FORMULATION OF HERBAL GEL FROM *CALENDULA OFFICINALIS* PLANT LEAF EXTRACT AND EVALUATE THE WOUND HEALING ACTIVITY

A Thesis Report Submitted To

ASSAM SCIENCE AND TECHNOLOGY UNIVERSITY, GUWAHATI, ASSAM

In the partial fulfillment of the requirement for award of degree of Master of Pharmacy (M.Pharm)

Submitted By

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I wish his all success in life….

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DECLARATION

I hereby declare that the thesis entitled **"Formulation of herbal gel from *Calendula officinalis* plant leaf extract and evaluate the wound healing activity"** a bonified and genuine research work carried out by me under the guidance and supervision of **Dr. Tapash Chakraborty,** Assistant professor, Department of Pharmaceutics, **Girijananda Chowdhury Institute of Pharmaceutical Science**, affiliated to **Assam Science and Technology University**, Guwahati, Assam. The work embodied in the thesis is original and has not been submitted in part or full for the award of degree, diploma, associate ship or fellowship to any other University or Institute.

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To Whom It May Concern

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Abstract:

Calendula officinalis belonging to the family Asteraceae, a commonly available medicinal plant, that has been traditionally found to have activity against several diseases counting skin infection, wounds, burns, and ulcer. Since the Calendula officinalis contains secondary metabolites like terpenoids, flavonoids, and carotenoids, which may have the potential activity in wound healing. It also possesses anti-cancer, anti-inflammatory, hepatoprotective, spasmolytic, and spasmogenic properties. Wound healing involves a composite series of interactions amid diverse cell types, cytokine mediators, and the extracellular matrix. The cascade of wound-healing actions starts with homeostasis, inflammatory cell conscription cell proliferation, and restoration. To compile existing knowledge on Calendula officinalis with literature survey and highlight its wound-healing function, we focused at its identification, phytochemistry, pre-formulation study, compatibility study, formulation of gel with Carbopol 934 and evaluation of gels. Conclusively, further in-vitro antimicrobial and in-vivo wound healing studies is necessary to determine whether Calendula has a potential role in standard wound management.

Keyword: Calendula officinalis, Wound healing, Burns, Ulcers, Flavonoids.

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Jakirul Islam

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INTRODUCTION

1. Introduction

1.1 Wound Healing

Wound healing can be simply defined as healing of the skin layers. The progression of wound healing starts instantly after any injury or cut to the skin's epidermal layer. Wounds exist as an important global growing medical problem requiring dedicated care services. Wounds have various pathophysiology, with sorting grouped according to periods, which is divided into acute, chronic, and burns wound Dermal wound healing is an important physiological process comprising of the association of many cell strains and their products. Attempts to restore the lesion tempted by local aggression start very early on in the inflammatory stage. Wound healing involves a composite series of interactions amid diverse cell types, cytokine mediators, and the extracellular matrix. The cascade of wound-healing actions starts with homeostasis, inflammatory cell conscription cell proliferation, and restoration. Acute wounds pass all stages and attain complete repossession, based on injury types and lesion size, typically in between 5 to 10 days. Chronic wounds need longer times than expected to heal, typically more than 4 weeks, due to failure to resolve one or more healing phases. Burn wounds are accounted as a separate group as they differ in their pathophysiology and natural course from other cutaneous wounds. [1-3]

1.1.1 Classification of Wounds

Wounds can be classified according to various criteria. Time is an important factor in injury management and wound repair. Thus, wounds can be clinically categorized as acute and chronic according to their time frame of healing.

1.1.1.1 Acute Wounds

Wounds that repair themselves and that proceed normally by following a timely and orderly healing pathway, with the result of both functional and anatomical restoration, are classified as acute wounds. The time course of healing usually ranges from 5 to 10 days, or within 30 days. Acute wounds can be acquired as a result of traumatic loss of tissue or a surgical procedure. For example, an operation to remove a soft tissue tumor located in the skin and underlying parenchyma can sometimes result in a large albeit noncontaminated wound that cannot be healed by primary intention, due to the large defect

within the tissue. Traumatic wounds are also frequently encountered. They may involve only the soft tissue or they might be associated with bone fractures. These combined injuries have been classified by the classification system of the AO Foundation (Arbeitsgemeinschaft fur Osteosynthesefragen/Association for the Study of Internal Fixation), which is one of the most comprehensive and widely used. Included within this classification system are closed and open fractures with the assessment of skin, muscle, tendon, and neurovascular injuries. A benefit of the AO Foundation's classification system is that the extent of damage to muscles and tendons is taken into account, as it determines the prognosis of the injured limb. [4-7]

1.1.1.2 Chronic Wound

Chronic wounds are those that fail to progress through the normal stages of healing and cannot be repaired in an orderly and timely manner. The healing process is incomplete and disturbed by various factors, which prolong one or more stages in the phases of hemostasis, inflammation, proliferation, or remodeling. These factors include infection, tissue hypoxia, necrosis, exudate, and excess levels of inflammatory cytokines. A continuous state of inflammation in the wound creates a cascade of tissue responses that together perpetuate a non-healing state. Because the healing then proceeds in an uncoordinated manner, functional and anatomical outcomes are poor and these wounds frequently relapse. Chronic wounds may result from various causes, including naturopathic, pressure, arterial and venous insufficiency, burns, and vasculitis. [4,8,9,10]

1.1.1.3 Complicated Wounds

A complicated wound is a special entity and is defined as a combination of an infection and a tissue defect. Infection poses a constant threat to the wound. The cause of the defect, in contrast, evolves due to the traumatic or post-infectious etiology, or a wide tissue resection (e.g. in tumor management). Every wound is contaminated irrespective of the cause, size, location, and management. Whether or not a manifest infection develops depends on the virulence, number, and type of microorganisms, as well as on the local blood supply and the patient's inherent resistance. Typical characteristics of infection are the five signs and symptoms that have been well documented: redness, heat, pain, edema,

and loss or limited function in the affected part. The frequency of wound infections depends on the type of surgical technique and the location of the wound. Other criteria taken into account during wound classification include etiology, degree of contamination, morphological characteristics, and communication with hollow or solid organs. etiology classifies wounds according to the trigger factor into contusions, abrasions, avulsions, lacerations, cuts, stab wounds, crush wounds, shot wounds, and burns. According to the degree of contamination, wounds are classified into three groups as follows: (i) aseptic wounds (bone and joint operations); (ii) contaminated wounds (abdominal and lung operations); and (iii) septic wounds (abscesses, bowel operations, etc.). Wounds may also be referred to as closed, where the underlying tissue has been traumatized but the skin has not been severed, or as open, where the skin layer has been damaged with the underlying tissue exposed. [4,9,10,12]

1.1.2 The Wound Healing Process

Wounding and wound healing take place in all tissues and organs of the body. Many of these repair processes are common to all tissues. Although the process of healing is continuous, it is arbitrarily divided into different phases to aid understanding of the physiological processes that are taking place in the wound and surrounding tissue. Healing is a complex process involving coordinated interactions between diverse immunological and biological systems. It involves a

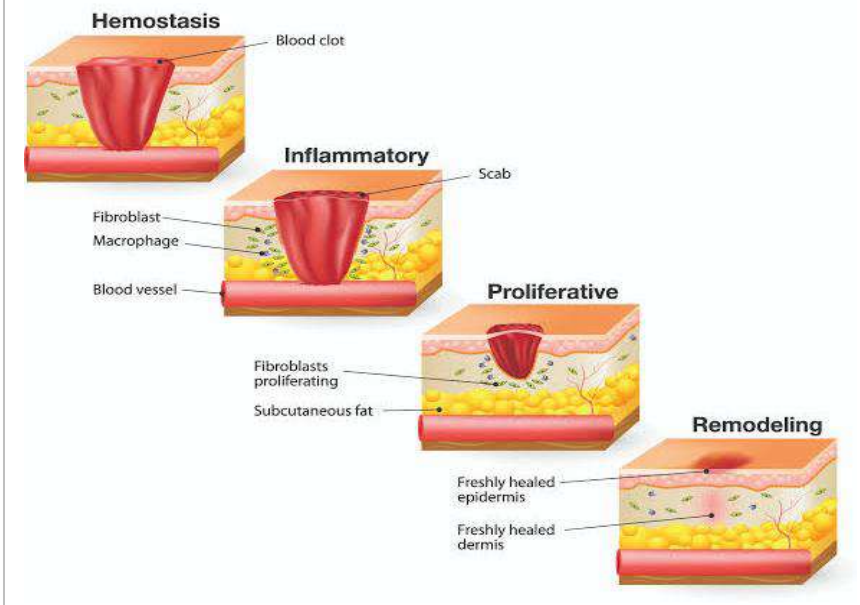

Fig 1: Phases of Wound Healing

cascade of carefully and precisely regulated steps and events that correlate with the appearance of various cell types in the wound bed during distinct phases of the healing process. Separate parts of a wound may be at different stages of healing at any one time. Timing and interactions between the components taking part in the wound healing process differ for acute and chronic wounds, although the main phases remain the same. The various processes of acute tissue repair, which are triggered by tissue injury, may be united into a sequence of four time-dependent phases: (i) coagulation and hemostasis, beginning immediately after injury; (ii) inflammation, which begins shortly thereafter; (iii) proliferation, which starts within days of the injury and encompasses the major healing processes; and (iv) wound remodeling, in which scar tissue formation takes place, and which may last up to a year or more. [4,11,13]

1.1.2.1 Coagulation and Hemostasis Phase

Immediately after injury, coagulation and hemostasis take place in the wound. The principal aim of these mechanisms is to prevent exsanguination. It is a way to protect the vascular system, keeping it intact so that the function of the vital organs remains unharmed despite the injury. A second aim is a long-term one, which is to provide a matrix for invading cells that are needed in the later phases of healing. A dynamic balance between endothelial cells, thrombocytes, coagulation, and fibrinolysis regulates hemostasis and determines the amount of fibrin deposited at the wound site, thereby influencing the progress of the reparative processes. Noxious insult causes microvascular injury and extravasation of blood into the wound. Owing to the neuronal reflex mechanism, injured vessels constrict rapidly due to the contraction of vascular smooth muscle cells in the circular muscle layer. The contraction is strong enough to prevent bleeding from an arteriole with a diameter of 0.5 cm. The process is, however, only effective in transversally interrupted vessels and may cause a complete cessation of blood leakage. In contrast, in longitudinally severed arterioles it increases the gap. Reflex vasoconstriction can temporarily reduce or even stop the amount of bleeding. The vascular smooth muscle tone is, however, only useful for a few minutes until hypoxia and acidosis in the wound wall cause their passive relaxation, and bleeding resumes. Were it not for the formation of an insoluble fibrin plug, the hemostatic mechanisms alone would be ineffective over the

longer term. Together with hemostatic events, the coagulation cascade is activated through extrinsic and intrinsic pathways, leading to platelet aggregation and clot formation to limit blood loss. As blood spills into the site of injury, the blood components and platelets come in contact with exposed collagen and other extracellular matrix components. This contact triggers the release of clotting factors from the platelets and the formation of a blood clot, composed of fibronectin, fibrin, vitronectin, and thrombospondin. The blood clot and platelets trapped within it are not only important for hemostasis, as the clot also provides a provisional matrix for cell migration in the subsequent phases of the hemostatic and inflammatory phases. The cytoplasm of platelets contains α-granules filled with growth factors and cytokines, such functions in the immediate inflammatory response. as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor, and insulin-like growth factors. These molecules act as promoters in the wound healing cascade by activating and attracting neutrophils and, later, macrophages, endothelial cells, and fibroblasts. Platelets also contain vasoactive amines, such as serotonin, that are stored in dense bodies and cause vasodilation and increased vascular permeability, leading to fluid extravasation in the tissue that results in edema which, in turn, potentiates itself during the following inflammatory phase. Eicosanoids and other products of arachidonic acid metabolism are released after injury to cell membranes and have potent biological functions in the immediate inflammatory response. [4,5,14,15]

1.1.2.2 Inflammatory Phase

The humoral and cellular inflammatory phase follows next, intending to establish an immune barrier against invading micro-organisms. It is divided into two separate phases, an early inflammatory phase, and a late inflammatory phase. Starting during the late phase of coagulation and shortly thereafter, the early inflammatory response has many functions. It activates the complement cascade and initiates molecular events, leading to infiltration of the wound site by neutrophils, whose main function is to prevent infection. The neutrophils start with the critical task of phagocytosis to destroy and remove bacteria, foreign particles, and damaged tissue. Phagocytotic activity is crucial for the subsequent processes because acute wounds that have a bacterial imbalance will not heal. The neutrophils begin to be attracted to the wound site within 24-36 h of injury by various

chemo-attractive agents, including TGF-β, complement components such as C3a and C5a, and formyl methionyl peptides produced by bacteria and platelet products.3 Due to alterations in the regulation of surface adhesion molecules, neutrophils become sticky and, through a process of margination, begin to adhere to the endothelial cells in the post-capillary venules surrounding the wound. Then, the neutrophils roll along the surface of the endothelium being pushed forward by the blood flow. These adhesions and rolling mechanisms are mediated by selectin-dependent interactions and are classified as weak attachments. Neutrophil activity gradually changes within a few days of wounding, once all the contaminating bacteria have been removed. Upon completing the task, the neutrophils must be eliminated from the wound before progression to the next phase of healing. Redundant cells are disposed of by extrusion to the wound surface as slough and by apoptosis, allowing elimination of the entire neutrophil population without tissue damage or potentiating the inflammatory response.16,26 The cell remnants and apoptotic bodies are then phagocytosed by macrophages.

