not involve the respiratory, alimentary, genital, or urinary tracts. Examples of clean wounds include an inguinal hernia repair or a thyroidectomy.

Class 2 wounds are categorized as clean-contaminated, which means they have a low level of contamination. These types of wounds involve entry into the respiratory, alimentary, genital, or urinary tracts but only under controlled circumstances.

Class 3 wounds are classified as contaminated and typically result from a breach in sterile techniques or leakage from the gastrointestinal tract. Incisions resulting from acute or nonpurulent inflammation are also considered Class 3 wounds.

Class 4 wounds are considered to be dirty or infected. These injuries usually occur from inadequate treatment of traumatic wounds, gross purulence, and evident infections. When tissues lose vitality, it can lead to Class 4 wounds. This is often caused by surgery or microorganisms found in perforated organs.

TYPES OF WOUNDS

OPEN WOUND

FIGURE-3

CLOSE WOUND

FIGURE-4

1.3 INTRODUCTION TO WOUND-HEALING

Wound healing is a natural process to a living organism's replacement of destroyed or damaged tissue by newly produced tissue.In undamaged skin, the epidermis (surface, epithelial layer) and dermis (deeper, connective layer) form a protective barrier against the external environment. When the barrier is broken, a regulated sequence of biochemical events is set into motion to repair the damage.This process is divided into predictable phases: blood clotting (hemostasis), inflammation, tissue growth (cell proliferation), and tissue remodeling (maturation and cell differentiation). Blood clotting may be considered to be part of the inflammation stage instead of a separate stage.

Primary healing

It’s also known as healing by primary intention, occurs when surgical sutures or tapes are used to close the wound with minimal tissue loss.

Secondary healing

It, on the other hand, occurs when there is more extensive tissue loss or large surface wounds. In this process, granulation tissue grows from the wound margins to complete the repair.

Tertiary healing

It’s also called healing by third intention, is a delayed form of primary wound healing that occurs after 4-6 days. This happens when the process of secondary intention healing is intentionally interrupted, and the wound is mechanically closed after granulation tissue has formed.

1.4 PHASES OF WOUND HEALING PROCESS

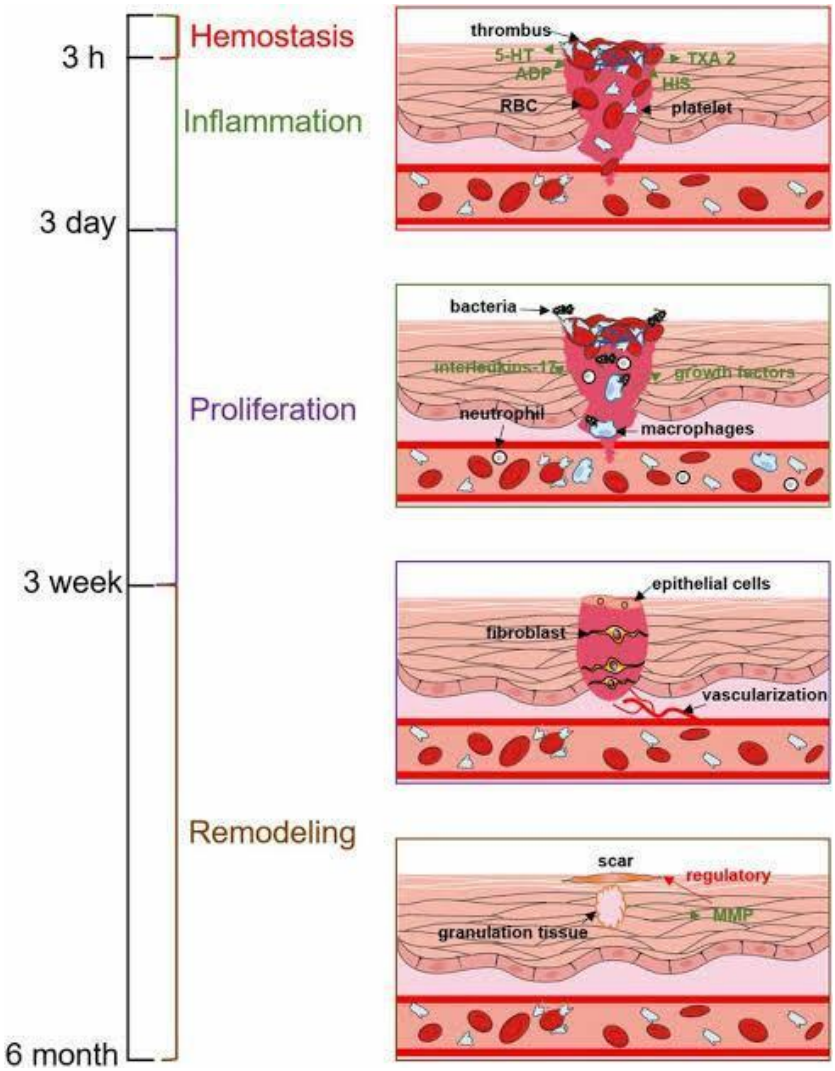


FIGURE-5

WOUND-HEALING PROCESS

Phase 1: Hemostasis Phase

Hemostasis, When an injury occurs, the initial phase is always an outpouring of lymphatic fluid and blood. It is during this process that adequate hemostasis is achieved. Both the extrinsic and intrinsic coagulation pathways are activated and play a role in stopping blood loss. Aggregation of platelets follows the arterial vasoconstriction to the damaged endothelial lining. A release of adenosine 5´ diphosphate (ADP) results in the clumping of platelets and initiates the process of thrombosis.

Phase 2: Defensive/Inflammatory Phase

The inflammatory phase begins with hemostasis and chemotaxis. Both the white cells and thrombocytes speed up the inflammatory process by releasing more mediators and cytokines. Besides the platelet-derived growth factor, other factors promote collagen degradation, the transformation of fibroblasts, the growth of new vessels, and re-epithelialization. All of the processes occur at the same time but in a synchronized fashion. Mediators like serotonin and histamine are released from platelets and increase cellular permeability. The platelet-derived growth factor attracts fibroblasts and, along with transforming growth factor, enhances the division and multiplication of fibroblasts. The fibroblasts, in turn, synthesize collagen.

Inflammatory cells, such as neutrophils, monocytes, and endothelial cells, adhere to a fibrin scaffold formed by platelet activation. The neutrophils enable phagocytosis of cellular debris and bacteria, allowing for decontamination of the wound.

Phase 3: Proliferative Phase

During this stage, The proliferative or granulation phase does not occur at a discrete time, but is ongoing all the time in the background. By days 5 through 7, the fibroblasts have started to lay down new collagen and glycosaminoglycans. These proteoglycans form the core of the wound and help stabilize the wound. Then, reepithelialization starts to occur with the migration of cells from the wound periphery and adjacent edges. Initially, only a thin superficial layer of epithelial cells is laid down, but a thicker and more durable layer of cells will bridge the wound over time. Once collagen fibers have been laid down on the fibrin framework, the wound starts to mature. The wound also begins to contract and is facilitated by continued deposition of fibroblasts and myofibroblasts.

Phase 4: Maturation Phase (Remodeling)

During the Maturation phase, The maturational or remodeling phase starts around week 3 and can last up to 12 months. The excess collagen degrades, and wound contraction also begins to peak around week 3. Wound contraction occurs to a much

greater extent in secondary healing than in primary healing. The maximal tensile strength of the incision wound occurs after about 11 to 14 weeks. The ultimate resulting scar will never have 100% of the original strength of the wound and only about 80% of the tensile strength.

TABLE-2

Phase	Cellular and Bio-physiologic Events
Hemostasis	1. vascular constriction 2. platelet aggregation, degranulation, and fibrin formation (thrombus).
Inflammation	1. neutrophil infiltration 2. monocyte infiltration and differentiation to macrophage.
Proliferation	1. re-epithelialization 2. angiogenesis 3. collagen synthesis 4. ECM formation
Remodeling	1. collagen remodeling 2. vascular maturation and regression.

1.5 INTRODUCTION TO VARIOUS PLANT USED FOR WOUND-HEALING PROCESS

1. Liquorice (*Glycyrrhiza glabra*) *Glycyrrhiza glabra* is one of the most popular medicinal plants belonging to the Fabaceae family (also known as Leguminosae), and its members are now commonly used as feed and food. The genus Glycyrrhiza is derived from the Greek words glykos (sweet) and rhiza (root).

2. Pomegranate (*Punica granatum*) *Punica granatum*, commonly known as pomegranate, has garnered interest in wound healing due to its rich content of bioactive compounds like polyphenols and flavonoids, which exhibit antioxidant, anti-inflammatory, and antimicrobial properties. Research suggests that pomegranate extracts can aid in wound healing by reducing inflammation, combating oxidative stress, preventing infections, stimulating collagen synthesis, and promoting angiogenesis, highlighting its potential as a natural remedy for supporting the various stages of the wound healing process. However, further clinical studies are necessary to fully understand its mechanisms and establish standardized protocols for therapeutic use in wound care.

3. Babool tree (*Acacia nilotica*) Nilotica pod extract enhances wound healing via angiogenesis, collagen deposition epithelisation and wound contractions. Nilotica bark and leaves have been used in traditional medicine for centuries to treat various ailments, including diarrhea, wounds, and malaria. The leaves part is employed to treat intestinal disorders and ulcers, to cure bronchitis, to heal fractures and to treat eye diseases.

4. Drumstick tree (*Moringa oleifera*) *Moringa oleifera* is known to possess wound healing activity. The present study evaluated the healing properties of methanolic extract of *Moringa oleifera* leaves in excision wounds infected with methicillin-resistant Staphylococcus aureus (MRSA) or P. aeruginosa in diabetic rats. An in vitro study was also carried out to determine the gene expression of VEGF and TGF-β1. Preliminary phytochemical and GC-MS analyses were carried out to determine different chemical constituents present in the extract. M. oleifera was applied locally as an ointment at two different concentrations. Wound contraction, period of epithelization, antioxidant enzyme activities and histological changes were determined.

5. Amla (*Phyllanthus emblica*) *Phyllanthus emblica* extracts possess strong antioxidant, antimicrobial, and anti-inflammatory properties. Antioxidants are thought to aid tissue repair during wound healing by helping to defend against oxidative stress and inflammation.The activity of *P. emblica* extract on wound healing has also been investigated in various studies, and in vitro studies have shown that *P. emblica* exhibits significant wound healing activity through the proliferation and mobilization of fibroblasts and keratinocytes. *P. emblica* has a high antioxidant capacity and improved endothelial wound healing at low concentrations.

6. Turmeric *(Curcuma longa)* Curcumin is a natural polyphenolic substance that has been used since ancient times in Ayurveda for its healing properties, as it reduces inflammation and acts on several healing stages. Several research studies for curcumin delivery at the wound site reported the effectiveness of curcumin in eradicating reactive oxygen species and its ability to enhance the deposition of collagen, granulation tissue formation, and finally, expedite wound contraction.

7. Taj powder (*Cinnamomum tamala*) *Cinnamomum tamala,* Several Cinnamomum species' barks are generally labeled as cinnamon, although only Cinnamomum verum carries the common name of true cinnamon. Cassia, a common name for a related species, is rarely used on labels; instead, various cassia types may also be labeled "cinnamon." Confusion of true cinnamon and cassia spices in foods generally does not present a risk to health, except possibly at the highest intake levels.