At the late inflammatory phase, 48-72 h after injury, macrophages appear in the wound and continue the process of phagocytosis. These cells are originally blood monocytes that undergo phenotypic changes on arrival into the wound to become tissue macrophages. Attracted to the wound site by a myriad of chemo-attractive agents, including clotting factors, complement components, cytokines such as PDGF, TGF-β, leukotriene B4, and platelet factor IV, as well as elastin and collagen breakdown products, macrophages have a longer lifespan than neutrophils and continue to work at a lower pH. These cells are fundamental for the late stages of the inflammatory response, acting as key regulatory cells and providing an abundant reservoir of potent tissue growth factors, particularly TGF-β, as well as other mediators (TGF-α, heparin-binding epidermal growth factor, fibroblast growth factor [FGF], collagenase), activating keratinocytes, fibroblasts, and endothelial cells. The depletion of monocytes and macrophages from the wound causes severe healing disturbances due to poor wound debridement, delayed fibroblast proliferation and maturation, as well as delayed angiogenesis, resulting in inadequate fibrosis and a more weakly repaired wound. The last cells to enter the wound site in the late inflammatory phase are lymphocytes, attracted 72 h after injury by the action of interleukin-1 (IL-1), complement components, and immunoglobulin G (IgG) breakdown products. The IL-1

plays an important role in collagenase regulation, which is later needed for collagen remodeling, production of extracellular matrix components, and their degradation. [4,5,16,17]

1.1.2.3 Proliferation Phase

As the inflammation subsides, proliferation becomes a major theme with the focus on covering the wound surface (i.e., re-epithelialization), restoring the vascular network, and forming granulation tissue. Re-epithelialization requires migration and proliferation of keratinocytes. In a few hours to 1 day after the injury, the existing wound-edge keratinocytes start to migrate. To generate more cells to cover the wound, keratinocytes at the basal layer of the wound edge and epithelial stem cells from nearby hair follicles or sweat glands start proliferating approximately 2–3 days after injury. Migration is triggered by the loss of contact inhibition and physical tension at cell adhesion structures, i.e., desmosomes and hemidesmosomes, which activate membrane-associated kinases, thus leading to increased membrane permeability for calcium. This is a signal for the reorganization of the cytoskeleton driving migration. Meanwhile, the migrating cells are released from their original sites by collagenase and elastase. Details for this process were summarized elsewhere. Migration stops when the cells get in contact and new adhesion structures are formed. Keratinocytes secrete proteins to rebuild the basement membrane. Re-epithelialization can be stimulated by a variety of wound-related signals, e.g., nitric oxide, which is mainly synthesized by macrophages, cytokines, and growth factors, including epidermal growth factor (EGF), KGF, IGF-1, and nerve growth factor (NGF), secreted from multiple cell types in the wounds.

Restoring the network of blood vessels is important since nutrients and oxygen are needed during wound repair. The process of new blood vessel formation, also known as ‘angiogenesis’, is initiated by growth factors, e.g., vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and the serine protease thrombin in the wounds, which activate the endothelial cells of existing vessels. After secreting proteolytic enzymes to dissolve the basal lamina, the endothelial cells escape from the existing vessels, proliferate, and migrate towards the source of the angiogenic stimulus. These sprouts form vessel lumen, differentiate into

arteries and venules, and mature by recruitment of pericytes and smooth muscle cells. In addition, bone marrow-derived endothelial progenitors can also form vessels de novo, a process is known as vasculogenesis.

In the proliferation phase, the provisional wound matrix formed during hemostasis is replaced by granulation tissue, consisting of a large number of fibroblasts, granulocytes, macrophages, blood vessels, in complex with collagen bundles, which partially recovers the structure and function of the wounded skin. Fibroblasts play a central role in the formation of the granulation tissue, which migrates mainly from the nearby dermis to the wound in response to cytokines and growth factors, e.g., PDGF, transforming growth factor (TGF)-β and bFGF, produced by platelets and macrophages in the wounds. If the wound condition is long-lasting, fibroblasts in the wounds may also originate from fibrocytes, which is a group of bone marrow-derived mesenchymal progenitor cells. Circulating fibrocytes migrate to regions of skin injury and promote healing not only by contributing to a subset of fibroblasts in the wounds, but also by producing cytokines, chemokines, and growth factors, serving as antigen-presenting cells as well as enhancing angiogenesis. After migrating into the provisional wound matrix, fibroblasts proliferate and produce proteinases, e.g., matrix metalloproteinases (MMPs), to degrade provisional matrix; while depositing collagen and other extracellular matrices (ECM) components, e.g., proteoglycans, hyaluronic acid, glycosaminoglycans, and fibronectin, to form granulation tissue, which fills up the wound gap and provide a scaffold for cell adhesion, migration, growth, and differentiation during wound repair. [18-21]

1.1.2.4 Remodeling Phase

The remodeling phase starts at the end of the granulation tissue development. Mechanical tension and cytokines, e.g., TGF-β, drive fibroblasts to differentiate into myofibroblasts, which express α-smooth muscle actin (SMA) and contract the wound. Myofibroblasts undergo apoptosis when healing is complete. At this phase, the quickly produced collagen III in the ECM is replaced by collagen I, which has higher tensile strength but takes a long time to deposit. The number of new blood vessels and the blood flow decline. A mature avascular and acellular environment is formed. Some skin components, e.g., hair follicles

and sweat glands, cannot be recovered after serious injury; and the healed skin can only achieve a maximum of 80 % of the original tensile strength. [18]

1.2 Calendula Officinalis

Calendula officinalis which is called pot marigold is belonging to the Compositae family (Asteraceae). The native name in Hindi is called Genda, Geographically It is broadly cultivated and distributed in Europe (Southern), North Africa, Asia, and America. The optimum time for cultivation and harvesting of marigold for Rainy season the Sowing time and Harvesting time is July to August and September to October respectively, for Winter season the Sowing time and Harvesting time is September to October and November to December respectively, and for the Summer season, the Sowing time and Harvesting time is January and March to April respectively. The production of the marigold plant around the world stands at around 600000 tonnes, of which India has a 75-80% of the total shares. India utilizes about 80% of its Production. The quality of marigolds of Indian origin is measured best in the world. The plant species has been testified to comprise several phytoconstituents, which include lipids compound carbohydrates group, phenolic compounds, steroids, quinones, terpenoids, and carotenoids. The concentrations of these compounds vary depending on geographical area. [22-24]

Fig 2: Calendula officinalis Plant

Calendula officinalis, a commonly available medicinal plant, has been used for eras to treat several diseases counting skin infection, wounds, burns, and ulcers of the duodenum. *Calendula officinalis* extract has been testified for its, antioxidant, anti-inflammatory, antibacterial, and wound healing activities. The efficacy of *Calendula officinalis* on wound healing helps for appropriate healing of the wound caused by any means in humans. It reduces the harmful effects (toxicities) of modern pharmaceutical products by reducing

their random use and applications. In Indian origin, the herbal compounds including *Calendula officinalis* are topically used to treat hemorrhoids. The Calendula cream formulation alone or amalgamation with other remedies is also a favorite homeopathic remedy to treat abrasions and minor burns. In Europe, the leaves part of the plant are measured as resolvent and diaphoretic whereas the flowers are used as a stimulant, antispasmodic, and emmenagogue. In England, the decoction of the part of the flower is considered as a posset drink for the treatment of smallpox and measles, and the juice of the fresh plant use as a remedy for maintaining high bilirubin levels in jaundice, constipation, and controlling flow.

1.2.1 Plant profile

Table 1: Taxonomy of *Calendula officinalis*:

Kingdom	**Plantae**
Subkingdom	Tracheobionta [29]
Division	Magnoliophyta [30]
Class	Magnoliopsida [31]
Subclass	Asteridae [30]
Order	Asterales [31]
Family	Asteraceae [31]
Tribe	Calenduleae [30]
Genus	Calendula [32]
Species	officinalis [32]

Various pharmacological studies have confirmed that *Calendula officinalis* exerts a wide range of biological properties, some of which are very interesting for probable future progress like Anti-inflammatory and anti-edematous activities, Anti-HIV activity, Antibacterial and antifungal activities, Anticancer and lymphocyte activation dual activities, Hepatoprotective activity, Immunostimulant activity, Antioxidant activity, Wound healing activity, Spasmolytic and spasmogenic dual activity, Insecticidal activity,

Inhibition of heart rate, Genotoxic, and antigenotoxic dual activities, Genotoxic, and antigenotoxic dual activities. [25-28]

1.2.2 Phytoconstituent of Calendula officinalis:

Table 2: Phytoconstituent of *Calendula officinalis*:

Plant part	Secondary Metabolites	Active constituents
Leaves	Quinones	Phylloquinone, α-tocopherol, Ubiquinone, Plastoquinone [33]
Flowers	Terpenoids	Lupeol [34], Erythrodiol [35], Calenduloside, *Calendula officinalis* glycoside A [36], *Calendula officinalis* glycoside B, Cornulacic acid acetate [37]
	Flavonoids	Isoquercitrin, Rutin, Calendoflavoside [36], Quercetin, Isorhamnetin, Isorhamnetin-3-O-β-D-glycoside, Narcissin [38]
	Coumarins	Esculetin, Scopoletin, Umbelliferone [39]
	Volatile oils	Cubenol, α-cardinal, Oplopanone, Methyllnoleate [40], Sabinene, Limonene, p-cymene, Carvacrol, Geraniol, Nerolidol [41]
Root	Terpenoid	Calenduloside A [42]
Petal and Pollens	Carotenoids	Flavoxanthin, Luteoxanthin, Auroxanthin, 9Z-antheraxanthin, Violaxanthin [43]

1.3 Herbal Drug

Natural plant products have been used throughout human history for various purposes. Many of these natural products have biological activity that can involve in drug discovery and drug design. The Indian system of medicine known as "Ayurveda" uses mainly plant-based drugs or formulations to treat various ailments, including cancer. Herbal drugs have great growth potential in the global market. Research work on the chemistry of natural products, pharmacognosy, pharmaceutics, pharmacology, and clinical therapeutics have been carried out on herbal drugs and most of the leading Pharmaceutical corporations have revised their strategies in favor of natural products. Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. The therapeutic value of *Calendula officinalis* commonly known as Marigold has been recognized in different systems of traditional medicine for the treatment of different human ailments. [23]

1.3.1 Herbal drug for wound healing

Ayurveda or herbal medicine has been in practice for a long time as one of the basic treatments for the cure of various diseases in India1. Many indigenous plants have been evaluated and used as a source of many effective and potent drugs against various diseases. Microbial infections represent an important set of ailments challenging human health throughout the world. Researchers have great keenness in the screening of medicinal plants for biochemical constituents and antimicrobial activities. The potential new therapeutics used as drugs obtained from plants are mostly secondary metabolites. Major groups of secondary metabolites include phenolics, tannins, alkaloids, flavonoids, steroids, and gums. [46]

In this era of modern science, herbal medicine still holds its potential existence in the mode of having without side effects. A wide variety of products from herbs are usually used by the Indians tribal community for initial treatment of incidents like cuts, wounds, and burns. The chemical composition obtained from herbs needs identification followed by formulation for the treatment and management of wounds. Pharmacological and chemical studies with medicinal plants have amplified in the last eras, not only associated with the

extraction of active principles, but also to the depiction of a new entity with pharmacological potential and nutraceutical property, needed for the use in food industries, as well as in cosmetics preparation and pharmacology. [4,5]

1.3.2 Why choose herbal over synthetic drug in Wounds

The wound healing process is known as interdependent cellular and biochemical stages which are in trying to improve the wound. Wound healing can be defined as stages that are done by the body and delayed wound healing increases the chance of microbial infection. The improved wound healing process can be performed by shortening the time needed for healing or lowering the inappropriate happens. The drugs were locally or systemically administrated to help wound healing. Antibiotics, antiseptics, desloughing agents, etc. have been used to wound healing. Some synthetic drugs are faced with limitations because of their side effects. Plants or combinations derived from plants are needed to investigate identify and formulate for treatment and management of wound healing. There is increasing interest to use medicinal plants in wound healing because of lower side effects and management of wounds over the years. Studies have shown that medicinal plants improve wound healing in diabetic, infected, and opened wounds. Different mechanisms have been reported to improve wound healing by medicinal plants.

The different agents are used to wound healing including antibiotics and antiseptics, desloughing agents (chemical debridement, e.g., hydrogen peroxide, eusol, and collagenase ointment, wound healing promoters, some substances such as tissue extracts, vitamins, and minerals, and several plant products. Medicinal plants heal the wound healing process by promoting blood clotting, fighting against infection, and accelerating wound healing. It can be stated plants and chemical agents obtained from plants improve treatment and manage wound healing. Medicinal plants show wound healing effects by the different mechanisms, such as modulation in wound healing, decreasing bacterial count, improving collagen deposition, increasing fibroblasts and fibrocytes. [47]

1.3.3 Wound healing activity of *Calendula officinalis*:

Burns: Healing of burned tissues is a compound process in which the process involves are re-epithelisation, formation of tissue granulation, and extracellular remodeling. Various

studies have shown that in thermal injury there are close relationships between secondary pathological changes and the peroxidative reaction of lipids. Studies show that severe burning not only affects the outer skin but it can also affect the internal organs. Common local burns produce oxidant-induced organs which leads to changes as evidenced by increased lipid peroxidation in the remote organs. The body's innate mechanism to protect itself from the deleterious effect of free radicals is antioxidants. Glutathione shows a significant role in the detoxification of foreign particles, hydrogen peroxide, and free radicals. In the burned tissue there is a significant increase in the content of glutathione, which may be due to the triggering of the antioxidant system. With the *Calendula officinalis* treatment, the level can be increased. Studies show that the effectiveness of extracts of *Calendula officinalis* enhancing the defense mechanism of antioxidant which decrease the burn injury. In burns, antioxidant therapy is used to prevent the harmful effect of oxygen-free radicals. [48,49]

Cesarean section wounds: Based on the study if we compared ointment of calendula concerning standard care, the cesarean healed wound on 3, 6, and 9 days after surgery is significantly accelerated. Till now, on cesarean wound healers, several studies have been healing abdominal surgery. The statistics of the current study on the effect of marigold ointment on wound healing in the cesarean section shows that the mean c-section wound healed 3 days after treatment (4 days after c-section) in marigold less the control group, respectively. Besides, the study explores the effects of calendula ointment on perineal healing of episiotomy, and the results indicated that the average level of wound healing of individuals using calendula ointment was less than that of the treatment group 5 days post of episiotomy. Although the influence of this medication on wound healing episiotomy has been investigated, it is consistent with the research. This result in the study regarding the wound healing impact of calendula ointment on c-section shows that the mean result between the two groups after the intervention was 6 days (7 days after c-section) was a statistically significant difference. According to the results of this study, there were no side effects from the dermal application of calendula ointment and, as a result, this could be used as a treatment option for cesarean wounds. [50]

Acute wounds: Through the use of *Calendula officinalis* in wounds it was found to be beneficial in the present studies, based on analysis and histological assessments performed on days 14 and 21. It is suggested that its efficacy in wounds is due to its approach to the reduction of inflammation and the management of environmental microcirculation. The *Calendula officinalis* (Marigold), which belongs to the family Asteraceae, is well acknowledged for its pharmaceutical and cosmeceutical use, contains two series of OL glycosides, i.e. glucosides and glucuronides. glucosides are mainly allelopathic and hemolytic agents and glucuronides are potent fungi-statics. [50,51]

Chronic wounds: Chronic wounds like venous leg ulcers (VLU) continue to be a challenging public health concern, despite recent improvements in management and treatment. The high number of patients with this complication stimulates research into alternative treatments that could be more successful than conventional approaches. Plenusdermax, the *Calendula officinalis* hydro glycolic extract studied in the current research, has been considered to be an effective therapeutic therapy for VLUs. VLUs reported a substantially faster mean healing time (13 weeks) in patients treated with the extract than in treated patients with standard therapy (22 weeks). For treating chronic wounds, conventional therapies based on medicinal herb extracts and ointments have been commonly used. The therapeutic efficacy of herbal extracts and ointments against VLUs has been documented in previous studies. The therapeutic efficacy of adjuvant therapies based on conduct. Though, none of these studies has considered the effect of this ointment on wound medicinal plants has been linked to several studies for the treatment of VLUs. Three weeks of *Calendula officinalis* ointment therapy supported a 42 percent reduction in the region of the bite, and 33 percent of the wounds treated were completely healed. [52-54]

1.3.4 Traditional uses of *Calendula officinalis*

According to the Ayurvedic and Unani systems of medicine, The leaves and flowers of *Calendula officinalis* have antipyretic, anti-inflammatory, antiepileptic, and antimicrobial properties. Calendula flowers have been historically used to treat liver obstructions, snake bites, and heart weakness. In the 18th century, it was used to treat headaches, jaundice, and

red eyes. During the Civil War, the plant was used to treat wounds and as a vaccine for measles, smallpox, and jaundice. [55,56]

Calendula officinalis (Pot marigold) has been utilized in the treatment of internal organ inflammation, gastrointestinal ulcers, and dysmenorrhea, and to treat convulsions and Diaphoresis. It's also used to treat wounds and burns, as well as inflammations of the oral and pharynx.[57]

Field marigold, Calendula arvensis Linn., has been used as a disinfectant, antispasmodic, and diuretic. The plant is being used as an anti-inflammatory, anticancer, and antipyretic agent in Italian traditional medicine. In Spain, the leaves are considered sudorific. Traditionally, it is used as a substance that stimulates or increases menstrual flow.[56]

1.4 Herbal gels

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired drug concentrations. The route of administration has a significant impact on the therapeutic outcome of a drug. Skin is one of the most readily accessible organs on the human body for topical administration and is the main route of the topical drug delivery system. Topical delivery can be defined as the application of a drug-containing formulation to the skin to directly treat the cutaneous infection or disorders like wounds, acne, or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effects of the drug to the surface of the skin or within the skin. The semi-solid formulation in all their diversity dominates the system for topical delivery, but foams, spray, medicated powders solutions, as well as medicated adhesive systems are also in use.

A gel is a solid or semisolid system of at least two constituents, consisting of a condensed mass enclosing and interpenetrated by a liquid. Gels and jellies are composed of a small number of solids dispersed in a relatively large amount of liquid, yet they possess a more solid-like than liquid-like character. The characteristic of gel and jelly is the presence of some form of cutaneous structure, which provides solid-like properties. [58]

1.4.1 Advantages of gels [58]

- Avoidance of the first-pass metabolism.
- Convenient and easy to apply.
- Avoidance of the risks and inconveniences of intravenous therapy and the varied conditions of absorption, like pH changes, presence of enzymes.
- Achievement of efficacy with lower total daily dosage of the drug by continuous drug input.
- Avoids fluctuation in drug levels, inter-and intrapatient variations.
- Ability to easily terminate the medications, when needed.
- A relatively large area of application in comparison with the buccal or nasal cavity
- Ability to deliver the drug more selectively to a specific site.
- Providing utilization of drugs with a short biological half-life,
- Improving physiological and pharmacological response.
- Improve patient compliance.
- Provide suitability for self-medication.

1.4.2 Disadvantages of gels [58]

- Skin irritation of contact dermatitis may occur due to the drug and/or excipients.
- Poor permeability of some drugs through the skin.
- Possibility of allergenic reactions.
- Can be used only for drugs that require very small plasma concentration for action
- The enzyme in the epidermis may denature the drugs
- Drugs of larger particle size are not easy to absorb through the skin

1.4.3 Method of preparation of herbal gels

1.4.3.1 Carbopol 940 gel

Potassium sorbate was dissolved in purified water 50°C. 0.5, 1, 1.5, and 2 g of carbopol 940 were dispersed in purified water at 40°C by a mixer at 1200 rpm for 30 min. Herbal extracts and essential oil were dispersed separately in PEG 400 and added to gel base and

mixed well. The pH was then adjusted to pH, 6 using triethanolamine and stirred slowly until a clear and transparent gel was obtained. [59]

1.4.3.2 Sodium carboxymethylcellulose gel

Potassium sorbate was dissolved in purified water 50°C. 1, 2, 3, 4, and 5 g of Na-CMC were dispersed in purified water 50°C by a mixer at 1200 rpm for 30 min. Herbal extracts and essential oil were dispersed separately in PEG 400 and added to gel base and mixed well. [59]

1.4.3.3 Hydroxypropyl methylcellulose gel

Potassium sorbate was dissolved in purified water 50°C. 3, 4, and 5 g of HPMC K4M were dispersed in the amounts of purified 60°C water by a magnetic mixer at 1200 rpm for 30 min until prepared homogenous dispersion. Then, the remaining amount of water was poured coldly and mixed well, and kept in the refrigerator for 24 h until homogenous gel was obtained (hot/cold technique). Herbal extracts and essential oil were dispersed separately in PEG 400 and added to gel base and mixed well. [59]

1.4.3.4 Carbopol and sodium carboxymethylcellulose gel

First, potassium sorbate was dissolved in purified water 50°C. Then, specified amounts of carbopol 940 and Na-CMC were dispersed at purified water 40°C and mixed well. Herbal extracts and essential oil were dispersed separately in PEG 400 and added to gel base and mixed well. [59]

CHAPTER-2

LITERATURE REVIEW

2. Literature review

Givol *et al.*, (2019) Stated that Use of complementary and alternative medicine for wound healing is influencing mainstream medical practice. This systematic review evaluates the role of Calendula officinalis flower extract as monotherapy compared to control for wound healing in vivo. Findings from the review on acute wound healing showed faster resolution of the inflammation phase with increased production of granulation tissue in the test groups treated with the extract. These findings were consistent in five animal studies and one randomized clinical trial. Chronic wound healing studies were varied. Two clinical control studies on venous ulcers demonstrated decreased ulcer surface area compared to controls. Another randomized clinical trial demonstrated no improvement for the calendula group in diabetic leg ulcer healing. Burn healing similarly showed mixed results. Two animal studies demonstrated a prophylactic effect for the administration of calendula extract before burn injury. A randomized clinical trial of patients suffering from partial to full-thickness burns demonstrated no benefit for topical application of calendula extract compared to controls. Two randomized clinical trials assessed the potential for the extract to prevent acute post-radiation dermatitis, with one study showing improvements compared to trolamine, while the other found no improvement compared to aqua gel cream. Animal studies provide moderate evidence for improved recovery from the inflammation phase and increased production of granulation tissue in calendula extract treatment groups. This review identified some evidence for the beneficial effects of Calendula officinalis extracts for wound healing, consistent with its role in traditional medicine. There is a need for larger, well-designed randomized control trials to assess the effect of calendula on wound healing including complications. [1]

Gunasekaran *et al.*, (2020) Studied the in vivo wound healing activity of herbal ointment prepared from Calendula officinalis Linn. on excision wounded rats and stated that Animals were divided into five groups of six animals in each. Group, I served as normal control, Group II served as excision wounded control without treatment, and Group III, IV served as excision wounded rats were treated with the herbal ointment of two different doses applied topically for 14 days and group V served as excision wounded animals treated with reference ointment. Healing potential was evaluated by the rate of wound

contraction, immunological markers like IL-6(Interleukin 6), TNF-alpha (Tumor necrosis factor -α), PDGF (Platelet-Derived Growth Factor) and EGF (Epidermal Growth Factor), lipid peroxide (LPO), superoxide dismutase (SOD), and biochemical parameters like hydroxyproline, hexosamine, and tissue protein. The topical application of herbal ointment treated groups showed an increase in the levels of growth factors such as PDGF and EGF hydroxyproline, hexosamine, tissue protein, SOD, and wound contraction and the ointment normalized the levels of lipid peroxide, IL-6, TNF-alpha compared to that of excision wounded animals. And it was concluded that the topical application of herbal ointment exhibited significant wound healing activity in excision wounded rats as evidenced by increased wound contraction and collagen synthesis also mentioned that Further investigation can be done for extracted compounds from Calendula officinalis can be studied for the different wound models on experimental animals. [44]

Nicolaus *et al.*, (2017) Stated that the effect of Calendula extracts on the new tissue formation phase of wound healing was evaluated by studying the migratory properties of these extracts, triterpene mixtures, and single compounds in human immortalized keratinocytes using the scratch assay. Finally, the effect of the extracts on the formation of granulation tissue in wound healing was studied using bacterial collagenase isolated from Clostridium histolyticum and the determination of soluble collagen in the supernatant of human dermal fibroblasts. The n-hexane and the ethanolic extracts from Calendula flowers influence the inflammatory phase by activating the transcription factor NF-κB and by increasing the amount of the chemokine IL-8, both at the transcriptional and protein level, in human immortalized keratinocytes. The migration of the keratinocytes during the new tissue formation phase was only marginally influenced in the scratch assay. However, it can be assumed that the granulation tissue was affected, as the ethanolic extract inhibited the activity of collagenase in vitro and enhanced the amount of collagen in the supernatant of human dermal fibroblasts and concluded that results contribute to a better understanding of the wound healing properties of the traditional medicinal plant Calendula officinalis. However, further studies are necessary to evaluate which of its known constituents are responsible for these effects. Triterpenes seem to play only a marginal role, but carotene and xanthophyll derivatives should garner more attention in future studies. [60]

Parente *et al.*, (2012) Stated that Calendula officinalis is an annual herb of Mediterranean origin which is popularly used in wound healing and as an anti-inflammatory agent. In this study, the ethanolic extract, the dichloromethane, and hexane fractions of the flowers from plants growing in Brazil were produced. The angiogenic activity of the extract and fractions was evaluated through the chorioallantoic membrane and cutaneous wounds in rat models. The healing activity of the extract was evaluated by the same cutaneous wounds model through macroscopic, morphometric, histopathologic, and immunohistochemical analysis. The antibacterial activity of the extract and fractions was also evaluated. This experimental study revealed that C. Officinalis presented anti-inflammatory and antibacterial activities as well as angiogenic and fibroblastic properties acting positively on the inflammatory and proliferative phases of the healing process. [61]

Dinda *et al.*, (2016) Stated that the active fraction and/or compounds of Calendula officinalis responsible for wound healing are not known yet. In this work, we studied the molecular target of C. Officinalis hydroethanolic extract (CEE) and its active fraction (water fraction of hydroethanolic extract, WCEE) on primary human dermal fibroblasts (HDF). In vivo, CEE or WCEE were topically applied on excisional wounds of BALB/c mice and the rate of wound contraction and immunohistological studies were carried out. We found that CEE and only its WCEE significantly stimulated the proliferation as well as the migration of HDF cells. And also they up-regulate the expression of connective tissue growth factor (CTGF) and α-smooth muscle actin (α-SMA) in vitro. In vivo, CEE or WCEE treated mice groups showed faster wound healing and increased expression of CTGF and α-SMA compared to the placebo control group. The increased expression of both the proteins during the granulation phase of wound repair demonstrated the potential role of C. Officinalis in wound healing. In addition, HPLC-ESI MS analysis of the active water fraction revealed the presence of two major compounds, rutin and quercetin-3-O-glucoside. Thus, our results showed that C. Officinalis potentiated wound healing by stimulating the expression of CTGF and α-SMA, and further we identified active compounds. [25]

Rigane *et al.*, (2013) stated that *Calendula officinalis* (Marigold) was characterized concerning its chemical composition, antioxidant potential, and antimicrobial activities.