1.6 INTRODUCTION TO SELECTED MATERIAL PLANTS

1. Liquorice (*Glycyrrhiza glabra*)

FIGURE-6

TABLE-3

Scientific name	***Glycyrrhiza glabra***
Family	Papilionaceae, Fabaceae
Local name	Jethi-madh and Mulaithi and sweet wood
Common name	**Liquorice**
Parts used	Bark powder
Domain	Punjab, Jammu and Kashmir and South India.
Chemical constituents	Chemical constituent of liquorice is glycyrrhizin (about 2 to 9 %), a triterpene saponin with low haemolytic index. Glycyrrhetinic (glycyrrhetic) acid (0.5 to 0.9%), the aglycone of glycyrrhizin is also present in the root.
Action	Expectorant, antiallergic, anti-inflammatory, spasmolytic, mild laxative, anti-stress, antidepressive, antiulcer, liver protective.

Traditional uses

It was traditionally used as an insecticide, laxative, anti-inflammatory, anti-ulcer, antibiotic, anti-arthritic, antiviral.
Its action as a monoamine oxidase (MAO) inhibitor, anticholinergic, antitussive, Anti-caries, hypolipidemic, antimycotic, estrogenic, antioxidant, anticancer, and anti-diuretic agent.

2. Pomegranate (*Punica granatum*)

FIGURE-7

TABLE-4

Scientific name	***Punica granatum***
Family	Punicaceae
Local name	Dadam chhal
Common name	**Pomegranate**
Parts used	Bark of stem and root.
Domain	Native to Iran, but cultivated throughout India.
Chemical constituents	Phenolic substances in pomegranate peel, including tannins, flavonoids, and phenolic acids. fibers, alkaloids, minerals and vitamins.
Action	Anthelmintic, antiemetic, antidiarrhoeal

Traditional uses

Used for generations in treating ulcers, diarrhea. could be used to improve gut microbiota, and therefore prevent obesity and diabetes,

3. Babool tree (*Acacia nilotica*)

FIGURE-8

TABLE-5

Scientific name	***Acacia nilotica***
Family	Mimosaceae
Local name	Baval, Black Babul, Indian Gum arabic
Common name	**Babool tree**
Parts used	Stembark
Domain	Throughout the drier parts of India.
Chemical constituents	Terpenoids, alkaloids, saponins & glycosides.
Action	Anti-inflammatory

Traditional uses

Gum-for inflammatory conditions, digestive and urinary tracts.
Seeds-hypoglycaemic.
Seed oil—antifungal. Flowers,

4. Drumstick tree (*Moringa oleifera*)

FIGURE-9

TABLE-6

Scientific name	***Moringa oleifera***
Family	Moringaceae
Local name	Saragavo, Horse-Radish
Common name	**Drumstick**
Parts used	leaves
Domain	cultivated in northern countries of South America, Southeast Asia.
Chemical constituents	phenolic acids, isothiocyanates, tannins, flavonoids, and saponins
Action	Antipyretic, antibacterial, diuretic, anti-inflammatory, analgesic, antifungal

Therapeutic uses

Dried root bark in goitre, glycosuria and lipid disorders. and effective against both Gram-Positive and Gram-Negative bacteria.

5. Amla (*Phyllanthus emblica*)

FIGURE-10

TABLE-7

Scientific name	***Phyllanthus emblica***
Family	Euphorbiaceae
Local name	Indian gooseberry
Common name	**Amla**
Parts used	Fruit
Domain	Native to tropical Southeast Asia; distributed throughout India,also planted in public parks.
Chemical constituents	Gallic acid, ascorbic, methanol extract found to be effective inflammation.
Action	Anti anaemic, diuretic, antidiabetic, carminative, antioxidant

Therapeutic uses

Used in jaundice, dyspepsia, bacillary dysentery, eye trouble and as a gastrointestinal tonic. Juice with turmeric powder and honey is prescribed in diabetes insipidus.
Seed—antibilious, anti-asthmatic.
Bark—astringent.
Leaf—juice is given in vomiting.

6. Turmeric (*Curcuma longa*)

FIGURE-11

TABLE-8

Scientific name	***Curcuma longa***
Family	Zingiberaceae
Local name	Haldi
Common name	**Turmeric**
Parts used	Rhizomes
Domain	West Bengal, Tamil Nadu and Maharashtra.
Chemical constituents	four phenolic diarylheptanoids, including curcumin and volatile oil, ketones, sugars, starch, resin.
Action	Anti-inflammatory, antioxidant, anti-asthmatic, antitumour, carminative

Therapeutic uses

Curcuminoids prevent the increases in liver enzymes, SGOT and SGPT; this validates the use of turmeric as a hepatoprotective drug in liver disorders.

7. Taj powder (*Cinnamomum tamala*)

FIGURE-12

TABLE-9

Scientific name	***Cinnamomum tamala***
Family	Lauraceae
Local name	Cinnamomum tamala, Dal chini
Common name	**Taj**
Parts used	Bark
Domain	Himalayas, Khasi and Jaintia Hills.
Chemical constituents	cinnamaldehyde, volatile oil, α and β pinene
Action	antidiarrhoeal, spasmolytic, antirheumatic, hypoglycaemic.

Therapeutic uses

Treating inflammation, intestinal infections, astringent, diuretic and diabetic complications (leaves).

1.7 SELECTED OIL MATERIAL

1. Til oil (*Sesamum indicum*)

FIGURE-13

TABLE-10

Scientific name	***Sesamum indicum***
Family	Pedaliaceae
Local name	Ghani oil
Common name	**Til oil**
Chemical constituents	Sesamin, sesamolin, tocopherols, PUFA, phytosterols, phytates and other phenolics.

Therapeutic uses

They have antioxidants, blood lipid regulation, anti-inflammatory, cardiovascular system protection.

2. Jatyadi tel

Jatyadi tel is a polyherbal formulation.

FIGURE-14

Chemical constituents

Major chemical compounds were 9,12-octadecadienoic acid or linoleic acid (24.86%), n-hexadecanoic acid or palmitic acid (17.17%), cis 13- octadecenoic acid (15.19%), cis-13,16-docosadienoic acid (5.29%), ar-turmerone (2.49%), alpha-turmerone (2.03%), beta-turmerone or curlone (1.84%), 9-octadecenoic acid (Z) methyl ester or oleic acid (1.46%), phytol (1.61%), and squalene (1.84%), while other compounds were present in minor quantities with peak areas ranging from 0.09 to 1.61%.

Therapeutic uses

Polyherbal formulations Jathyadi Thailam and Jatyadi Ghritam (JT) are used in Indian traditional medicine for diabetic chronic wounds, fistula, and burn management.

2. LITERATURE REVIEW

2.1 LITERATURE REVIEW RELATED TO WOUND HEALING ACTIVITY

Gonzalez AC et al.(2016) suggested that Regeneration and tissue repair processes consist of a sequence of molecular and cellular events which occur after the onset of a tissue lesion in order to restore the damaged tissue. The exsudative, proliferative, and extracellular matrix remodeling phases are sequential events that occur through the integration of dynamic processes involving soluble mediators, blood cells, and parenchymal cells. Exsudative phenomena that take place after injury contribute to the development of tissue edema. The proliferative stage seeks to reduce the area of tissue injury by contracting myofibroblasts and fibroplasia. At this stage, angiogenesis and reepithelialization processes can still be observed. Endothelial cells are able to differentiate into mesenchymal components, and this difference appears to be finely orchestrated by a set of signaling proteins that have been studied in the literature. This pathway is known as Hedgehog. The purpose of this review is to describe the various cellular and molecular aspects involved in the skin healing process.

Velnar T et al. (2009) Wound healing remains a challenging clinical problem and correct, efficient wound management is essential. Much effort has been focused on wound care with an emphasis on new therapeutic approaches and the development of technologies for acute and chronic wound management. Wound healing involves multiple cell populations, the extracellular matrix and the action of soluble mediators such as growth factors and cytokines. Although the process of healing is continuous, it may be arbitrarily divided into four phases: (i) coagulation and haemostasis; (ii) inflammation; (iii) proliferation; and (iv) wound remodelling with scar tissue formation. The correct approach to wound management may effectively influence the clinical outcome. This review discusses wound classification, the physiology of the wound healing process and the methods used in wound management.

J C Bleacher et al. (1993) Oct. The fibrosis and scar formation that characterize adult wound healing are also the cause of clinical problems; scar contracture, hypertrophic scar, and pulmonary and hepatic fibrosis are only a few examples. Studies of fetal wound healing can provide an insight into the initiation and regulation of a scarless repair process akin to regeneration. Studies of fetal repair have already suggested mechanisms that might favorably alter adult healing. Topical application of hyaluronic acid to wounds in adult diabetic rats leads to enhanced epithelial migration. It has been recognized that the addition of TGF-beta to fetal wounds causes an adultlike healing response with fibrosis and inflammation. A subsequent study using neutralizing antibody to TGF-beta in adult wounds showed enhanced healing with a more normal dermal architecture with fewer macrophages, fewer blood vessels, and less collagen. As our understanding of regenerative tissue repair increases, the opportunities to modulate adult fibrotic conditions should expand.

Martin P. (1997) The healing of an adult skin wound is a complex process requiring the collaborative efforts of many different tissues and cell lineages. The behavior of each of the contributing cell types during the phases of proliferation, migration, matrix synthesis, and contraction, as well as the growth factor and matrix signals present at a wound site, are now roughly understood. Details of how these signals control wound cell activities are beginning to emerge, and studies of healing in embryos have begun to show how the normal adult repair process might be readjusted to make it less like patching up and more like regeneration.

Smith's 2020 study focuses on the role of bioactive peptides in wound healing processes. The research investigates how these peptides contribute to cell proliferation, angiogenesis, and extracellular matrix remodeling, ultimately promoting faster wound closure and tissue regeneration. Smith's work provides valuable insights into the potential applications of bioactive peptides as therapeutic agents for enhancing wound healing.

MADHURI A. THENG (2017) A large number of plants are used by folklore traditions in India for treatment of cuts, wounds and burns. The drugs selected for this work were Acacia arabica and Butea monosperma. These two important herbs are reported to have significant antibacterial, immunomodulatory and anti-inflammatory activities which are complementary to wound healing process. The growing popularity of natural and herbal medications, easy availability of raw materials, cost-effectiveness and the paucity of reported adverse reaction, prompted us to formulate a polyherbal topical preparation and assess its wound healing ability. The combination is used in order to enhance the wound healing activity.

2.2 Literature review of *Glycyrrhiza glabra*

Metar Siriwattanasatorn et al 2020 Eight Thailand medicinal plants, most commonly used to treat wounds, were evaluated for their in vitro biological activities such as antioxidation by NBT assay, anti-inflammation by production inhibition of NO, promoting fibroblast cell proliferation, and wound closure activities. Plant materials were extracted with 95% ethanol or distilled water and then concentrated and dried. Three established markers from these three plants were selected according to the selection criteria. Alpha-mangostin, glycyrrhizin, and thymoquinone were found to be the active markers for wound closure activities. The ethanolic extracts of G. mangostana, G. glabra, and N. sativa could scavenge superoxide anion and inhibit the production of nitric oxide; therefore these extracts could assist in surpassing the inflammatory phase and protected the cells surrounding the wound area. Most importantly, these extracts also increased the proliferation and accelerated wound closure, indicating that these plant extracts could be promoting wound healing processes and support the use of TTM.