Five compounds were identified and quantified by LC/MS and HPLC in leaves and flowers of aqueous-methanolic extracts. Total flavonoids ranged between 44.91 and 76.44 mg QE/g dry weight in leaf and flower extracts, respectively. Rutin, quercetin-3-O-glucoside, scopoletin-7-O-glucoside, isorhamnetin-3-O-glucoside and gallic acid were tentatively identified in this plant. The highest antioxidant activities using two methods, DPPH and FRAP assays were obtained with aqueous-methanol flower extract from C. Officinalis (0.35 mg.mL-1 and 28.37 mM of Trolox). The potential antimicrobial activity of the leaf and flower aqueous-methanol extracts from the C. Officinalis was screened against three bacteria and two pathogenic fungi, using the cellulosic disc method. A strong inhibited activity against the five microorganisms is obtained. This study could provide useful information for the industry to produce potentially bioactive plant extract. [45]

Shafeie *et al.*, (2015) Stated that Lots of biological dressings and indigenous medicines have been reported to possess wound healing properties. Calendula officinalis (marigold) has many pharmacological properties. It is used for the treatment of skin disorders, pain and also as a bactericide, antiseptic and anti-inflammatory. In this investigation, the effects of different concentrations of Calendula officinalis gel on histological and biomechanical changes of the skin are studied. Seventy-five mature male rats were randomly divided into three groups (control, placebo, and treatment group). Under sterile conditions, a 2×2-cm piece of cervical skin for histopathological groups and a rectangular shape with a metal ruler from the cervical to the lumbar region for biomechanical groups were excised in each animal. The treatment group received a daily topical application of 5%, 7%, and 10% C.officinalis gel, the placebo group received a daily topical application of the base gel, and the control group received no treatment during this experimental study. Fourteen and 21 days later, the rats were euthanized and biopsies were taken from the site of the initial incisions, and samples were collected for histopathological and biomechanical investigation. Histopathological and biomechanical restorations in the group treated with 7% gel were significantly more than the placebo and control groups. Upper and lower doses seem to be less effective, although the reasons for this remain unclear. [62]

John *et al.*, (2017) Stated that Calendula officinalis L. (Marigold) is globally known for its medicinal importance containing various phytochemicals including carbohydrates, amino

acids, lipids, fatty acids, carotenoids, terpenoids, flavonoids, quinones, coumarins, and other constituents, showing some important biological activities like wound healing, immuno-stimulant, spasmogenic and spasmolytic, hepatoprotective, genotoxic and antigenotoxic, anti-amylase, anti-inflammatory, anti-oedematous, anti-bacterial and anti-fungal, antioxidant, antidiabetic, anti-HIV and anti-cancerous, nephron-protective, prevention of oropharyngeal mucositis, hypoglycemic and gastroprotective activities with no toxic effect. In this review, a detailed account of different phytochemicals and their medicinal properties of Calendula officinalis have been addressed. [63]

Buzzi *et al.*, (2016) Stated that Non-healing venous leg ulcers (VLUs) have a significant effect on patients' quality of life and substantially increase expenditures in healthcare systems. Patients treated with Calendula officinalis extract (n=38) and control patients (n=19) were evaluated every two weeks for 30 weeks or until their ulcers healed. Assessments included determination of the wound area by planimetry, infection control, and evaluation of the clinical aspects of the wounds. The percentage of healing velocity per week (%HVw), taking the initial area at baseline into account, was also determined. The proportion of the treatment patients achieving complete epithelialization was 72 % and 32 % in the treatment and control groups, respectively. The average healing time was approximately 12 weeks in the treatment group and 25 % in control patients. Patients with ulcers treated with Calendula officinalis extract had a significant 4-fold increase in percentage healing velocity per week, 7.4 %, compared with 1.7 % in the control group. No adverse events were observed during the Calendula officinalis extract treatment. These findings indicate that Calendula officinalis extract is an effective treatment for VLUs.

The earliest people used to treat diseases by unconventional methods using plants, animal products, and minerals, of the plants were given major priority. Each secondary metabolite has unique pharmacological action, mechanism of action, and therapeutic uses. So, plants or products isolated from them have been used to treat infections, health disorders, or diseases from centuries ago till date. The current review article gives the importance and various pharmacological actions of five plants namely Calendula officinalis, Tagetes erecta, Carica papaya, Hypericum perforatum, and Salvia officinalis which have certain activities in common based on the literature survey. All of them have been used in a holistic

system of medicine and many scientific experiments have been done to prove their activities. Most of them have a good amount of flavonoids that make a suitable antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, and nephroprotective agents in general. Apart from them, they have other individual constituents responsible for their unique pharmacological activities. Combinations of such plants based on the activity needed can be formulated to a new synergistic formulation instead of extracting the individual constituent fractions from it. They are thus been studied extensively to get a safe and efficacious product. [22]

Jafari *et al.*, (2017) Stated that Infectious diseases have always been one of the important concerns of humans and have continuously attracted the attention of a large number of various medical and laboratory professionals. On the other hand, treatment with antibiotics has other problems such as drug resistance and side effects, so the use of new herbal medicines with fewer side effects can be a great help in treating these types of infections. The objective of this study was to investigate the antibacterial activity of marigold (*Calendula officinalis*) extract on four reference strains including *Staphylococcus aureus, Bacillus cereus, Escherichia coli,* and *Pseudomonas aeruginosa*. In this study, the antimicrobial effects of methanolic extract of the marigold plant were experimented on four above-mentioned reference strains after extraction by the Soxhlet method. Methanolic extract with concentrations of 20 mg/ml, 30 mg/ml, 50 mg/ml and 400 mg/ml was prepared by solvent dimethyl sulfoxide. Then, their antimicrobial effects were investigated using well diffusion and tubular dilution methods. The data were analyzed using the analysis of Variance (ANOVA) and Chi-square test at $P<0.01$. The results showed that the methanolic extract of the marigold plant inhibits the growth of *S. aureus*, *B. cereus,* and *E. coli*, which also increased their antimicrobial activity by increasing the concentration. As well, the concentration of 1000 μg/ml essential oil of this plant leaves also showed an inhibitory effect on *S. aureus*, *B. cereus,* and *E. coli*. The results of this study showed that the extract of the marigold plant has antimicrobial effect. [64]

CHAPTER-3

AIM & OBJECTIVES

3. Aim and Objectives

3.1 Aim of the study:

This study aims to formulate the herbal gel from *Calendula officinalis* plant leaf extract and to evaluate the wound healing activity

3.2 Objectives:

The objective of the study is

- To formulate the herbal gel containing leaf extract of the *Calendula officinalis* plant intended for wound healing activities.
- Phytochemical screening and evaluation of wound healing activity through in-vitro antimicrobial and in-vivo wound healing studies.

3.3 Plan of work

1. Literature review

2. Preparation of plant extract: Collect the plant leaves, collected leaves are cleaned and shade-dried and make the powder of dried leaf using a mechanical grinder and passed through a 20-mesh sieve. Then a weight amount of powdered leaf undergoes extraction with ethanol as solvent using Soxhlet apparatus. The extraction is carried out for 24 hours at room temperature with mild shaking. The extracts were filtered and concentrated at 35° C and evaporate the remaining solvent using a Rotary dry evaporator or Freeze-drying techniques.

3. Identification

i. Absorption maxima
ii. Fourier transform infrared spectroscopy (FTIR)
iii. Phytochemical Screening: Flavonoids test for flavonoids, Mayer's test for alkaloids, test for triterpenoids, Millon s test for protein, Salkowski test for steroids, etc.

4. Pre-formulation Study:

Pre-formulation studies are needed to ensure the development of a stable as well as the effective and safe dosage form. It is a stage of development during which the pharmacist characterizes the physic-chemical properties of the drug substances and their interaction with various formulation components. Goals of Pre-formulation study:

- To determine the necessary physicochemical parameter of a new drug substance.
- To establish its incompatibility with excipients of the formulation.

4. Formulation of gel: Preparation of gel with Carbopol 934

5. Evaluation of Gel Formulation: Physicochemical evaluations like Physical appearance, Measurement of pH, Spreadability, Viscosity are performed to make safe and efficacious dosage forms.

6. In-vitro Antimicrobial Study: In-vitro antimicrobial activity can be studied with the Disc diffusion method by calculating the Minimum inhibitory concentration (MIC) against some selective bacterial species.

7. In-vivo Study for Wound healing: The present study aims to determine the efficacy of Calendula officinalis for wound healing activity. Animal models are the next step when assessing product efficacy. Animal models are beneficial to wound research because they comply with wound healing studies.

In-vivo Wound healing study can be performed using an Animal model (Albino rats) by calculating the contraction wounds (which is previously developed by making an incision) after application of a formulated gel containing the extract.

CHAPTER-4

MATERIALS & METHODS

4. Materials and Methods:

4.1 Materials used

All the chemicals are used of analytical grade and the manufacturers for respective chemicals are listed below:

Table 3: List of chemicals used

Sl no	Chemicals	Suppliers
1.	Ethanol	Infinity solution
2.	Chloroform	Infinity solution
3.	Petroleum ether	Infinity solution
4.	Agar	B S Trading, Kolkata
5.	Peptone	B S Trading, Kolkata
6.	Sulphuric acid	Infinity solution
7.	Ferric Chloride	Infinity solution
8.	Lead acetate	Infinity solution
9.	Sodium hydroxide	Infinity solution
10.	Glacial acetic acid	Rankem, Mumbai.
11.	Hydrochloric acid	Rankem, Mumbai.
12.	Silica Gel	B S Trading, Kolkata
13.	Carbopol 934	B S Trading, Kolkata
14.	Methylparaben	Rankem, Mumbai.
15.	Propylparaben	Rankem, Mumbai.
16.	Triethanolamine	Infinity solution
17.	Propylene glycol 400	B S Trading, Kolkata
18.	Disodium hydrogen phosphate	Infinity solution
19.	Potassium dihydrogen phosphate	Infinity solution

4.2 Instruments used:

Instruments required for the practical works conducted throughout the project and the respective manufacturers are enlisted below:

Table 4: List of instruments used

Sl no	Instruments	Company name
1	Digital weighing balance	Denver Instrument, USA
	UV Visible Spectrophotometer	Shimadju, Model No: UV 1800 240V, Japan
	Hot air oven	International Commercial Traders, 18, Kolkata-700001
	FT-IR	Alpha, Bruker Model No: 10059736, Germany
	Thermostate water bath	JSGW
	Digital Brookfield Viscometer	DVE viscometer
	Refrigerator	Godrej, India
	Magnetic stirrer with a hot plate	Rolex, India
	pH meter	Systronic
	Lyophilizer freeze dryer	IIC Corporation
	Mechanical grinder	USHA

4.3 Preparation of plant extract

Fresh *Calendula officinalis* leaves were collected from the locality. The leaves were separated, washed, and dried in shade and make the powder of dried leaf using a mechanical grinder and passed through a 20-mesh sieve. 50 grams of dried and grounded leaves were transferred into a Soxhlet apparatus containing ethanol as solvent. The extraction is carried out for 24 hours at room temperature with mild shaking. The extracts were filtered and concentrated using a water bath. Then stored in a freezer for 24 hours followed by evaporates the remaining solvent using a laboratory Freeze dryer.

4.4 Identification

4.4.1 Absorption maxima:

10 mg of extract was weighed accurately and dissolved in 10 ml of ethanol in a volumetric flask (stock solution 1 mg/ml). The stock solution is diluted to make a concentration of 10mg/ml. The spectrum of this solution was run in the 300-800 nm range in a UV-visible spectrophotometer.

4.4.2 FTIR of the extract:

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. Solvent extracts of plant material were used for FTIR analysis. A minute amount of extract sample was positioned in contact with the attenuated total reflectance (ATR) plate. And after the specimen was loaded in FTIR Spectroscopy, run the scan with a scan range from 400 to 4000 cm-1 with a resolution of 4cm-1.

4.4.3 Phytochemical Screening:

4.4.3.1 Test for flavonoids:

Ferric chloride test: To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. The appearance of green color indicates the presence of flavonoids.

Lead acetate solution test: Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

4.4.3.2 Test for alkaloids

Dragendroff's test: Dissolve extract of the herbal drug in chloroform. Evaporate chloroform and acidify their side by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). The appearance of an orange-red precipitate indicates the presence of alkaloids.

Mayer's test: 2-3 ml of filtrate with few drops of Mayer's reagent gives ppt.

Wagner'stest: 2-3 ml of filtrate with few drops of Wagner's reagent gives reddish-brown color.

4.4.3.3 Test for proteins:

Million's test: Test solution treated with million's reagent and heated on a water bath.

Xanthoprotein test: Test solution treated with conc. nitric acid and on boiling gives yellow precipitate.

Ninhydrin test: Test solution treated with Ninhydrine reagent gives blue color.

4.4.3.4 Test for steroids:

Salkowski reaction: To 2 ml of extract, add 2 ml chloroform and 2 ml of H2SO4. Shake well. The chloroform layer appears red and the acid layer shows greenish-yellow fluorescence.

Liebermann's reaction: Mix 3 ml extract with 3 ml acetic and Heat and cool. Add few drops of conc. H2SO4. The blue color appears.

4.4.3.5 Test for triterpenoids

The dry crude plant extract (5 mg) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. The formation of reddish-violet color shows the presence of triterpenoids.

4.4.3.6 Test for glycosides:

Keller-killiani test: The test solution with few drops of glacial acetic acid in 2 ml of ferric chloride solution and conc. sulphuric acid is added from the sides of the test tube which shows the separation between two layers, the lower layer shows reddish-brown and the upper layer turns bluish-green.

Bromine water test: Test solution dissolved in Bromine water gives yellow precipitate.

4.4.3.7 Test for carbohydrates:

Fehling's test: Mix 1ml. Fehling's A and 1ml. Fehling's B solutions boil for one minute. Add an equal volume of test extract solution. Heat in boiling water bath for 5-10 min. The appearance of an orange-red precipitate indicates the presence of carbohydrates.

4.5 Pre-formulations study

4.5.1 Organoleptic property

This includes the physical characteristics that can be examined merely by sense organs such as color, odor, taste, and touch. Colour is closely related to the composition of every material; mostly the extracts are dark brown or black. Some compounds have a specific odor. Drugs containing sulfur may give an unpleasant odor of sulfur after burning. Similarly, taste and touch are correlated with chemical nature. In the case of herbomineral drugs, the presence of metallic particles in bhasma gives a specific metallic taste. Such bhasma is considered unripe or incompletely incinerated and able to cause nausea, vomiting, gastric irritation, various skin diseases, and major harm to vital organs if administered internally for a longer duration.

4.5.2 Compatibility study:

Study of interaction of the extract with excipients by FTIR study: Each excipient used in the formulations was blended with the drug levels that are realistic concerning the final dosage form. Each excipient was thoroughly blended with drug extract at a 1:1 ratio to increase drug-excipients molecular contacts and also to accelerate the reaction if possible. Each drug extract excipients blend was taken separately into vials and kept for 24 hours at room temperature. After 24 hours drug extract with excipients samples was observed under FTIR study and compared with each other and with the FTIR of extract.

4.5.3 Purity:

This is another important aspect of pre-formulation studies. For every compound, depending on its dose and toxicity, the limit of impurity is defined. Until and unless the purity of the drug is assured other studies like stability, degradation, and toxicity cannot be performed. Various parameters which are considered to find the purity of the drug

substance are pesticide residue, TLC, HPLC, UV absorption, IR spectra. All poisonous herbs, highly potent drugs, and all metals-minerals are advised to use only after proper purification. According to the chemical point of view, Ayurvedic purification methods may result in depletion of percent purity but according to the therapeutic point of view, these purification methods remove some toxins and make the metals minerals suitable for further processing.

4.5.4 Hygroscopicity: This may be considered instability studies. The herbal extracts are very much prone to changes in their structure due to moisture in the atmosphere. They may either be hygroscopic or effervescent. It may have serious implications during the manufacturing process, environmental controls on the manufacturing, packaging, and most importantly on the stability and microbial qualities of the material and formulations.

4.6 Formulation of gel:

Preparation of gel with Carbopol 934: Accurately weighed Carbopol 934 was taken in a beaker and dispersed in 50 ml of distilled Kept the beaker aside to swell the Carbopol for half an hour and then stirring should be done using mechanical/lab stirrer at 1200 rpm for 30 min. Take5 ml of propylene glycol and the required quantity of Extract. Take 5 ml propylene glycol in another beaker and add weighed quantity of propylparaben or methylparaben to it and stirred properly. After all, Carbopol dispersed, 1 gm extract, and preservatives solutions were added with constant stirring. Finally, volume made up to 100 ml by adding remaining distilled water and Triethanolamine was added dropwise to the formulations for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency.

Table 5: Gel formulations with Carbopol 934

Formulation Code	Extract (g)	Carbopol 934 (g)	Propylene Glycol (ml)	Triethanolamine (g)	Methyl-paraben (%)	Dist. water
F1	0.5	1	5	q.s	0.2	q.s
F2	1	1	5	q.s	0.2	q.s
F3	1.5	1	5	q.s	0.2	q.s
F4	2	1	5	q.s	0.2	q.s

4.7 Evaluation of gel:

4.7.1 Physical appearance:

The prepared gel formulations containing extract of *Calendula officinalis* were inspected visually for their color, homogeneity, consistency, and phase separation.

4.7.2 Measurement of pH:

The pH of developed gel formulations was determined using a digital pH meter. 1 gm of gel was dissolved in 100 ml distilled water and kept aside for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.

4.7.3 Spreadability:

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability is measured based on slip and drag characteristics of gels. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of a fixed ground slide and provided with the hook. One kg weight was placed on the top of the two slides for 5 min. to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scraped off from the edges. The top plate was then subjected to a pull of 80 gm. With the help of string attached to the hook and the time (in sec.) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability.

Spreadability was calculated using the following formula:

$S = M \times L / T$

Where, S= Spreadability,
M= weight in the pan (tied to upper slide),
L= Length moved by the slide,
T= Time (in sec.)

4.7.4 Extrudability:

The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. Weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percentage of the extruded gel was calculated (>90% extrudability: excellent,>80% extrudability: good, >70% extrudability: fair).

4.7.5 Rheological Study:

The viscosity of the developed gel formulations was determined by using a Brookfield viscometer with spindle No. 7.

4.8 In-vitro antimicrobial Study

In vitro antimicrobial activity of extracts of *Calendula officinalis* can be determined by standard agar well diffusion assay. Mueller Hinton agar and Sabouraud dextrose agar media are used for antibacterial and antifungal activity respectively. Molten Mueller Hinton agar/ Sabouraud dextrose agar (40-42°C) were seeded with 200 µl of inoculum (1 × 108 CFU/ml) and poured into Petri dishes. The media was allowed to solidify and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). Extracts are dissolved in 100% DMSO at a concentration of 20 mg/ml, from this 100µl of extracts were added into the sterile 8.5 mm diameter well. The plates are incubated at 37°C and 28°C for 24 and 48 h for bacteria and fungi, respectively. DMSO is used as a negative control. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well in mm. The experiment was done in triplicate and the average values were calculated for antibacterial activity. [65]

4.9 In-vivo wound healing study:

4.9.1 Animals required

Species and Strain: Wistar albino rats
Age and Weight: Adult (150-200) rats
Gender: Male

Table 6: Number of a group of animals to be used:

Test/animals	Animal group	No. of animals	Study type
Wound healing study/ Wister albino rats	Group A	6	Control (Gel base)
	Group B	6	Standard (Marketed formulation)
	Group C	6	Test (Prepared Formulation)

4.9.2 Wound healing study:

The animals will be anesthetized before the infliction of the experimental wound. Then the skin wound on experimental rats will be developed by shaving the skin hair followed by A 1-cm-long, full-thickness incision wound will create in the shaved and cleaned area and observed the animals for a period of time. Then, the 3 groups can be applied with equal aliquots of formulation, standard, and blank. Each group of rats should receive a particular treatment regimen observed the wound activity of the gel formulation against the experimental wounds on the experimental rats up to 28 days and measures the wound contraction, house the rats separately in a ventilated cage with appropriate bedding, food, and water. Rats need to check twice daily during wounds and treatment to ensure no adverse reactions and closely. [66]

CHAPTER-5

RESULTS & DISCUSSION

5. Result and Discussion

5.1 Extractive yield value:

The yield value of each extract was calculated as:

$$\frac{\text{Extracts Obtained}}{\text{The total amount of crude Drug}} \text{X } 100\%$$

$$= \frac{6.21}{50} \text{X } 100\%$$

$$= 12.42\ \%$$

The yield value of soxhlet extraction of the *Calendula officinalis* was found to be

= 12.42 %

5.2 Phytochemical screening of the extract

The results of phytochemical screening were obtained as follows:

Table 7: Phytochemical screening

Sl. no	Phytochemical Test	Reagents used (test performed)	Interference	Result
1	Flavonoids	Ferric chloride test	The appearance of green color	+ve
		Lead acetate solution test	Gives yellow precipitate.	+ve
2	Test for carbohydrates	Fehling's test	The appearance of orange-red precipitate	+ve
3.	Test for triterpenoids.	Triterpenoids test	Gives reddish-violet color	+ve
4	Test for proteins	Xanthoprotein test	The appearance of yellow precipitate.	-ve
		Ninhydrin test:	Gives blue color.	-ve
5	Test for glycosides .	Keller-killiani test	The lower layer shows reddish-brown and the	+ve

			upper layer turns bluish-green.	
		Bromine water test	Gives yellow precipitate	+ve
6	Test for steroids	Salkowski reaction	The chloroform layer appears red and the acid layer shows greenish-yellow fluorescence	-ve
		Liebermann's reaction	Appearance blue color	-ve

5.2 Pre-formulation Study

5.2.1 Organoleptic properties

The organoleptic properties like color, odor, and taste were observed and the results were compared with the official requirement and found to be acceptable. The results are-

Table 8: Organoleptic properties of the extract

Sample	Taste	Colour	Odor
C. Officinalis Extract	Bitter	Dark green	Characteristics

5.2.2 Determination of absorption maxima for the drug in phosphate buffer pH 6.8

The spectrum of the *C. officinalis* extract solution was run in the 300-800 nm range in a UV-visible spectrometer. The maximum *C. officinalis* extract maxima was found to be 342.5 nm.

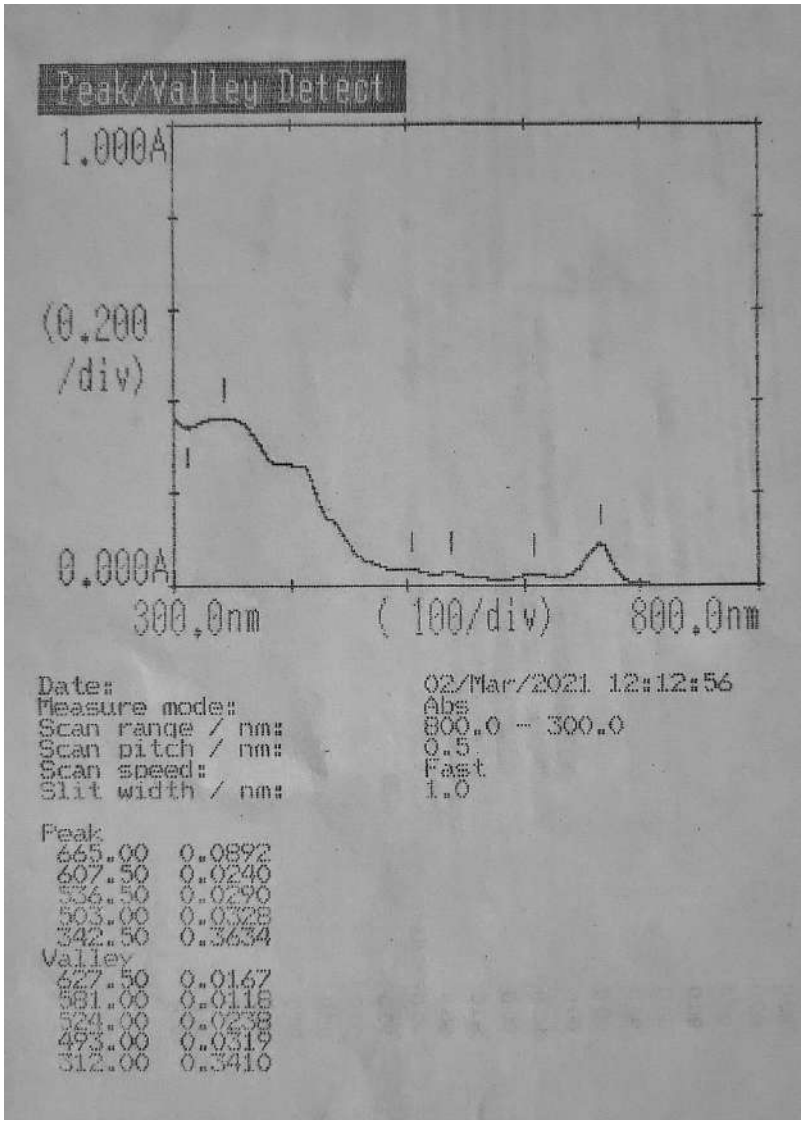

Fig 3: Absorption maxima of *Calendula officinalis*

5.2.3 Preparation of standard calibration curve of *C. officinalis* extract in phosphate buffer pH 6.8

The standard curve was obtained by plotting the observed absorbance with the respective concentrations. The absorbance increases with the increase of concentrations and the curves obtained were linear with the equation $y = 0.0036x + 0.0125$ and R^2 value was found to be 0.9939 for *C. officinalis* extract.