Doaa H Assar et al. 2021 histopathological findings detected complete re-epithelialization with increasing collagen synthesis while IHC results revealed a significant enhancement in the expression of α-SMA, PDGFR-α, FGFR1 and Cytokeratin 14 in licorice treated groups compared with the control group. Licorice extract supplementation accelerated wound healing by increasing angiogenesis and collagen deposition through up-regulation of bFGF, VEGF and TGF-β gene expression levels compared with the control group. UPLC-PDA-MS/MS aided to authenticate the studied Glycyrrihza species and recognized 101 potential constituents that may be responsible for licorice-exhibited potentials. Based on our observations we concluded that licorice enhanced cutaneous wound healing via its free radical-scavenging potential, potent antioxidant activities, and anti-inflammatory actions. Therefore, licorice could be used as a potential alternative therapy for wound injury which could overcome the associated limitations of modern therapeutic products.

H Akamatsu et al 1991 The effect of glycyrrhizin on inflammatory mediators such as neutrophil functions including reactive oxygen species (ROS) generation was examined. Glycyrrhizin significantly decreased neutrophil-generated O2-, H2O2 and OH in a dose-dependent manner. However, the drug did not reduce any of the ROS generated in a cell-free, xanthine-xanthine oxidase system. The drug did not affect neutrophil chemotaxis or phagocytosis, either. The present study indicates that glycyrrhizin is not an ROS scavenger but exerts an anti-inflammatory action by inhibiting the generation of ROS by neutrophils, the most potent inflammatory mediator at the site of inflammation.

2.3 Literature review of *Punica granatum*

Shivananda B Nayak et al. 2013 The skin of the fruit and the bark of Punica granatum are used as a traditional remedy against diarrhea, dysentery, and intestinal parasites. The fruit skin extract of P. granatum was tested for its wound healing activity in rats using an excision wound model. The animals were divided into three groups of six each. The experimental group of animals was topically treated with P. granatum at a dose of 100 mg/kg every day for 15 days, while the controls and standard group animals were treated with petroleum jelly and mupirocin ointment, respectively. Phytochemical analysis of the extract revealed the presence of saponins, triterpenes, tannins, alkaloids, flavonoids, and cardiac glycosides. Extract-treated animals exhibited 95% reduction in the wound area when compared with controls (84%), which was statistically significant (P<.01). The extract-treated wounds were found to epithelize faster compared with controls. The hydroxyproline content of extract-treated animals was significantly higher than controls (P<.05). The fruit skin extract did not show any antimicrobial activity against the microrganisms tested. P. granatum promotes significant wound healing in rats and further evaluation of this activity in humans is suggested.

E A Hayouni et al. 2011 in vivo wound healing potential of Punica granatum L. peels. A 5% (w/w) methanolic extract based-ointment was formulated and evaluated for its wound healing in guinea pigs. The ointment was applied in vivo on the paravertebral area of twelve excised wounded models once a day for 10 consecutive days. The ointment significantly enhanced the wound contraction and the period of epithelialization as assessed by the mechanical (contraction rate, tensile strength), the biochemical (increasing of collagen, DNA and proteins synthesis) and the histopathological characteristics. Such investigation was encouraged by the efficiency of the methanolic extract as antimicrobial and antioxidant. Indeed, the extract showed antioxidant activity as strong as natural and synthetic compounds (Trolox, BHA, Quercetin).

M Cuccioloni et al 2009 Pomegranate (Punica granatum) is an important source of polyphenols with assessed antioxidant properties. The aims of this study were: (i) the characterization of the monomeric phenolic variability on each isolated fruit component (endocarp, mesocarp, aril); (ii) the study on the effect of pomegranate fruit components on human thrombin amidolytic activity. Collectively, our data show that pomegranate components contain bioactive metabolites (mainly ellagic acid) and suggest a potential role for the pomegranate extract in the regulation of a number of physio-pathological processes involving thrombin (or thrombin-like proteinase).

2.4 *Literature review of Acacia nilotica*

Rushda Saeedi 2020, *Acacia nilotica* plays an important role as free radical scavenging properties due to a rich source of antioxidants like flavonoids, phenolics, tannins, curcumin, and terpenoids. They can reduce the contact of oxidants and other toxic molecules due to their ability to scavenge oxygen-nitrogen-derived free radicals by donating hydrogen atom or an electron, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases. A. nilotica ingredient shows an effective role in the management of cancer through the regulation of cell signalling pathways. It modulates the activity of various tumour suppressor genes, angiogenesis, and apoptosis. A. nilotica also plays a role as an anti-inflammatory via regulation of pro-inflammatory enzyme activities including cyclooxygenase and lipoxygenase enzyme.

Nimra Riasat 10 May 2024 A. nilotica leaves extract; A. nilotica bark extract (ANB-E), and A. nilotica stem extract were prepared using methanol-chloroform (1:1). Phytochemical analysis was performed using gallic acid equivalent total phenolic content, quercetin equivalent total flavonoid content assays and High-performance liquid chromatography. In vitro antioxidant potential (free radical scavenging activity, total antioxidant capacity, and ferric reducing antioxidant power assay), antibacterial activity and hemolytic analysis was carried out. Wound healing proficiency of ANB-E was determined by wound excision model followed by estimating hydroxyproline content and endogenous antioxidant markers.

Mshelia et al. 2022, The stem bark CME of A. nilotica reveals the presence of tannins, saponins, glycosides, alkaloids, and terpenoids. The much likely presence of phenolic compounds here is of great significance in terms of the wound-healing potential of A. nilotica. For instance, it has been noted that tannins act by iron deprivation, hydrogen bonding, or specific interaction with proteins such as enzymes, cell envelopes, and complex formation with polysaccharides. A tannin molecule (palas tannin) isolated from Butea Gum had been reported to possess wound-healing properties. More so, the saponins were reported to possess antibacterial properties. Their mode of action was attributed to their ability to cause leakage of proteins and certain enzymes from bacterial cells. Consequently, these compounds have been reported to be biologically active and hence may contribute singly as antibacterial, antifungal, and wound healing agents/enhancers in the total wound healing activity exhibited. Medicinal plants heal the wound healing process by promoting blood clotting, fighting against infection, and accelerating wound healing. It can be stated that plants and chemical agents obtained from plants improve treatment and manage wound healing. Wound healing effects by different mechanisms, such as modulation in wound healing, decreasing bacterial count, improving collagen deposition, increasing fibroblasts and fibrocytes, etc.

2.5 Literature review of *Moringa oleifera*

Nurmaziah Mohammad Shafie et al. 2022. Moringa oleifera (locally known as merunggai in Malaysia) has been traditionally used in various ailments, including for wound management. To evaluate the wound healing properties in M. oleifera,total, 18 in vivo studies were included, which involved the leaves, while the remaining 5 studies involved other plant parts tested on excision, incision, dead space, abrasion, and burn-induced wound models. All studies reported significant wound healing abilities. Most studies used different topical formulations of aqueous leaves extract. The accumulation of collagen content and underlying wound healing mechanism through antimicrobial, antioxidant, and anti-inflammatory activities may be contributed by its bioactive phytochemical content, which has the potential to accelerate the wound contraction, increase the rate of epithelialization, and protect tissues against oxidative damage. In conclusion, M. oleifera showed wound healing potential but further studies are warranted to determine the main bioactive phytocompounds and safety.

Aaliya Ali et al. 2020 at seeds of Moringa oleifera which is the essential ethnomedicinal plant, were studied for wound healing efficacy. The study was planned for the assessment of in vitro (antioxidant and antimicrobial activities) and in vivo (excision and incision wound healing models) wound healing efficacy of n-hexane extract and hydrogels of Moringa oleifera seeds.In excision and incision wound models, Swiss albino mice were used for wound healing efficacy of hydrogels, i.e., 5% and 10% hexane extracts of Moringa oleifera seeds. The n-hexane extract showed antioxidant as well as antibacterial activities. The hydrogels formulated using n-hexane extract of Moringa oleifera seeds showed significant wound healing activity compared to both control and standard until the end of the protocol in both the models. Furthermore, the histopathological investigation confirmed the findings of accelerated regeneration of tissue accompanied by a decrease in inflammatory cells and increased vascularity of the immediate skin. The results (both in vitro and in vivo) claimed conclusively that our n-hexane hydrogel formulation of Moringa oleifera seeds might serve as an alternative therapy in skin restoration during wound healing.

Abdullah A Al-Ghanayem et al. 2022 at The present study investigated the wound healing activity of Moringa oleifera leaf extract on an infected excision wound model in rats.The methanol extract of M. oleifera leaves was analyzed for the presence of phytochemicals by LCMS. The antimicrobial activity of the extract was also determined. Wound contraction, days for epithelization, antioxidant enzyme activities, epidermal height, angiogenesis, and collagen deposition were studied. M. oleifera showed an antimicrobial effect and significantly improved wound contraction, reduced epithelization period, increased antioxidant enzymes activity, and reduced capillary density. Effect of the extract was less in wounds infected with P. aeruginosa when compared to MRSA. The VEGF and TGF-β1 gene expression was increased by M. oleifera.

2.6 Literature review of *Phyllanthus emblica*

Linda Chularojmontri et al. 2013 at In this study, we examined the antioxidant constituents and capacity of *Phyllanthus emblica* L. (PE) fruit in freeze-dried power form. The pharmacological properties of PE were investigated using human umbilical vein endothelial cells (HUVECs) in the aspects of endothelial cell proliferation, nitric oxide (NO) production, wound healing, cell migration, in vitro angiogenesis, and VEGF gene expression. PE significantly promoted NO production, endothelial wound closure, endothelial sprouting, and VEGF mRNA expression. Therefore, PE is a candidate for antioxidant supplement that promotes endothelial function and restores wound healing competency.

Gefei Li et al. 2023 at The fruit of *Phyllanthus emblica* Linn., which mainly grows in tropical and subtropical regions, is well-known for its medicine and food homology properties. It has a distinctive flavor, great nutritional content, and potent antioxidant, anti-inflammatory, anti-cancer and immunoregulatory effects. Phyllanthus emblica fruit can promote saliva secretion, regulate the balance of the oral microecology, prevent and treat oral cancer early, promote alveolar bone remodeling and aid mucosal wound healing. Thus, it plays a specific role in the prevention and treatment of common oral disorders, producing surprising results.For instance, enhancing the effectiveness of scaling and root planing in the treatment of periodontitis, relieving mucosal inflammation caused by radiotherapy for oral cancer, and regulating the blood glucose metabolism to alleviate oral discomfort. Herein, we systematically review the latest research on the use of Phyllanthus emblica fruit in the management of oral health and examine the challenges and future research directions based on its chemical composition and characteristics.

Ipek Canatar et al. 2024 at One plant with remarkable healing properties is *Phyllanthus emblica* Linn (P. emblica), which is described as having potent antioxidant, antimicrobial and anti-inflammatory properties. The aim of this study is to evaluate the biocompatibility of P. emblica-loaded polyvinyl alcohol/gelatin-based cryogels (PVA/Gel/P.emblica) through cytotoxicity and proliferation tests in HaCaT cells and examine their potential in wound dressing applications.P. emblica enhances cell proliferation, increases cell number, and improves cell viability. Based on the scanning electron microscope, immunofluorescence, and Giemsa staining images, it is observed that P. emblica promotes cell attachment, proliferation, and penetration. These findings confirm that PVA/Gel/P.emblica cryogels are suitable for use as wound dressing materials and can be developed with further studies.