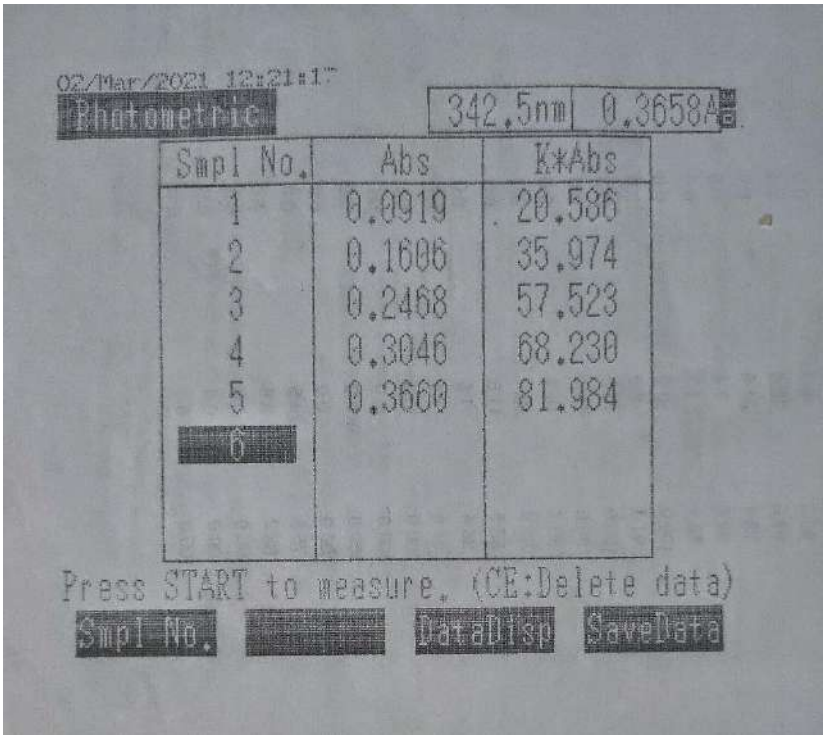

Fig 4: UV data for calibration curve of *C. officinalis*

Table 9: Data of calibration curve of *C. officinalis* extract in Phosphate buffer 6.8

Concentration(µg/ml)	Absorbance(nm)
20	0.0919
40	0.1606
60	0.2468
80	0.3046
100	0.3660

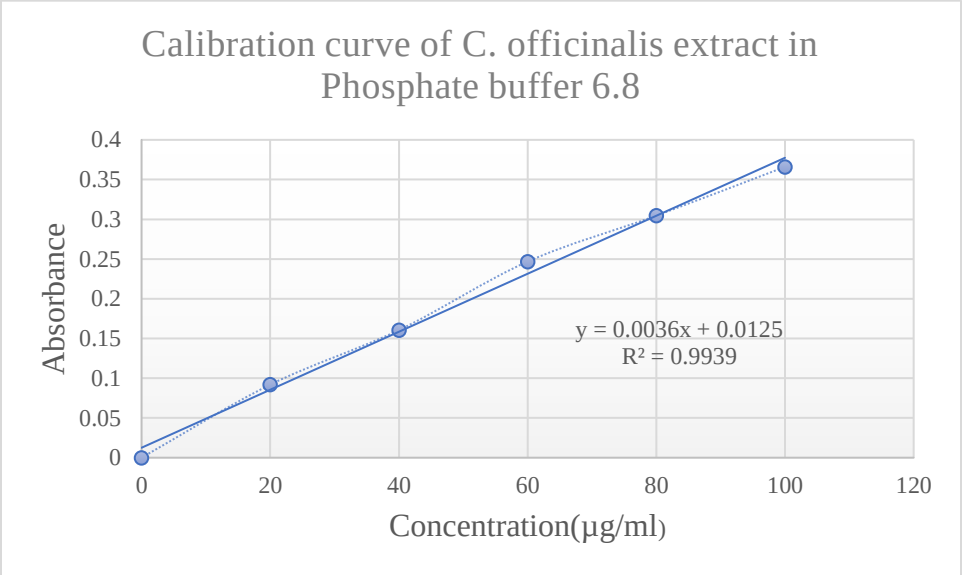

Fig 5: Calibration curve of *Calendula officinalis*

5.2.4 Compatibility study:

Study of interaction of the extract with excipients by FTIR study:

Table 10: Interpretation of FTIR data

Functional group	**Wavenumbers observed (cm^{-1})**		
	C. officinalis extract	**Extract + Carbopol 934**	**Extract + Carbopol 934+ Triethanolamine**
C-H (Stretch)	2921.48	2973.65	2926.60
C=O (Stretch)	1708.37	1704.94	1723.74
C=C (Stretch)	1452.98	1450.22	1453.44
C-O (Stretch)	1173.44	1172.96	1159.19
C-H (Bend)	812.91	877.27	879.08

The physicochemical compatibility study was carried out by infrared spectroscopy. IR spectral analysis of *C. Officinalis* extract showed peaks at 2921.48 cm^{-1} (C-H stretching), 2850.78 cm^{-1} (C-H stretching), 1708.37 cm^{-1} (C=O stretching), 1452.98 cm^{-1} (C=C stretching), 1173.44 cm^{-1} C-O (stretching), 812.91.85 cm^{-1} (C-H bending), confirmed the purity of the drug with literature data. The IR spectra of Carbopol 934 showed peaks at 2926.48 cm^{-1} (O-H stretching), 1688.88 (cm^{-1} C=O stretching), 1160.50 cm^{-1} (C-O stretching), 792.78 cm^{-1} (=C-H bending), 656.50 cm^{-1} (-C=C-H:C-H bending). The IR spectra of Triethanolamine (TEA) showed peaks at 3301.86 cm^{-1} (C-H stretching), 1658.58 cm^{-1} (C=C stretching), 1152.36 cm^{-1} (C-O stretching), 1027.06 cm^{-1} (C-O stretching). The IR study of the physical mixture showed the peaks of the extract with some variations in the same range and indicating no chemical interaction.