2.7 Literature review of *Curcuma longa*

Silvia Tejada et al 2016 Since antiquity, vegetable substances have been used as phytotherapeutic agents for wound healing, and more recently natural substances of vegetable origin have been studied with the attempt to show their beneficial effect on wound treatment. Curcumin, the most active component of rhizome of Curcuma longa L. (common name: turmeric), has been studied for many years due to its bio-functional properties, especially antioxidant, radical scavenger, antimicrobial and anti-inflammatory activities, which play a crucial role in the wound healing process. Moreover, curcumin stimulated the production of the growth factors involved in the wound healing process, and so curcumin also accelerated the management of wound restoration.

Dania Akbik et al. 2014 Turmeric (Curcuma longa) is a popular Indian spice that has been used for centuries in herbal medicines for the treatment of a variety of ailments such as rheumatism, diabetic ulcers, anorexia, cough and sinusitis. Curcumin (diferuloylmethane) is the main curcuminoid present in turmeric and responsible for its yellow color. Curcumin has been shown to possess significant anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-mutagenic, anticoagulant and anti-infective effects. Curcumin has also been shown to have significant wound healing properties. It acts on various stages of the natural wound healing process to hasten healing. This review summarizes and discusses recently published papers on the effects of curcumin on skin wound healing. The highlighted studies in the review provide evidence of the ability of curcumin to reduce the body's natural response to cutaneous wounds such as inflammation and oxidation. The recent literature on the wound healing properties of curcumin also provides evidence for its ability to enhance granulation tissue formation, collagen deposition, tissue remodeling and wound contraction. It has become evident that optimizing the topical application of curcumin through altering its formulation is essential to ensure the maximum therapeutical effects of curcumin on skin wounds.

Chandana Mohanty et al. 2017 Oxidative damage and inflammation have been identified, through clinical and preclinical studies, as the main causes of nonhealing chronic wounds. Reduction of persistent chronic inflammation by application of antioxidant and anti-inflammatory agents such as curcumin has been well studied. However, low aqueous solubility, poor tissue absorption, rapid metabolism and short plasma half-life have made curcumin unsuitable for systemic administration for better wound healing. Recently, various topical formulations of curcumin such as films, fibers, emulsion, hydrogels and different nanoformulations have been developed for targeted delivery of curcumin at wounded sites. In this review, we summarize and discuss different topical formulations of curcumin with emphasis on their wound-healing properties in animal models.

2.8 Literature review of *Cinnamomum tamala*

Dr. Shashank tiwari et al. 2020 *Cinnamomum tamala* is a multipurpose evergreen plant it is a native to India. The plant is commonly known as Indian cassia, Tejpatta, Indian bay leaf etc. All parts of plant possess many major bioactive constituent due to the presence of major phytoconstituent it is useful for the treatment of various diseases or disorders such as Cancer, cardiac diseases, diabetes, Anxiety, depression, ulcer, GI diseases and possess many pharmacological activity includes anti-oxidant, anti-hypercholesterolemia, anti-diarrhoeal, anti-inflammatory, anti-fungal, anti-bacterial etc. In Ancient time the plant was also used for its medicinal value and it contains an aromatic property due to the presence of these properties. It is used in the perfumery industry and used as mouth refreshing, useful for removal of bad odour from body, mouth and also used in pharmaceutical industries. The leaves of the plant possess a flavouring agent property; it is used as a flavouring agent in food, curry, fast food, pickles and used as a spice. The main aim of this review/study was to promote and upgrade the knowledge of the use of this multipurpose evergreen plant

RUPESH SONI 2013. The ethanolic extract of *Cinnamomum tamala* leaves was evaluated for wound healing activity in diabetic rats. The all four phases (hemostasis, inflammation, granulation and remodeling) of wound healing studied by excision, incision and dead space wound models. The high blood glucose level is the root cause of delayed wound healing in patients of diabetes. The treatment of ethanolic extract of C. tamala leaves promotes wound healing by decrease in blood glucose level, faster contraction of wound and increased granulation of tissue with increased tensile strength. This action may be due to antidiabetic, antioxidant and antimicrobial activities of phytoconstituents like phenolics and tannins which present in ethanolic extract of Cinnamomum tamala leaves. Further studies are needed to identify active compound responsible for faster wound healing activity with detailed mechanism of action.

Donia Waleed Khaled1 2023. The results have shown that a dry cinnamomum powder extracts in ethanol can be used in therapeutic fields. Cinnamomum extract have shown good inhibitory effects against the growth of escherichia coli, klebsiella sp, Staphylococcus epidermidis and Staphylococcus aureus, as well as Candida albicans. The greatest effect of cinnamomum extract was obtained against the Gram positive bacterial strains S. epidermidis and S. aureus. Furthermore, cinnamomum extract has shown to be a good wound healing agent, in which rats whom treated with cinnamomum extract have shown a faster healing compared to control rats starting from the day. These effects make cinnamomum to be very useful in the medical field generally, and in inflammation line specifically.

2.9 Literature review of Nanoemulgel

Gururaj C Aithal et al. Curr Pharm Des. 2020. Recently, the delivery of hydrophobic/ poorly water-soluble drugs has been a challenging task. Various strategies have been developed to counter the former along with other prime issues, such as stability, bioavailability etc. However, only few formulations have been successful in addressing the problems and nanoemulgel is a standout among them. Nanoemulgel are appropriate candidates for drug delivery because of their dual character i.e. the presence of an emulsion in the nano scale and a gel base, both combined as a single formulation. The nanoemulsion component of the nanoemulgel conforms protection to the active moiety by preventing the enzymatic degradation and certain reactions like hydrolysis. The gel base attributes thermodynamic stability to the emulsion by increasing the viscosity of the aqueous phase by decreasing the interfacial and surface tension. Nanoemulgel possess rheological characteristics which are suited especially for topical delivery and other forms such as dental delivery with the aid of better patient acceptance. As the globule size is present in the nano form alongside the employment of certain penetration enhancers can increase the effectiveness of the formulation by enhancing the permeability and diffusibility. Reports suggest that certain commercially available topical dosage forms have a low spreading coefficient in comparison with the nanoemulgel thereby focusing on the application of nanoemulgel in the field of dermatology, although paving way for various other fields have not been thoroughly exploited. This comprehensive review highlights the benefits of nanoemulgel as a potential carrier for drug delivery with an overview of a few illustrations supporting the cause.

Kumar Anand et al. Recent Pat Antiinfect Drug Discov. 2019. enormous efforts for different drug discovery processes have led to a number of drug molecules available today to overcome different challenges of the health care system. Unfortunately, more than half of these drugs are listed in either BCS (biopharmaceutical classification system) class II/ IV or both are eliminated from the development pipeline due to their limited clinical use. A nanotechnological approach bears much hope and lipoidal fabrication is found to be suitable for the delivery of such drugs. Nanoemulsion based gel i.e. nanoemulgel out of different nanolipoidal formulations has been found to be a suitable approach to successful drug delivery through topical routes. In past few years many herbal and synthetic active pharmaceutical ingredients (APIs) have been patented as nano sized emulsified gel for various therapeutic activities.

Hira Choudhury et al. J Pharm Sci. 2017 July. Being an emerging transdermal delivery tool, nanoemulgel, has proved to show surprising upshots for the lipophilic drugs over other formulations. This lipophilic nature of majority of the newer drugs developed in this modern era resulting in poor oral bioavailability, erratic absorption, and pharmacokinetic variations. Therefore, this novel transdermal delivery system has been proved to be advantageous over other oral and topical drug delivery to avoid such disturbances. These nanoemulgels are basically oil-in-water nanoemulsions gelled with the use of some gelling agent in it. This gel phase in the formulation is nongreasy, which favors user compliance and stabilizes the formulation through reduction in surface as well as interfacial tension. Simultaneously, it can be targeted more specifically to the site of action and can avoid first-pass metabolism and relieve the user from gastric/systemic incompatibilities. This brief review is focused on nanoemulgel as a better topical drug delivery system including its components screening, formulation method, and recent pharmacokinetic and pharmacodynamic advancement in research studies carried out by scientists all over the world. Therefore, at the end of this survey it could be inferred that nanoemulgel can be a better and effective drug delivery tool for the topical system.

20PHUSM046
20PHUOS050 Development and evaluation of wound healing polyherbal formulations.
20PHUBS063

3. <u>OBJECTIVES</u>

1. Identification of potential medicinal plants for wound-healing activity.

2. Collection processing, preparation of extract & phytochemical screening of the plants.

3. Development of stable herbal formulation & it's evaluation.

4. Evaluation of wound-healing potential of prepared formulation.

4. MATERIAL AND METHODS

4.1 List of apparatus, instruments and material used in the experiment

TABLE-11

Apparatus	Instrument
Beaker	Brookfield viscometer
Flask	pH meter
Pipette	Hot plate
Petri dish	BOD incubator
Test tube	Hot air oven
China dish	Weigh balance
Measuring Cylinder	Rotary shaker
Glass rod	Ultrasonicator
Whatmann filter paper	Dessicator
Dropper	UV Spectrophotometer
Spatula	Electric water bath
Test tube	Homogenizer
Funnel	Thermometer
Watch glass	Water bath

TABLE-12

Ingredients	Methanol
Glycyrrhiza glabra powder	Distilled water
Punica granatum powder	Carbopol 934
Acacia nilotica powder	Propylene glycol
Moringa oleifera powder	Triethanolamine
Phyllanthus emblica powder	Span 80
Curcuma longa powder	Tween 80
Cinnamomum tamala powder	Detergent
Sesamum indicum oil	**Preservative**
Jatyadi tel	Methyl Paraben Sodium

4.2 PREPARATION OF EXTRACT

Extracts of formulation A

Powder of root of glycyrrhiza glabra and peel of moringa olifera were taken from the authenticated source and thus the oil of Sesamum indicum were taken and directly used in the formulation.

A. Preparation of *Glycyrrhiza glabra* extract

Aqueous extract was prepared with this powder of glycyrrhiza glabra root.Weighing 50g of standard powder of glycyrrhiza glabra was added to 500ml of water as a solvent to form solution (1:10).
The solution was then filtered with filter paper and poured in a petri dish which then by drying in a hot air oven at suitable temperature. After complete drying the dry powder was collected and stored in a suitable vessel.

B. Preparation of *Moringa oleifera* extract

Aqueous extract (1:10) was prepared using this ingredient. Weighing 50g of standard powder of Moringa oleifera was added to a solution of 500ml water.The mixture then filtered and poured in petri dish which then dried on a hot air oven at suitable temperature. After drying the dry powder collected and stored in a suitable vessel.

Extracts of formulation B

A. Preparation of *Glycyrrhiza glabra* extract

Aqueous extract was prepared with this powder of glycyrrhiza glabra root.Weighing 50g of standard powder of glycyrrhiza glabra was added to 500ml of water as a solvent to form solution (1:10). The solution was then filtered with filter paper and poured in a petri dish which then by drying in a hot air oven at suitable temperature. After complete drying the dry powder was collected and stored in a suitable vessel.

B. Preparation of Acacia nilotica (M)

Methanolic extract prepared by adding 50 gm of powder in 500 ml of methanol.The mixture then filtered and poured in petri dish which was then dried by water bath and dried powder collected and stored.

Extracts of formulation C

Used extracts,
A. *Cinnamomum tamala* (Methanolic)
B. *Phyllanthus emblica*
C. *Punica granatum*
D. *Curcuma longa*

Preparation is the same as above mentioned for Aqueous and Methanolic extract.

FIGURE-15

Filtration

Methanolic and Aqueous

Hot air oven

Extract

4.3 PREPARATION OF NANOEMULGEL

4.3.1 Preparation of nanoemulsion

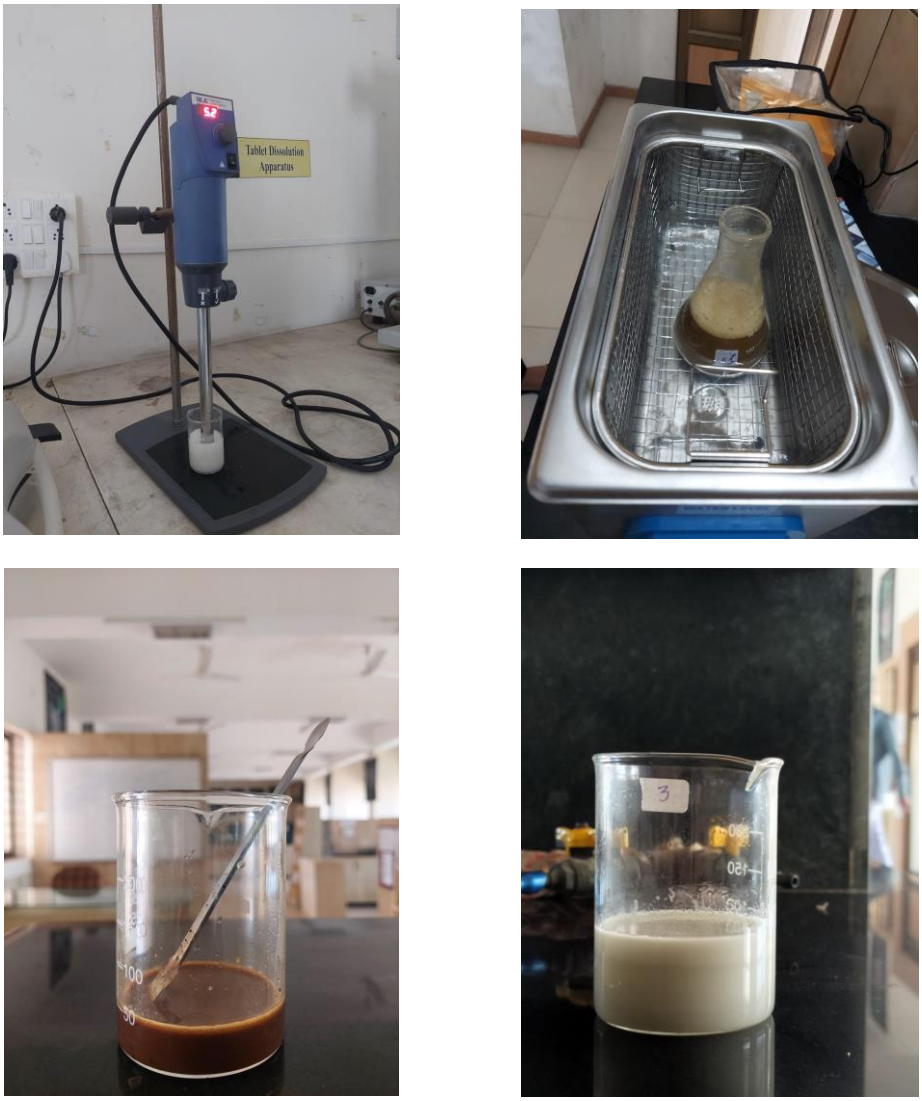
The aqueous phase was prepared by heating 5g Tween 80 and 85 g deionised water in at 60 °C on a hotplate stirrer, mixing at 500 rpm for 10 min.

The lipid phase was prepared by mixing a 2.5 g of Span 80 and 7.5 g essential oil under the same conditions as Tween 80.

The two phases were mixed by adding the aqueous phase to the lipid phase under vigorous stirring at 800 rpm and 60 °C for a 5 min on a hotplate stirrer.

This coarse milky emulsion was homogenised at 25,000 rpm for 10 min using a high shear homogeniser available in Pharmaceutics instrument room at the 1st floor.

FIGURE-16



(Homogeniser, Sonicator, Nanoemulsion)

4.3.2 Preparation of hydrogel

Take given quantity of polymers (take at least 1 concentration from each, so total 3 gels) in 99.85 ml of water. Mix well. Add 0.15 ml (one drop) of TEA at last and left it overnight.

Briefly, the defined polymer was dispersed slowly in deionised water under continuous stirring until homogenous gel formation.

Triethanolamine was added to neutralise the pH of gel, which had been left overnight to complete cross-linking, and gelation, as well as expel any trapped air bubbles within gel.

FIGURE-17

Carbopol 934

TABLE-13

Polymer	Concentration %w/w	Mixing rate (rpm)	Processing Temperature	Triethanolamine (ml)
Carbopol 934	0.5 0.75 1	400	25	0.15
Xanthan gum	1 1.5 2 2.5	400	40	0.25
HPMC	1 2	400	40	0.25

4.3.3 Preparation of nanoemulgel

Nanoemulgel was prepared by mixing the nanoemulsion with hydrogel at a 1:1 ratio, under continuous slow stirring (125 rpm) at 25 °C until visually homogeneous product formation.

FIGURE-18

Nanoemulgel (Formulation)

4.4 QUALITATIVE PHYTOCHEMICAL SCREENING

1. TEST FOR ALKALOID

Test solution: For chloroform, methanol, n-hexane, ethyl acetate extract/fraction. Crush with dilute acid (10% acetic acid or 1 to 5% hydrochloric acid). Filter. Take 0.5 to 1 mL filtrate + add 1 to 2 mL of following reagents;

TABLE-14

S/N	TEST	PROCEDURE	INFERENCE
1.	Mayer's test	Solution I: dissolve 1.36 g $HgCl_2$ in 60mL water. Solution II: dissolve 5 g KI in 10mL water. Procedure: combine the two solutions and dilute with water to 100mL.	:White of buff color precipitates
2.	Drangendorff's test	(a): Dissolve 0.85 g basic bismuth nitrate in 10 ml glacial acetic acid and 40 ml water under heating. If necessary, filter. Solution. (b): Dissolve 13.33 g potassium iodide in 30 ml water. Mix a & b.	:Bright orange red precipitates
3.	Wagner' test	Solution: dissolve 1.27 g I_2 (sublimed) and 2 g KI in 20mL water, and make up with water to 100 mL.	: Brown precipitates
4.	Hager's test	Dissolve 1.3 g picric acid in distilled water.	: yellow precipitates

FIGURE-19

2. TEST FOR FLAVONOID

Test solution: For chloroform, methanol, n-hexane, ethyl acetate extract/fraction, dissolve 20 to 30 mg extract in 10 mL methanol/water extract dissolve in water. Take 1 to 2 mL of test solution and follow the following steps;

TABLE-15

S/N	TEST	PROCEDURE	INFERENCE
1.	Shinoda test (With Magnesium metal)	An alcoholic solution of the sample, add magnesium powder and a few drops of concentrated HCl.	Orange, pink, red to purple colors
2.	Sulfuric acid test	Take 1-2 mL test solution and add few drops of conc. sulfuric acid from the wall of test tubes.	Produce red or red-bluish solutions.

3. TEST FOR SAPONIN

Take 0.1 to 0.2 g of extract, add 10 mL distilled water, shake vigorously. Stabilize frothing for 10 to 15 minutes indicates presence of saponin.

4. TEST FOR STEROL

Mostly non-polar extracts like chloroform, n-hexane, petroleum ether, sometimes methanol give this test positive.

TABLE-16

S/N	TEST	PROCEDURE	INFERENCE
1.	Salkowaski's test	The crude extract (about 50 to 100 mg) was separately shaken with chloroform (2 mL) followed by the addition of concentrated H2SO4 (2 mL) along the side of the test tube.	A reddish brown coloration of the interface indicates the presence of terpenoid/sterol.

5. TEST FOR SUGARS

Mostly polar extracts/fractions like methanol, water, ethyl acetate and n-butanol give this test positive.

TABLE-17

S/N	TEST	PROCEDURE	INFERENCE
1.	Molisch's Test	Test tube containing 0.5 ml of water, and it was mixed with two drops of Molisch's reagent. To this solution, was added 1 ml of concentrated sulphuric acid from the side of the inclined test tube, so that the acid formed a layer beneath the aqueous solution without mixing.	Red brown/ violet ring appears

6. TEST FOR TANNINS

The test residue of each extract was taken separately in water and filtered. Tests were carried out with the filtrate using following reagent;

TABLE-18

S/N	TEST	PROCEDURE	INFERENCE
1.	Ferric Chloride Test	Prepare 5 % solution of ferric chloride in 90 % methanol.	Dark green or deep blue color is obtained.
2.	Lead Acetate Test	Prepare 10 % w/v solution of basic lead acetate in distilled water.	precipitate is obtained.

4.5 EVALUATION PARAMETERS

4.5.1 Physical Characteristics :

Odour, color, homogenicity , consistency, texture and other physical characteristics are checked visually for patient compliance for formulation developed. Clarity, are visually checked for presence of any unwanted or foreign particle.

4.5.2 Washability :

Washability of formulation determined by applying a small amount of prepared formulation over the skin and after some time washed it with water and by checking it manually formulation was evaluated.

4.5.3 Irritancy :

Irritancy test of skin are determined to check potential of formulation for irritation. Small amount of formulation applied to the small part of skin of subject for particular period of time. After that it is washed with acetone and result is observed and further evaluated.

4.5.4 Stability :

Stability testing of formulation determined by placing small amount of formulation at room temperature for 3 days to check its stability. After 3 days stability of formulation checked and observed if any presence of fungus or other foreign material and maintains its homogenicity.

4.5.5 pH :

Determination of pH is essential for different suitability of the skin to the formulation. Thus, by putting little wet pH paper on the formulation surface or by applying formulation on pH paper it gives certain color. Checking pH chart to match the observed colour of certain pH.

4.5.6 Viscosity :

Viscosity of the formulation was determined by using Brookfield viscometer. As the system is non-Newtonian spindle no. 63 is used. Viscosity was measured for the fixed time 3 min for 10 rpm.

4.6 DETERMINATION OF PHENOLIC CONTENT

- Total phenolic content was measured by the Folin-ciocalteu (FC) method.
- Take 1ml of FC reagent and diluted with distilled water (1:10) mixture.
- Take 2 ml of previously diluted FC reagent and mix it with the 1.6ml of sodium carbonate solution (7.5% w/v).
- By adding 0.4 ml of various concentration of different extract or gallic acid.
- It is incubated for at least 2 hours.
- Uv absorbance at 765 nm taken in a UV spectrophotometer by using water as a blank.

4.7 ANTI-MICROBIAL STUDY

In-vitro anti-microbial study was determined for these extracts(Cinnamomum tamala and Acacia nilotica) was checked for the anti-microbial activity against S.aureus as a test organism using disk-diffusion method.

4.7.1 Procedure :

1. Preparation of agar solution :

Firstly sterilize the vessel used for preparation. Add 10 g of nutrient agar into 500 ml of distilled water and keep in a water bath at 50°C and allow solution to boil until agar completely dissolved. Put these agar solution into conical flask which were already sterilize into autoclave at standard condition (15 psi pressure , 15 min at 121°C temperature). After this remove the conical flask and pour hot solution into petri dish.

2. Preparation of nutrient broth :

Nutrient broth is a liquid medium used for cultivation of wide range of organism. It is prepared by weighing 1gm of nutrient broth and dissolved it in 50 ml of water and keep it water bath at 50°C and allow to completely dissolve the nutrient broth. Keep this solution in 500ml conical flask and autoclave it at standard condition (15 psi,15min,15°C).Remove conical flask from autoclave and fill this solution into different test tubes.Then inoculate test microorganism by using inoculating loop to test tubes. Incubate these test tubes for 24 hours using BOD incubator.