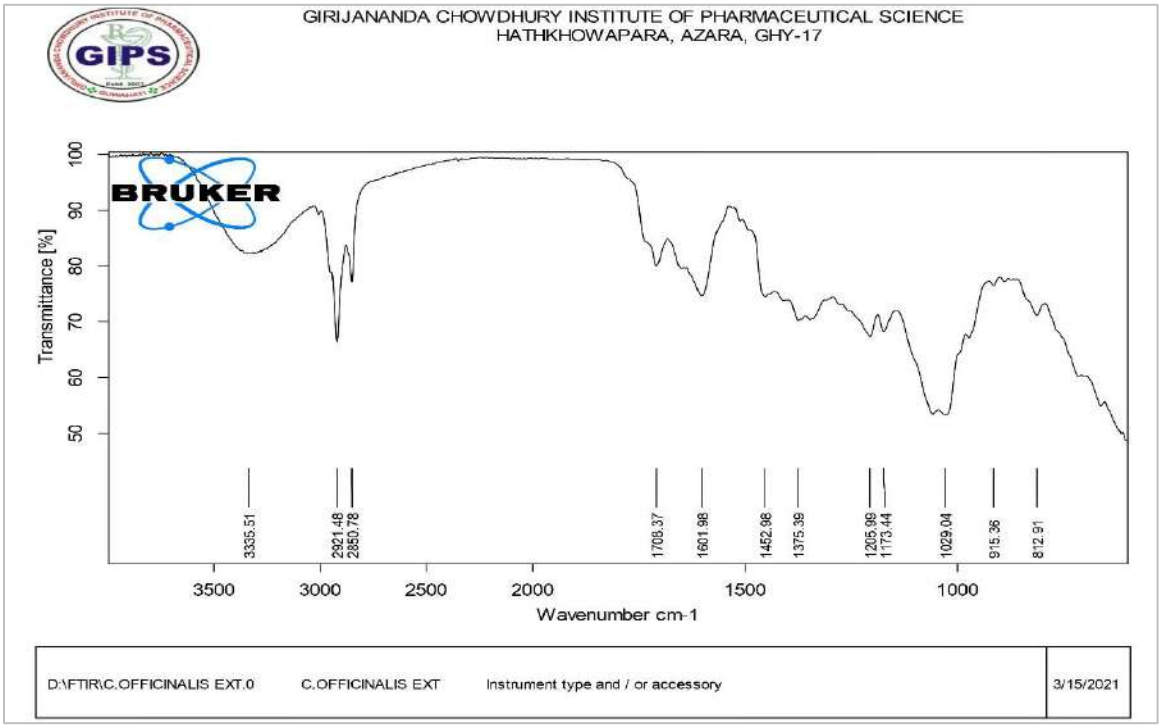

Fig 6: FT-IR spectra of *C. officinalis* extract

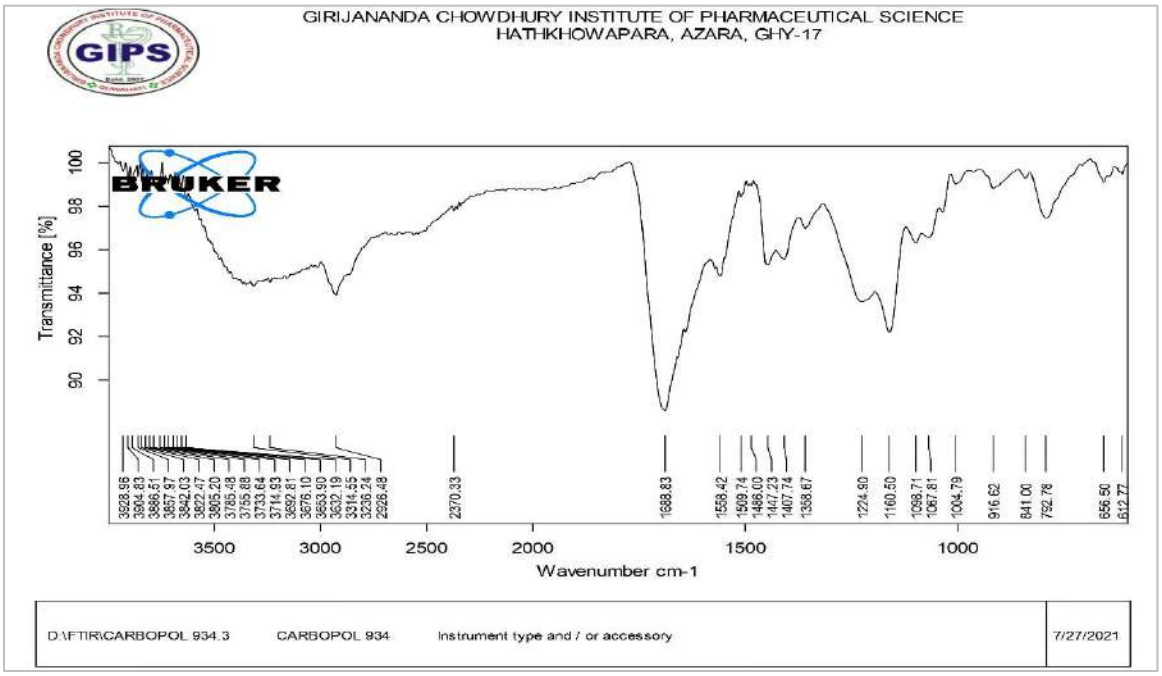

Fig 7: FT-IR spectra of Carbopol 934

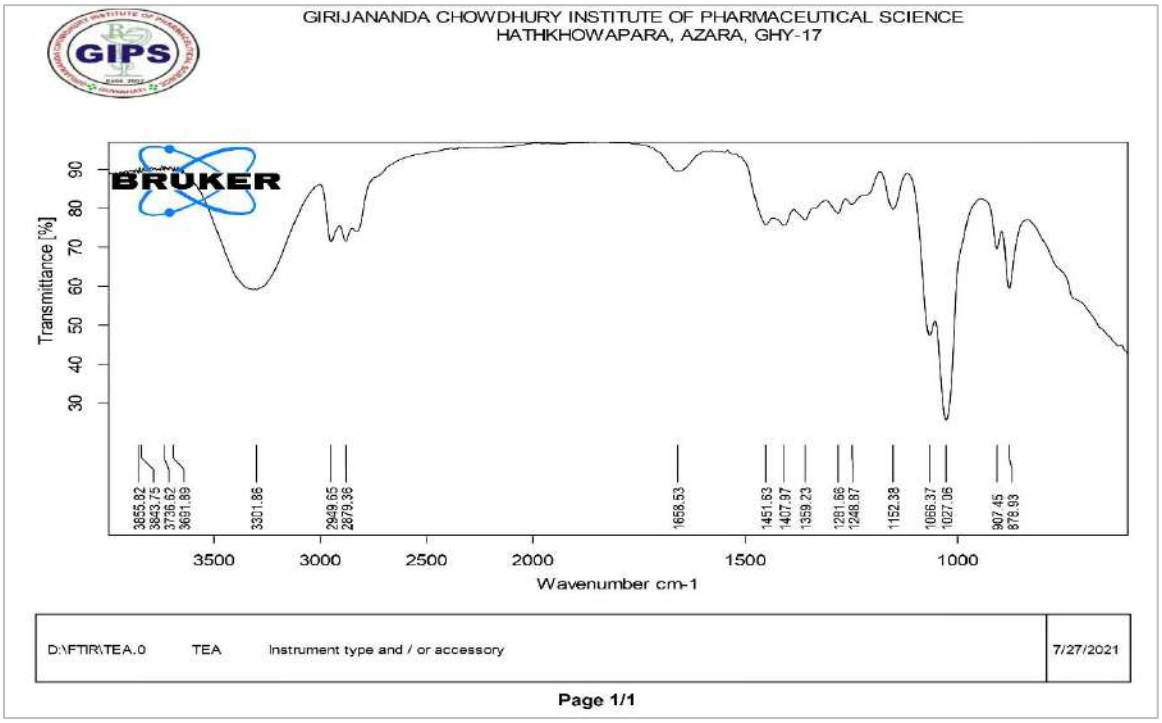

Fig 8: FT-IR spectra Triethanolamine

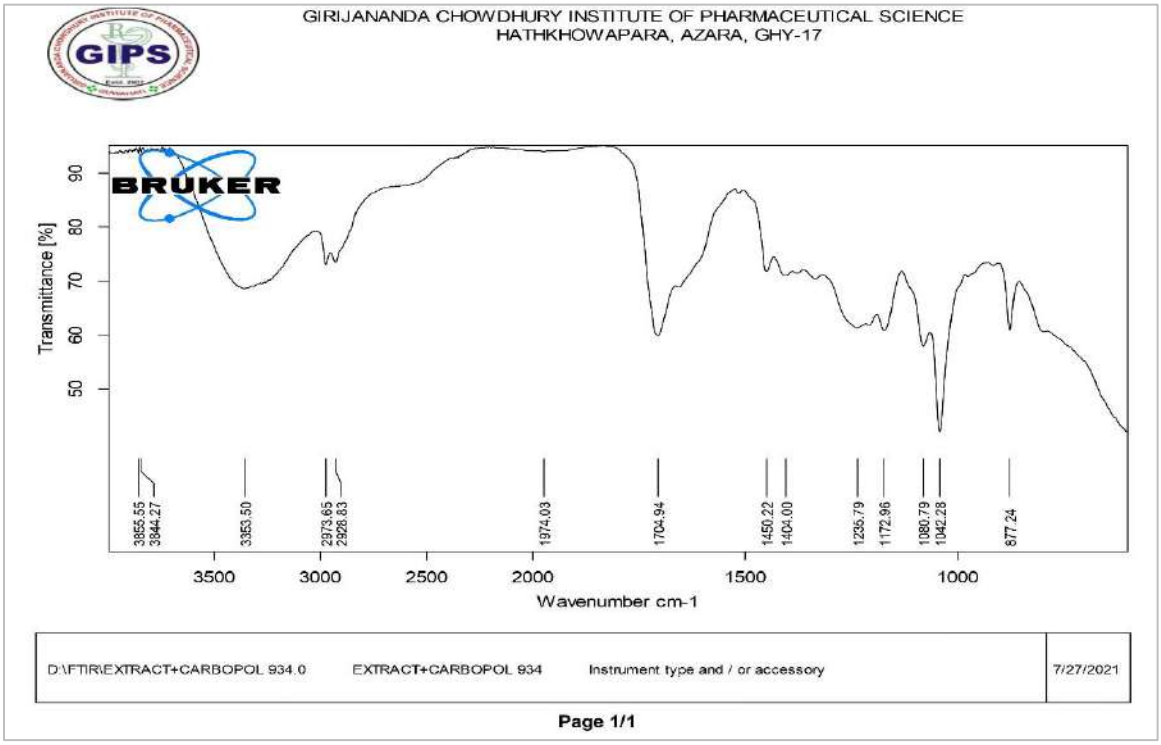

Fig 9: FT-IR spectra of *C. officinalis* extract + Carbopol 934

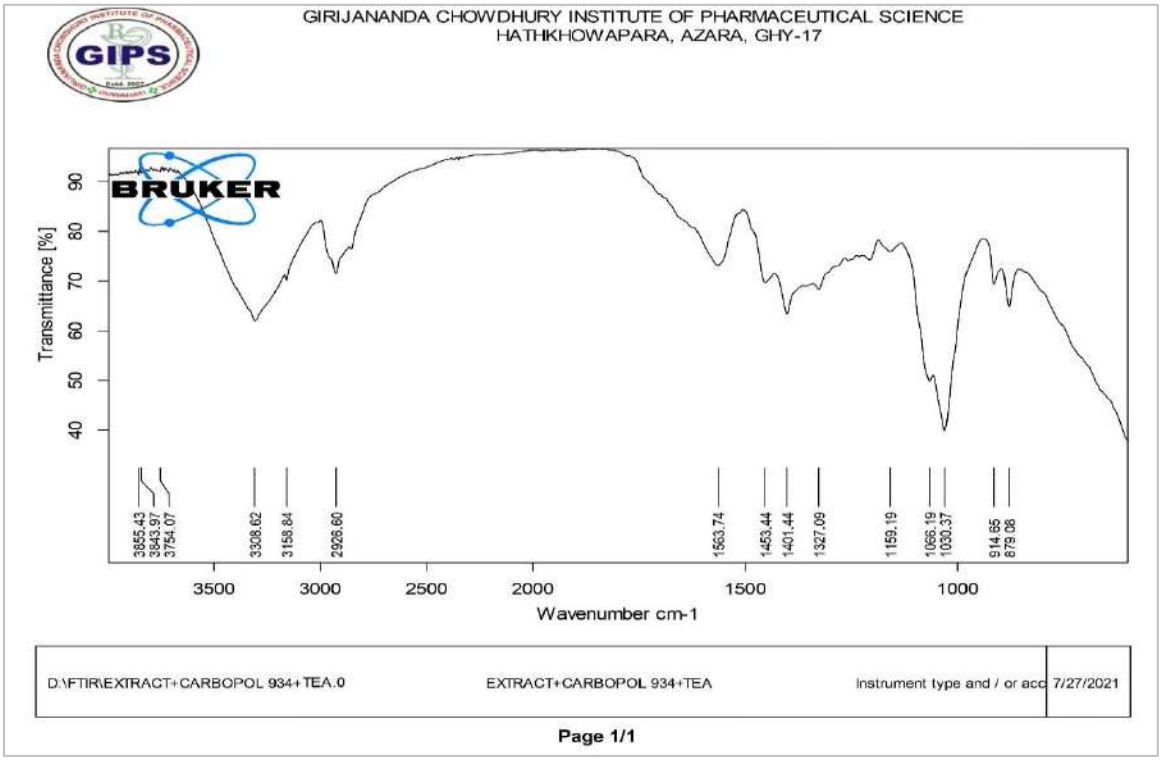

Fig 10: FT-IR spectra of *C. officinalis* extract + Carbopol 934 + Triethanolamine

5.3 Evaluation of gel

5.3.1 Physical appearance

Prepared formulation batches were found to be homogeneous green gel preparations.

5.3.2 Measurement of pH

Table 11: pH of prepared formulations

Formulations	pH
F1	6.7
F2	6.9
F3	6.8
F4	7.1

5.3.3 Spreadability

Table 12: Spreadibility study of prepared formulations

Formulations	Spreadibility (gm cm/sec)
F1	31.20
F2	32.15
F3	34.61
F4	33.50

5.3.4 Viscosity Study

Table 13: Viscosity of prepared formulations

Formulations	Viscosity (cp)
F1	0.3852
F2	0.3672
F3	0.3587
F4	0.3795

5.3.5 Extrudability study

This method was used to analyze the force required to squeeze the content out of the tube and the amount of content squeezed. All the formulations showed a good extrudability. There is no significant change in the extrudability.

CHAPTER-6

CONCLUSION

6. Conclusions

The utility of gel-based herbal drug delivery systems is being employed in the recent past for the therapeutic effectiveness of the topically applied formulation. The drugs were locally or systemically administrated to help wound healing. Antibiotics, antiseptics agents, etc. have been used to wound healing. Some synthetic drugs are faced with limitations because of their side effects. There is increasing interest to use medicinal plants in wound healing because of lower side effects and management of wounds over the years. *Calendula officinalis* is being traditionally used in wound management. It has anti, anti-oxidative properties. The topical route for *Calendula officinalis* was selected up to avoid the first-pass metabolism, avoids fluctuation in drug levels, the ability to deliver the drug more selectively to a specific site, and maximize the drug concentration at the site of action. In the present study, an attempt was made to formulate and evaluate topical herbal gels of *Calendula officinalis* plant leaf extract. In our preliminary study, the standardization of *Calendula officinalis* was carried out for purity and identity by FT-IR study. The pre-formulation studies include extraction, phytochemical screening of extract, organoleptic properties, determination of absorption maxima, preparation of standard calibration curve, compatibility study with excipients were carried out. In the phytochemical investigation, it is found have that the *Calendula officinalis* plant leaf extract consists of flavonoids, glycosides, triterpenoids, carbohydrates. The flavonoids phytoconstituents include Quercetin, Isoquercetin, Rutin, Isorhamnetin, Calendoflavoside, Narcissin. These phytoconstituents possess antiproliferative, anti-inflammatory, antiviral, antioxidant activities. In the compatibility study with FT-IR of the extract with excipient used in the formulation indicating no chemical interaction. Physicochemical evaluations like physical appearance, measurement of pH, spreadability, viscosity are performed and found to be optimum. After performing phytochemical screening and with the context of literature review we can expect that the *Calendula officinalis* plant leaf extract possesses the wound healing activity. Further in-vitro antimicrobial activity studies and in-vivo wound healing studies with suitable animal models is necessary to determine whether Calendula officinalis has a potential role in standard wound management

BIBLIOGRAPHY

Bibliography:

1. Givol O, Kornhaber R, Visentin D. A systematic review of Calendula of fi cinalis extract for wound healing. 2019;1–14.

2. Cristina A, Gonzalez DO. Wound healing - A literature review *. (Figure 1):614–20.

3. Preethi KC, Kuttan R. WOUND HEALING ACTIVITY OF FLOWER EXTRACT. :73–80.

4. Velnar, Tomaz, T. Bailey, and V. Smrkolj. 2009. "The Wound Healing Process: An Overview of the Cellular and Molecular Mechanisms." Journal of International Medical Research 37(5):1528–42. doi: 10.1177/147323000903700531.

5. Robson MC, Steed DL, Franz MG: Wound healing: biologic features and approaches to maximize healing trajectories. Curr Probl Surg 2001; 38: 72 – 140.

6. Lazurus GS, Cooper DM, Knighton DR, et al: Definitions and guidelines for assessment of wounds and evaluation of healing. Arch Dermatol

7. AO Foundation (Arbeitsgemeinschaft fuer Osteosynthesefragen/Association for the Study of Internal Fixation). Available at: http://www.aofoundation.org/wps/portal/.

8. Szycher M, Lee SJ: Modern wound dressings: a systematic approach to wound healing. J Biomater Appl 1992; 7: 142 – 213.

9. Vanwijck R: Surgical biology of wound healing. Bull Mem Acad R Med Belg 2001; 115: 175 – 184.

10. Degreef H: How to heal a wound fast. Dermatol Clin 1998; 16: 365 – 375.

11. Bischoff M, Kinzl L, Schmelz A: The complicated wound. Unfallchirurg 1999; 102: 797 – 804 [in German].

12. Attinger CE, Janis JE, Steinberg J, et al: Clinical approach to wounds: debridement and wound bed preparation including the use of dressings and wound-healing adjuvants. Plast Reconstr Surg 2006: 117(7 suppl): 72S – 109S.

13. Broughton G 2nd, Janis JE, Attinger CE: Wound healing: an overview. Plast Reconstr Surg 2006; 117(7 suppl): 1e-S – 32e-S.