3. Preparation of extract solution :

Take 2 test tubes and add 1 extract (50mg) in 1 test tube and add 1 ml distilled water to test-tube and diluted it to get desired concentration.

Extract 1 : Containing C.tamala in 1 test tube and follow above procedure.
Extract 2 : Containing A.nilotica in another test tube by following above procedure.

4. Preparation of agar plate :

Solidified agar plate were streak with inoculating loop containing culture media of microorganism. Then take Whatmann filter paper cut it in circular disc of 6mm diameter. Placed test extract of desired concentration on filter paper. These further placed on the solidified agar plate containing culture and incubate it. This extract diffuses into agar and inhibits the growth of microorganism. By disk-diffusion method antimicrobial activity is measured in terms of zone of inhibition which measured in mm.

5. ANIMAL STUDY

Our objective of the animal study was to evaluate the topical potency of different formulations on wound healing. For animal study the selected animal was wistar species of rats (Approved by IAEC).Following study of animal was based on the incision model on healthy rats. The rats was classified as the following:

TABLE-19

No.	Name of Group	Treatment Type	No. of Animal
1.	No treatment	No Treatment Given	2 Animal
2.	Control	Blank formulation	2 Animal
3.	Group A	Fromulation A	2 Animal
4.	Group B	Formulation B	2 Animal
5.	Group C	Fromulation C	2 Animal

FIGURE-20

5.1 Procedure for animal study:

1. Animal husbandry :

FIGURE-21

All 10 healthy wistar rats used in study were weighed on weighing balance. After weighing based on their average weights they were classified into different groups which were mentioned in the above table. They were placed in the individual polycarbonate cages which contain bedding material as a cardboard under controlled condition of temperature of 25°C and normal 12 hours day and night cycle. Before 7 days of study each animal was fed with commercial rat feed and tap water in a water bottle to settle it with the environment.

2. Inducing wounds on healthy rats and treatment process.

Before wound infliction on the rats they were anaesthetize by Diethyl ether in sufficient quantity in the desiccator. After this immediately the anesthetized rats were shaved with razor and trimmer at both side of dorsal part of back. On shaved skin a 6mm cut was inflicted by using Bard Parker blade (B.P. blade) with sufficient depth in epithelial layer approx 2-3 mm. Wounds created were remained open and right during Day 0 they were treated with different formulations once everyday as grouping showed in table. Thus following this treatment for 8 days and parameters which were observed was:

A. Daily food and water intake of rats during experiment
B. Total inspection of wound after treatments of different formulation
C. Photography of wound in each group

STEP 1
(ANAESTHETIZE)

STEP 2
(SHAVING HAIR)

STEP 3
(CREATE WOUND)

STEP 4
(APPLY FORMULATION)

FIGURE-22

6. RESULTS

6.1 Percentage yield of extracts : The Percentage extractability of different extracts are depicted below;

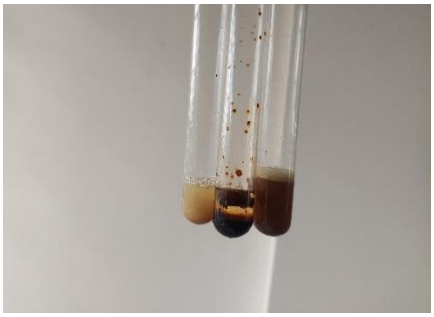
TABLE-20

SR.No.	Name of Plant	Name of Extract	Extractability
1.	*Glycyrrhiza glabra*	Aqueous	12%
2.	*Punica granatum*	Aqueous	16.57%
3.	*Acacia nilotica*	Methanolic	15%
4.	*Moringa oleifera*	Aqueous	12.5%
5.	*Phyllanthus emblica*	Aqueous	8.48%
6.	*Curcuma longa*	Aqueous	10.88%
7.	*Cinnamomum tamala*	Methanolic	10.52%

6.2 Qualitative Phytochemical Screening :

TABLE-21

No.	Name	Alkaloids	Flavonoid	Sterol	Saponin	Tannins	Sugar
1.	**G. glabra**	+	-	-	-	-	+
2.	**Punica granatum**	+	+	+	+	+	+
3.	**Acacia nilotica**	+	+	+	-	-	+
4.	**Moringa oleifera**	-	+	+	+	-	+
5.	**P. emblica**	+	+	+	-	-	-
6.	**Curcuma longa**	+	+	-	+	-	+
7.	**Cinnamomum t.**	-	+	+	+	-	-



6.3 Evaluation parameter :

FORMULATION A

TABLE-22

SR.No.	Parameters	Results
1.	Physical Characteristics	Color- Creamy yellowish Odour- Sapodilla fruit like aroma
2.	Washability	Washable
3.	Stability	No separation observed
4.	pH of formulation	7
5.	Skin Irritancy	No irritation

FORMULATION B

TABLE-23

SR.No.	Parameters	Results
1.	Physical Characteristics	Color- Light brown Odour- Sapodilla fruit like aroma
2.	Washability	Washable
3.	Stability	No separation observed
4.	pH of formulation	6
5.	Skin Irritancy	No irritation

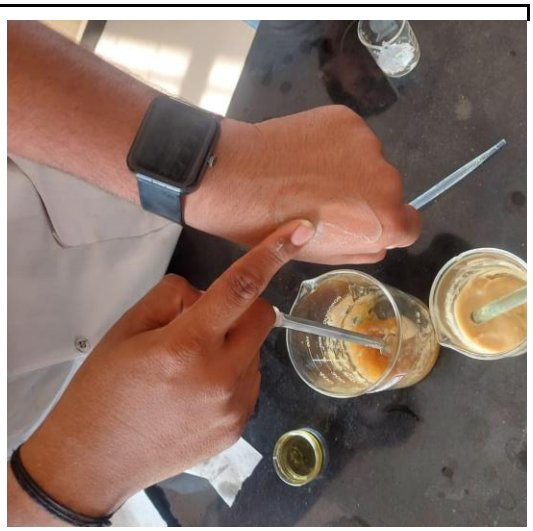
FORMULATION C

TABLE-24

SR.No.	Parameters	Results
1.	Physical Characteristics	Color- Light brown Odour- Sapodilla fruit like aroma
2.	Washability	Washable
3.	Stability	No separation observed
4.	pH of formulation	7
5.	Skin Irritancy	No irritation

TABLE-25

SR.No.	Parameters	Observation
1.	Physical Characteristics	
2.	Washability	
3.	Stability	 AFTER 3 DAYS 

4.	pH of formulation	
5.	Viscosity	
6.	Skin Irritancy	

6.4 Determination of Phenolic Content :

TABLE-26

Name of Extract	Concentration (10ug/ml)	Concentration (50ug/ml)
Glycyrrhiza glabra	0.663	0.486
Cinnamomum cassia	0.065	0.148

6.4.1 Calibration curve of gallic acid :

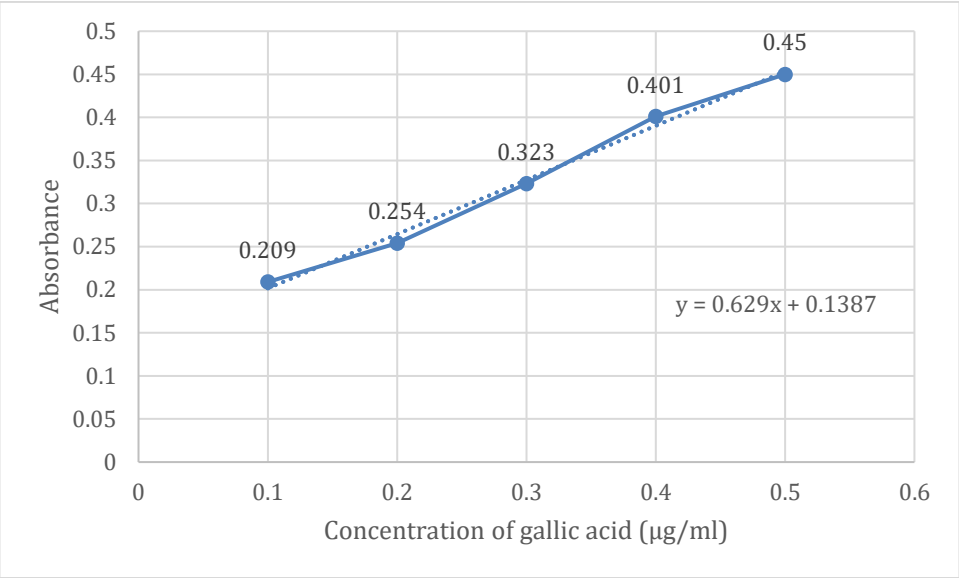


FIGURE-24

6.5 Anti-microbial Study :

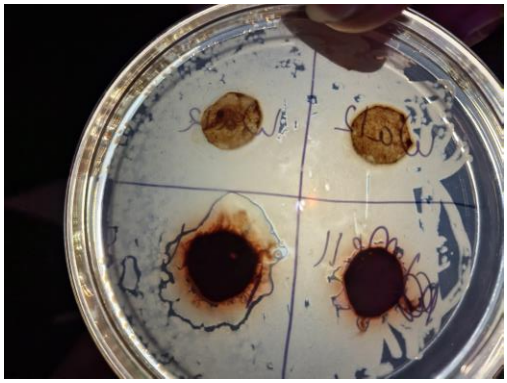
Zone of inhibition

S.aureus was used in the In-vitro Antibacterial activity

TABLE -27

Sr.no.	Name of Extract	Zoi (mm) Methanolic Extract
1.	*Acacia nilotica*	15.5mm
2.	*Cinammomum cassia*	15mm
3.	*Moringa oleifera*	ND

FIGURE 25

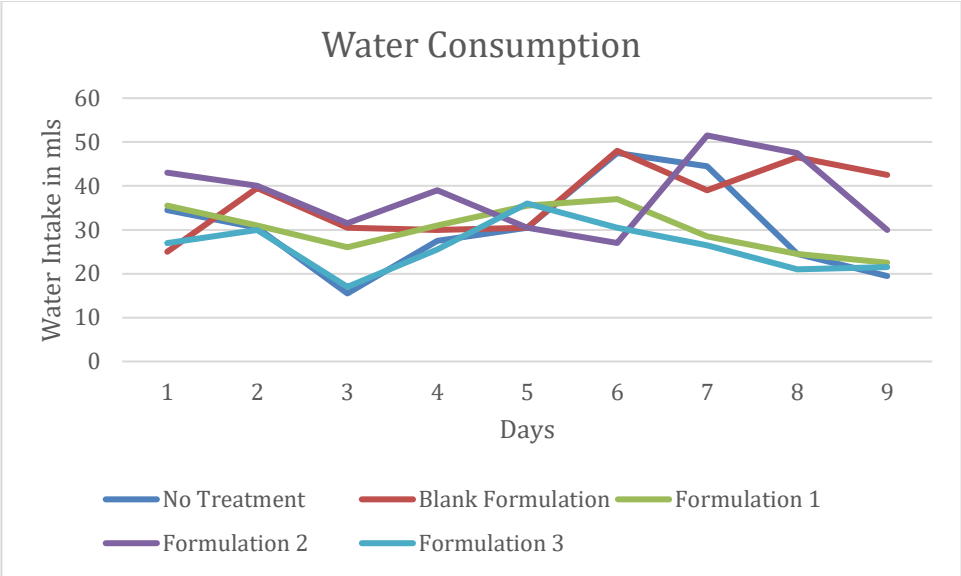


Invitro Antibacterial activity

6.6.1 Wound healing activity :

There was substantial wound healing observed in animals which are treated with formulations. Various scoring is given to different groups according to their healing pattern and scar formation. Thus it shows rapid wound healing activity in the treatment group on day 9 as compared to the blank formulation and no treatment group. These may occur due to presence of different wound healing ingredients present in the formulation . On the basis of study conducted on rats, upon treatment with different formulations has significant wound healing activity thus it can be concluded that these polyherbal formulations have noteworthy wound healing properties. Other observations made were on the feed and water consumption of rats.

1. Feed and Water Consumption of Wistar albino rats during experiment



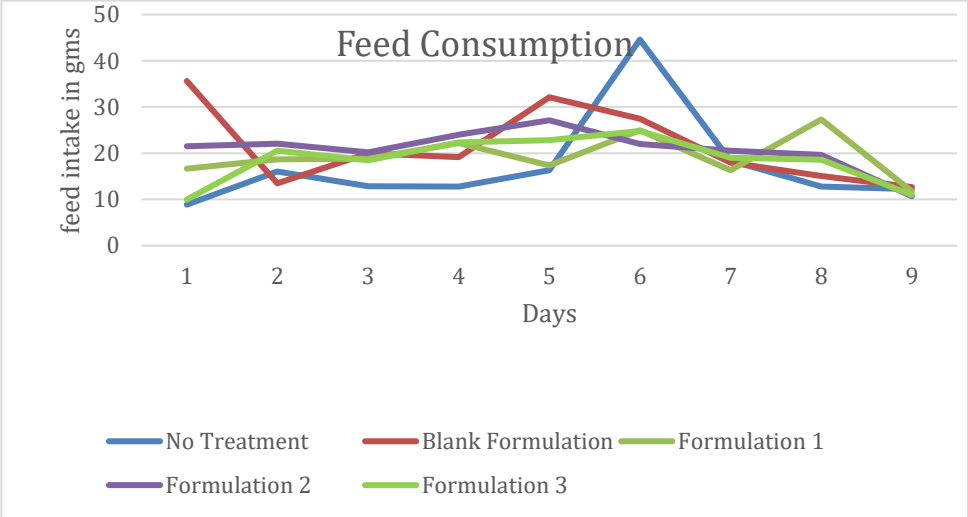


FIGURE-26

6.6.2 GROSS OBSERVATION

TABLE-28

Gross observation of wounds of various treatments at different time intervals

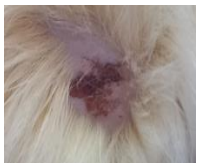
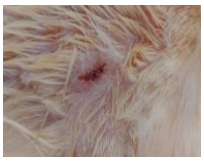
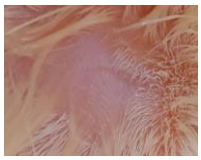
Day	Type of treatments	Suppuration	Scar Formation	Shape, irregularity and colour of the wound	Epithelization
3	No treatment	Not observed	-	Irregular, reddish	-
	Blank formulation	Not observed	+	Irregular, reddish	-
	Formulation-1	Not observed	++	Irregular, reddish	+
	Formulation-2	Observed	++	Irregular, reddish	+
	Formulation-3	No observed	++	Irregular, reddish	++
6	No treatment	Not observed	+	Irregular, reddish	-
	Blank formulation	Not observed	+	Irregular, reddish	+
	Formulation-1	Not observed	++	Round, contracted	++
	Formulation-2	Not observed	++	Round, brownish	++
	Formulation-3	Not observed	++	Contracted,brown	++
9	No treatment	Not observed	+	Contracted,reddis	+
	Blank formulation	Not observed	++	Contracted, white	++
	Formulation-1	Not observed	++	Contracted, white	+++
	Formulation-2	Not observed	+++	Contracted, white	++
	Formulation-3	Not observed	++	Contracted, white	+++

Scar Formation and Epithelization grading:

(- = None; + = Mild; ++ = Moderate; +++ = High; ++++ = Very high)

6.6.3 Photographs of the observations of Incision wound on days 0, 3, 6, 9

TABLE-29

Day	No treatment	Blank formulation	Formulation-1	Formulation-2	Formulation-3
0					
3					
6					
9					

7. DISCUSSION

Aleksandra Shedoeva et al. (2019) studied that Indigenous and traditional medicines make extensive use of natural products and derivatives of natural products and provide more than half of all medicines consumed today throughout the world. Recognising the important role traditional medicine continues to play, we have undertaken an extensive survey of literature reporting the use of medical plants and plant-based products for cutaneous wounds. We describe the active ingredients, bioactivities, clinical uses, formulations, methods of preparation, and clinical value of 36 medical plant species. Several species stand out, including *Centella asiatica*, *Curcuma longa*, and *Paeonia suffruticosa*, which are popular wound healing products used by several cultures and ethnic groups. The popularity and evidence of continued use clearly indicates that there are still lessons to be learned from traditional practices.

Abubakar Amali Muhammad et al. (2013) Researched that The results of our study demonstrated that aqueous fraction of *M. oleifera* significantly enhanced proliferation and viability as well as migration of human dermal fibroblast (HDF) cells compared to the untreated control and other fractions. The HPLC and LC-MS/MS studies revealed kaempferol and quercetin compounds in the crude methanolic extract and a major bioactive compound Vicenin-2 was identified in the bioactive aqueous fraction which was confirmed with standard Vicenin-2 using HPLC and UV spectroscopic methods. These findings suggest that bioactive fraction of M. oleifera containing Vicenin-2 compound may enhance faster wound healing in vitro.

Kotade Kiran et al. (2008) studied that The seeds of *S. indicum* L (Pedaliaceae) are used traditionally in the folklore for the treatment of various kinds of wounds. The present study was undertaken to verify the effect of S. indicum seeds and its oil on experimentally induced excision wound, incision wound, burn wound and dead space wound models in rats. Aloe vera was used as standard wound healing agent. A formulation of seeds and oil was prepared in carbopol at 2.5% and 5% concentrations and applied to the wounds. In the excision and burn wound models, the so treated animals showed significant reduction in period of epithelization and wound contraction (50%). In the incision wound model a significant increase in the breaking strength was observed.

Recently many researchers had proved that the *M.olifera, Glyrrzhia glabra* have antioxidant, anti microbial and wound healing properties having various bioactive compounds . Above discussion meet the criteria for our research work on polyherbal drug on wound healing

8. CONCLUSION

The phytochemical studied was done for our study to determined the presence of bioactive components in the various extracts of herbal plants.Upon Nanoemulgel formulation prepared by using *M.olifera* (aqueous extract), *Glycyrrhiza* glabra (aqueous extract) and *Seasamum indicum* oil showed the wound healing properties . Further we conducted various study on the nanoemulgel formulations of different plants extract for various activities of formulations. It shows the good antimicrobial, antioxidant activity of extract that are important for the wound healing.The experiment was conducted on rats suggested that nanoemulgel formulation has significant wound healing activities. From the observations we can conclude that polyherbal nanoemulgel formulations of plant extracts can be suitable for the good wound activity. It requires further evaluation of nanoemulgel formulation for safety and efficacy on human and clinical use.

9. BIBLIOGRAPHY

[1]. E. A. Davis Company 1915 Arch Street Philadelphia, PA 19103

[2]. Nagle SM, Stevens KA, Wilbraham SC. Wound Assessment. [Updated 2023 Jun 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482198/

[3]. Herman TF, Bordoni B. Wound Classification. [Updated 2023 Aug 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554456/

[4]. Gonzalez AC, Costa TF, Andrade ZA, Medrado AR. Wound healing - A literature review. An Bras Dermatol. 2016 Sep-Oct;91(5):614-620. doi: 10.1590/abd1806-4841.20164741. PMID: 27828635; PMCID: PMC5087220.

[5]. Guo, S., & Dipietro, L. A. (2010). Factors affecting wound healing. Journal of dental research, 89(3), 219–229. https://doi.org/10.1177/0022034509359125

[6]. Wallace HA, Basehore BM, Zito PM. Wound Healing Phases. [Updated 2023 Jun 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470443/

[7]. Doaa H. Assar, Nagwan Elhabashi, Abd-Allah A. Mokhbatly, Amany E. Ragab, Zizy I. Elbialy, Sally A. Rizk, Aishah E. Albalawi, Norah A. Althobaiti, Soad Al Jaouni, Ayman Atiba, Wound healing potential of licorice extract in rat model: Antioxidants, histopathological, immunohistochemical and gene expression evidences, Biomedicine & Pharmacotherapy, Volume 143, 2021,112151, ISSN 0753-3322, https://doi.org/10.1016/j.biopha.2021.112151.

[8]. Luqman Jameel Rather, Shahid-ul-Islam, Faqeer Mohammad, Acacia nilotica (L.): A review of its traditional uses, phytochemistry, and pharmacology, Sustainable Chemistry and Pharmacy, Volume 2,2015,Pages 12-30,ISSN 2352-5541,https://doi.org/10.1016/j.scp.2015.08.002.

[9]. Fontoura, Guilherme & Rodrigues, Liliane & Reis, Aramys & Berretta, Andresa & Maciel, Márcia. (2022). Therapeutic potential of Punica granatum in wound healing: An overview. Revista Colombiana de Ciencias Químico-Farmacéuticas. 51. 10.15446/rcciquifa.v51n2.99252.

[10]. Al-Ghanayem, A. A., Alhussaini, M. S., Asad, M., & Joseph, B. (2022). Moringa oleifera Leaf Extract Promotes Healing of Infected Wounds in Diabetic Rats: Evidence of Antimicrobial, Antioxidant and Proliferative Properties. Pharmaceuticals (Basel, Switzerland), 15(5), 528. https://doi.org/10.3390/ph15050528

[11]. Canatar, S. Özdaş, G. Baydemir Peşint, Phyllanthus emblica-Loaded Cryogels for Improved Wound Care: Characterization and In Vitro Studies. Macromol. Mater. Eng. 2024, 309, 2300404. https://doi.org/10.1002/mame.202300404

[12]. Kumari, A., Raina, N., Wahi, A., Goh, K. W., Sharma, P., Nagpal, R., Jain, A., Ming, L. C., & Gupta, M. (2022). Wound-Healing Effects of

Curcumin and Its Nanoformulations: A Comprehensive Review. Pharmaceutics, 14(11), 2288. https://doi.org/10.3390/pharmaceutics14112288

[13]. Oketch-Rabah, H. A., Marles, R. J., & Brinckmann, J. A. (2018). Cinnamon and Cassia Nomenclature Confusion: A Challenge to the Applicability of Clinical Data. Clinical pharmacology and therapeutics, 104(3), 435–445. https://doi.org/10.1002/cpt.1162

[14]. Gonzalez, A. C., Costa, T. F., Andrade, Z. A., & Medrado, A. R. (2016). Wound healing - A literature review. Anais brasileiros de dermatologia, 91(5), 614–620. https://doi.org/10.1590/abd1806-4841.20164741

[15]. Velnar, T., Bailey, T., & Smrkolj, V. (2009). The wound healing process: an overview of the cellular and molecular mechanisms. The Journal of international medical research, 37(5), 1528–1542. https://doi.org/10.1177/147323000903700531

[16]. Bleacher, J. C., Adolph, V. R., Dillon, P. W., & Krummel, T. M. (1993). Fetal tissue repair and wound healing. Dermatologic clinics, 11(4), 677–683.

[17]. Martin P. (1997). Wound healing--aiming for perfect skin regeneration. Science (New York, N.Y.), 276(5309), 75–81. https://doi.org/10.1126/science.276.5309.75

[18]. An, J., Tsopmejio, I. S. N., Wang, Z., & Li, W. (2023). Review on Extraction, Modification, and Synthesis of Natural Peptides and Their Beneficial Effects on Skin. Molecules (Basel, Switzerland), 28(2), 908. https://doi.org/10.3390/molecules28020908

[19]. Theng, Madhuri & Sitaphale, G. & Biyani, K.. (2017). EVALUATION OF WOUND HEALING ACTIVITY OF POLYHERBAL FORMULATION. International Journal of Current Pharmaceutical Research. 9. 12. 10.22159/ijcpr.2017v9i6.23420.

[20]. Siriwattanasatorn, M., Itharat, A., Thongdeeying, P., & Ooraikul, B. (2020). In Vitro Wound Healing Activities of Three Most Commonly Used Thai Medicinal Plants and Their Three Markers. Evidence-based complementary and alternative medicine : eCAM, 2020, 6795383. https://doi.org/10.1155/2020/6795383

[21]. Assar, D. H., Elhabashi, N., Mokhbatly, A. A., Ragab, A. E., Elbialy, Z. I., Rizk, S. A., Albalawi, A. E., Althobaiti, N. A., Al Jaouni, S., & Atiba, A. (2021). Wound healing potential of licorice extract in rat model: Antioxidants, histopathological, immunohistochemical and gene expression evidences. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 143, 112151. https://doi.org/10.1016/j.biopha.2021.112151

[22]. Akamatsu, H., Komura, J., Asada, Y., & Niwa, Y. (1991). Mechanism of anti-inflammatory action of glycyrrhizin: effect on neutrophil functions including reactive oxygen species generation. Planta medica, 57(2), 119–121. https://doi.org/10.1055/s-2006-960045

[23]. Nayak, S. B., Rodrigues, V., Maharaj, S., & Bhogadi, V. S. (2013). Wound healing activity of the fruit skin of Punica granatum. Journal of medicinal food, 16(9), 857–861. https://doi.org/10.1089/jmf.2012.0229

[24]. Hayouni, E. A., Miled, K., Boubaker, S., Bellasfar, Z., Abedrabba, M., Iwaski, H., Oku, H., Matsui, T., Limam, F., & Hamdi, M. (2011). Hydroalcoholic extract based-ointment from Punica granatum L. peels with enhanced in vivo healing potential on dermal wounds. Phytomedicine : international journal of phytotherapy and phytopharmacology, 18(11), 976–984. https://doi.org/10.1016/j.phymed.2011.02.011

[25]. Cuccioloni, M., Mozzicafreddo, M., Sparapani, L., Spina, M., Eleuteri, A. M., Fioretti, E., & Angeletti, M. (2009). Pomegranate fruit components modulate human thrombin. Fitoterapia, 80(5), 301–305. https://doi.org/10.1016/j.fitote.2009.03.009

[26]. Saeedi, Rushda & Sultana, Arshiya & Raheman, Khaleeq. (2020). MEDICINAL PROPERTIES OF DIFFERENT PARTS OF ACACIA NILOTICA LINN (BABUL), ITS PHYTOCONSTITUENTS AND DIVERSE PHARMACOLOGICAL ACTIVITIES. International Journal of Pharmacy and Pharmaceutical Sciences. 8-14. 10.22159/ijpps.2020v12i2.35672.

[27]. Riasat, N., Jadoon, M., Akhtar, N., Kiani, M. N., Fatima, H., Abdel-Maksoud, M. A., Ali, S. M., Alfuraydi, A. A., Dar, M. J., & Ul Haq, I. (2024). Polyphenolic characterization and biological assessment of Acacia nilotica (L.) wild. Ex delilie: An In vitro and In vivo appraisal of wound healing potential. Journal of ethnopharmacology, 325, 117842. https://doi.org/10.1016/j.jep.2024.117842

[28]. Mohammad Shafie, Nurmaziah & Shah, Raja & Krishnan, Puspawathy & Haleem, Noorashikin & Tan, Terence. (2022). Scoping Review: Evaluation of Moringa oleifera (Lam.) for Potential Wound Healing in In Vivo Studies. Molecules. 27. 5541. 10.3390/molecules27175541.

[29]. Ali, A., Garg, P., Goyal, R., Kaur, G., Li, X., Negi, P., Valis, M., Kuca, K., & Kulshrestha, S. (2020). A Novel Herbal Hydrogel Formulation of Moringa oleifera for Wound Healing. Plants (Basel, Switzerland), 10(1), 25. https://doi.org/10.3390/plants10010025

[30]. Al-Ghanayem, A. A., Alhussaini, M. S., Asad, M., & Joseph, B. (2022). Effect of Moringa oleifera Leaf Extract on Excision Wound Infections in Rats: Antioxidant, Antimicrobial, and Gene Expression Analysis. Molecules (Basel, Switzerland), 27(14), 4481. https://doi.org/10.3390/molecules27144481

[31]. Chularojmontri, L., Suwatronnakorn, M., & Wattanapitayakul, S. K. (2013). Phyllanthus emblica L. Enhances Human Umbilical Vein Endothelial Wound Healing and Sprouting. Evidence-based complementary and alternative medicine : eCAM, 2013, 720728. https://doi.org/10.1155/2013/720728

[32]. CANATAR, İpek & ÖZDAŞ, SİBEL & Baydemir Peşint, Gözde. (2024). Phyllanthus emblica Loaded Cryogels for Improved Wound Care: Characterization and In Vitro Studies. 10.20944/preprints202311.1288.v2.

[33]. Tejada, S., Manayi, A., Daglia, M., Nabavi, S. F., Sureda, A., Hajheydari, Z., Gortzi, O., Pazoki-Toroudi, H., & Nabavi, S. M. (2016). Wound Healing Effect of Curcumin: A Review. Current pharmaceutical biotechnology, Advance online publication.

[34]. Akbik, D., Ghadiri, M., Chrzanowski, W., & Rohanizadeh, R. (2014). Curcumin as a wound healing agent. Life sciences, 116(1), 1–7. https://doi.org/10.1016/j.lfs.2014.08.016

[35]. Mohanty, C., & Sahoo, S. K. (2017). Curcumin and its topical formulations for wound healing applications. Drug discovery today, 22(10), 1582–1592. https://doi.org/10.1016/j.drudis.2017.07.001

[36]. Tiwari, Dr & Talreja, Shreya. (2020). Importance of Cinnamomum Tamala in the Treatment of Various Diseases. Pharmacognosy Journal. 12. 1792-1796. 10.5530/pj.2020.12.241.

[37]. Aithal, G. C., Narayan, R., & Nayak, U. Y. (2020). Nanoemulgel: A Promising Phase in Drug Delivery. Current pharmaceutical design, 26(2), 279–291. https://doi.org/10.2174/1381612826666191226100241

[38]. Anand, K., Ray, S., Rahman, M., Shaharyar, A., Bhowmik, R., Bera, R., & Karmakar, S. (2019). Nano-emulgel: Emerging as a Smarter Topical Lipidic Emulsion-based Nanocarrier for Skin Healthcare Applications. Recent patents on anti-infective drug discovery, 14(1), 16–35. https://doi.org/10.2174/1574891X14666190717111531

[39]. Choudhury, H., Gorain, B., Pandey, M., Chatterjee, L. A., Sengupta, P., Das, A., Molugulu, N., & Kesharwani, P. (2017). Recent Update on Nanoemulgel as Topical Drug Delivery System. Journal of pharmaceutical sciences, 106(7), 1736–1751. https://doi.org/10.1016/j.xphs.2017.03.042

[40]. Alhasso, B., Ghori, M. U., & Conway, B. R. (2023). Development of a Nanoemulgel for the Topical Application of Mupirocin. Pharmaceutics, 15(10), 2387. https://doi.org/10.3390/pharmaceutics15102387

[41]. Alhasso, B., Ghori, M. U., & Conway, B. R. (2023). Development of Nanoemulsions for Topical Application of Mupirocin. Pharmaceutics, 15(2), 378. https://doi.org/10.3390/pharmaceutics15020378

[42]. Shedoeva, A., Leavesley, D., Upton, Z., & Fan, C. (2019). Wound Healing and the Use of Medicinal Plants. Evidence-based complementary and alternative medicine : eCAM, 2019, 2684108. https://doi.org/10.1155/2019/2684108

[43]. Muhammad, A. A., Pauzi, N. A., Arulselvan, P., Abas, F., & Fakurazi, S. (2013). In vitro wound healing potential and identification of bioactive compounds from Moringa oleifera Lam. BioMed research international, 2013, 974580. https://doi.org/10.1155/2013/974580

[44]. Kiran, K., & Asad, M. (2008). Wound healing activity of Sesamum indicum L seed and oil in rats. Indian journal of experimental biology, 46(11), 777–782.

CERTIFICATE

This is to certify that, below mentioned medicinal plant samples submitted by Ms. Roshni Balsara, Mr. Smit Jyani and Mr. Yash Patel are authenticated and voucher specimens have been submitted to the Department of Pharmacology, Faculty of Pharmacy, Dharmsinh Desai University, Nadiad (Gujarat) India.

Sr. No.	Botanical Name	Family	Vernacular Name	Part of plant used	Voucher Specimen Number
1	*Glycyrrhiza glabra*	Fabaceae	Jethimadh	Bark	FOP/PCOL/08/2023-24
2	*Punica granatum*	Punicaceae	Dadam chhal	Chhal	FOP/PCOL/09/2023-24
3	*Acacia nilotica*	Mimosaceae	Baval	Stembark	FOP/PCOL/10/2023-24
4	*Moringa oleifera*	Moringaceae	Saragavo	Leaves	FOP/PCOL/11/2023-24
5	*Phyllanthus emblica*	Euphorbiaceae	Amla	Fruit	FOP/PCOL/12/2023-24
6	*Curcuma longa*	Zingiberaceae	Haldi	Rhizomes	FOP/PCOL/13/2023-24
7	*Cinnamomum tamala*	Lauroceae	Dal chini	Bark	FOP/PCOL/14/2023-24

Prof. Punitkumar R. Bhatt
Assistant Professor
Department of Pharmacology
Faculty of Pharmacy
Dharmsinh Desai University, (Nadiad) Gujarat

FACULTY OF PHARMACY
Dharmsinh Desai University
College Road, **Nadiad** - 387001, Gujarat, India.
Ph. : +91 0268 2520502 Fax : +91 0268 2520501 Website : www.ddu.ac.in

NAAC ACCREDITED

CERTIFICATE

This is to certify that the project proposal no **DDU/FOP/07/2023** entitled **"Development and evaluation of novel wound healing herbal formulation for veterinary use"** submitted by **Nishit D. Patel** has been approved/recommended by the IAEC of **Faculty of Pharmacy, Dharmsinh Desai University, Nadiad (Gujarat)** India in its meeting held on **30/10/2023** and **45 Wistar albino Rats** have been sanctioned under this proposal for a duration of next **12** months.

Authorized by	Name	Signature	Date
Chairman	Dr. Tejal G Soni		30/10/23
Member Secretary	Dr.Vashisth P Bhavsar	V.P.Bhavsar	30/10/23
Main Nominee of CPCSEA	Dr. Varsha J. Galani		30/10/23