14. Strecker-McGraw MK, Jones TR, Baer DG: Soft tissue wounds and principles of healing. Emerg Med Clin North Am 2007; 25: 1 – 22.

15. Lawrence WT: Physiology of the acute wound. Clin Plast Surg 1998; 25: 321 – 340.

16. SJ, Beasley RW, Thorne CH, eds). Philadelphia: Lippincott–Raven, 1997; pp 3 – 12.

17. Diegelmann RF, Evans MC: Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci 2004; 1: 283 – 289.

18. Landén, Ning Xu, Dongqing Li, and Mona Ståhle. 2016. "Transition from Inflammation to Proliferation: A Critical Step during Wound Healing." Cellular and Molecular Life Sciences 73(20):3861–85. doi: 10.1007/s00018-016-2268-0.

19. Lau K, Paus R, Tiede S, Day P, Bayat A (2009) Exploring the role of stem cells in cutaneous wound healing. Exp Dermatol 18(11):921–933

20. Jacinto A, Martinez-Arias A, Martin P (2001) Mechanisms of epithelial fusion and repair. Nat Cell Biol 3(5):E117–E123

21. Schultz GS, Wysocki A (2009) Interactions between extracellular matrix and growth factors in wound healing. Wound Repair Regen 17(2):153–162

22. Jyothi RB, Naganathan L, Chaitanya B, Naga A, Sunitha V. Review Article Various Pharmacological Actions of Calendula officinalis , Tagetes erecta , Carica papaya , Hypericum perforatum and Salvia officinalis. 2019;59(08):42–51.

23. Singh Y, Gupta A, Kannojia P. Tagetes erecta (Marigold) - A review on its phytochemical and medicinal properties. 2020;4(1):1–6.

24. Jan N, Andrabi KI, John R. Calendula officinalis - An Important Medicinal Plant with Potential Biological Properties. 2017;(4):769–87.

25. Dinda M, Mazumdar S, Das S, Ganguly D, Dasgupta UB, Dutta A, et al. The Water Fraction of Calendula officinalis Hydroethanol Extract Stimulates In Vitro and In Vivo Proliferation of Dermal Fibroblasts in Wound Healing. 2016;(June).

26. Paul S, Rahman ANMA, Al Mahmud MA, Uzzal AR, Das ZC. Wound healing by marigold (Calendula officinalis) and turmeric (Curcuma longa) paste: A comparative approach. J Adv Vet Anim Res. 2017;4(4):333–42.

27. Braun L. Calendula - Calendula officinalis. J Complement Med [Internet]. 2005;4(6):78–81.

28. Vinod M, Singh M, Pradhan M, Iyer SK, Tripathi DK. Phytochemical constituents and pharmacological activities of betula alba linn.- A review. Int J PharmTech Res. 2012;4(2):643–7.

29. World Health Organization. Herba Andrographidis. WHO monographs on selected medicinal plants, Volume 2. 2002. p. 12–24.

30. Order DC, Magnoliopsida M, Lamiaceae L, Rosmarinus R. Master data ingredient / monograph. Bhp 1996. 2007;1–5.

31. Baset MA. Digital Herbarium of Crop Plants. Digit Herb Crop Plants Establ [Internet]. 2017;2–6. Available from: http://dhcrop.bsmrau.net/?doing_wp_cron=1580991160.7416119575500488281250

32. Parente LML, Andrade MA, Brito LAB, De Dignan VMSB, Miguel MP, De Lino RS, et al. Angiogenic activity of Calendula officinalis flowers L. in rats. Acta Cir Bras.

33. Index-calendula KW. QUINONES AND a-TOCOPHEROL. 1976;15:125–7.

34. Zitterl-Eglseer K, Sosa S, Jurenitsch J, Schubert-Zsilavecz M, Della Loggia R, Tubaro A, et al. Anti-oedematous activities of the main triterpendiol esters of marigold (Calendula officinalis L.). J Ethnopharmacol. 1997;57(2):139–44.

35. Wojciechowski Z, Jelonkiewicz-Konador A, Tomaszewski M, Jankowski J, Kasprzyk Z. The structure of glycosides of oleanolic acid isolated from the roots of Calendula officinalis. Phytochemistry. 1971;10(5):1121–4.

36. Ukiya M, Akihisa T, Yasukawa K, Tokuda H, Suzuki T, Kimura Y. Anti-Inflammatory , Anti-Tumor-Promoting , and Cytotoxic Activities of Constituents of. J Nat Prod. 2006;69(12):1692–6.

37. Naved T, Ansari SH, Mukhtar HM, Ali M. New triterpenic esters of oleanene-series from the flowers of Calendula officinalis Linn. Indian J Chem - Sect B Org Med Chem. 2005;44(5):1088–91.

38. Vidal-ollivier E, Balansard G, Faure R, Babadjamian A. Revised structures of triterpenoid saponins from the flowers of calendula officinalis. J Nat Prod. 1989;52(5):1156–9.

39. Derkach AI, Komissarenko NF, Chernobai VT. Coumarins of the inflorescences of Calendula officinalis and Helichrysum arenarium. Chem Nat Compd. 1987;

40. Crabas N, Marongiu B, Piras A, Pivetta T, Porcedda S. Extraction, separation and isolation of volatiles and dyes from calendula officinalis l. and aloysia triphylla (l'her.) britton by supercritical co2. J Essent Oil Res. 2003;15(4):272–7.

41. Khalid K, Teixeira da Silva J. Biology of Calendula officinalis Linn.: focus on pharmacology, biological activities and agronomic practices. Med Aromat Plant Sci Biotechnol. 2012;

42. Yatsyno AI, Belova LF, Lipkina GS. On the pharmacology of Calendulozide B - a ned triterpene glycoside obtained from rhizomes of Calendula officinalis. Farmakol i Toksikol. 1978;

43. Bakó E, Deli J, Tóth G. HPLC study on the carotenoid composition of Calendula products. J Biochem Biophys Methods. 2002;53(1–3):241–50.

44. Gunasekaran S, Arul A, Nayagam J, Natarajan R. Wound healing potentials of herbal ointment containing Calendula officinalis Linn . on the alteration of immunological markers and biochemical parameters in excision wounded animals, 2020.

45. Younes B, Salem B. Investigation into the biological activities and chemical composition of Calendula officinalis L . growing in Tunisia, 2013; 20(6): 3001–7.

46. Bhat, S. K., and S. K. Bhat. 2012. "Bhat S. K et Al. IRJP 2012, 3 (11) INTERNATIONAL RESEARCH JOURNAL OF PHARMACY." 3(11):114–15.

47. Ruano, Paula, Lismet Lazo Delgado, Sergio Picco, Liliana Villegas, Franco Tonelli, Mario Merlo, Javier Rigau, Dario Diaz, and Martin Masuelli. 2016. "We Are IntechOpen , the World ' s Leading Publisher of Open Access Books Built by Scientists , for Scientists TOP 1 %." Intech (tourism):13.

48. Chandran PK, Kuttan R. Effect of Calendula officinalis flower extract on acute phase proteins, antioxidant defense mechanism and granuloma formation during thermal burns. J Clin Biochem Nutr, 2008; 43(2): 58–64.

49. Zhou ZB, Ding HQ, Qin FJ, Liu L, Cheng S. Effect of Zn7-metallothionein on oxidative stress in liver of rats with severe thermal injury. Acta Pharmacol Sin, 2003; 24(8): 764–70.

50. Jahdi F, Khabbaz AH, Kashian M, Taghizadeh M, Haghani H. The impact of calendula ointment on cesarean wound healing: A randomized controlled clinical trial. J Fam Med Prim care, 2018.

51. Kasprzyk Z, Wojciechowski Z. The structure of triterpenic glycosides from the flowers of Calendula officinalis L. Phytochemistry, 1967.

52. Avijgan M. Phytotherapy: an alternative treatment for non-healing ulcers. J Wound Care, 2004.

53. Buzzi M, Freitas F De, Winter MDB, Doctor M, Bioativos SA. Therapeutic effectiveness of a. J Wound Care, 2016; 25(12): 732–9.

54. Duran V, Matic M, Javanović M, Mimica N, Gajinov Z, Poljački M, et al. Results of the clinical examination of an ointment with marigold (Calendula Officinalis) extract in the treatment of venous leg ulcers. Int J Tissue React, 2005.

55. Kasiram K, Sakharkar PR, Patil AT. Antifungal activity of Calendula officinalis. Vol. 62, Indian Journal of Pharmaceutical Sciences, 2000; 464–6.

56. Arora D, Rani A, Sharma A. A review on phytochemistry and ethnopharmacological aspects of genus Calendula. Pharmacogn Rev, 2013; 7(14): 179–87.

57. Yoshikawa M, Murakami T, Kishi A, Kageura T, Matsuda H. Medicinal flowers. III. Marigold. (1): Hypoglycemic, gastric emptying inhibitory, and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A, B, C, and D, from egyptian Calendula officinalis. Chem Pharm Bull, 2001; 49(7): 863–70.

58. Yamini, K., and T. Onesimus. 2013. "Preparation and Evaluation of Herbal Anti-Acne Gel." International Journal of Pharma and Bio Sciences 4(2):1–30.

59. Aslani, Abolfazl, Behzad Zolfaghari, and Yeganeh Fereidani. 2018. "Design, Formulation, and Evaluation of a Herbal Gel Contains Melissa, Sumac, Licorice, Rosemary, and Geranium for Treatment of Recurrent Labial Herpes Infections." Dental Research Journal 15(3):191–200. doi: 10.4103/1735-3327.231865.

60. Nicolaus, C., Junghanns, S., Hartmann, A., Murillo, R., Ganzera, M., & Merfort, I. (2017). In vitro studies to evaluate the wound healing properties of Calendula officinalis extracts.Journal of Ethnopharmacology, 196(July 2016), 94–103. https://doi.org/10.1016/j.jep.2016.12.006

61. Parente, L. M. L., Lino Júnior, R. D. S., Tresvenzol, L. M. F., Vinaud, M. C., De Paula, J. R., & Paulo, N. M. (2012). Wound healing and anti-inflammatory effect in animal models of calendula officinalis L. growing in Brazil. Evidence-Based Complementary and Alternative Medicine, 2012. https://doi.org/10.1155/2012/375671

62. Shafeie N, Naini AT, Jahromi HK. Comparison of different concentrations of calendula officinalis gel on cutaneous wound healing. Biomed Pharmacol J, 2015; 8(2): 979–92.

63. Jan N, Andrabi KI, John R. Calendula officinalis - An Important Medicinal Plant with Potential Biological Properties, 2017; (4): 769–87.

64. Jafari, Behboud, and Changhiz Ahmadizadeh. 2017. "The In Vitro Study of Antimicrobial Effect of Marigold (Calendula Officinalis) Extract on Infectious Microorganisms." 13(4):348–52.

65. Padalia, Hemali, and Sumitra Chanda. 2015. "Antimicrobial Efficacy of Different Solvent Extracts of Tagetes Erecta L . Flower , Alone and in Combination with Antibiotics Applied Microbiology : Open Access." 1(1):1–10. doi: 10.4172/2471-9315.1000106.

66. Iraqui P, Chakraborty T, Das MK, Yadav R. Herbal antimicrobial gel with leaf extract of Cassia alata L. JDDT [Internet]. 15May2019 [cited 10Feb.2021];9(3):82-4. Available from: http://www.jddtonline.info/index.php/jddt/article/view/2527

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- Bordoloi SS, Chakraborty T, Das A, **Islam J**, Rynjah D and Baishya B.: The Applicability of Palm Trees in Pharmaceuticals as Excipients with a special emphasis on Palm Sugar: A Review. World Journal of Pharmaceutical Research. 2021; 10(6): 1778-1792.
- Baishya B, Rahman SS, Rynjah D, Bordoloi SS, **Islam J** and Hasan N.: Enhancing of Oral Bioavailability of Poorly water-soluble antihypertensive drugs. International Journal of Current Pharmaceutical Research. 2021; 13(4): 42-47.

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THE WOUND HEALING ACTIVITY OF CALENDULA OFFICINALIS: A REVIEW

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ABSTRACT

Calendula officinalis belonging to the family Asteraceae, a commonly available medicinal plant, that has been traditionally found to have activity against several diseases counting skin infection, wounds, burns, and ulcers. Since the Calendula officinalis contains secondary metabolites like terpenoids, flavonoids, and carotenoids, which may have the potential activity in wound healing. It also possesses anti-cancer, anti-inflammatory, hepatoprotective, spasmolytic, and spasmogenic properties. Wound healing involves a composite series of interactions amid diverse cell types, cytokine mediators, and the extracellular matrix. The cascade of wound-healing actions starts with homeostasis, inflammatory cell conscription cell proliferation, and restoration. To compile existing knowledge on Calendula officinalis and highlight its wound-healing function, we focused at its phytochemistry, wound healing activities, including burns and ulcers, and toxicological data in this study. Conclusively, further research is necessary to determine whether Calendula has a potential role in standard wound management.

KEYWORD: Calendula officinalis, Wound healing, Burns, Ulcers.

INTRODUCTION

Wound healing can be simply defined as healing of the skin layers. The progression of wound healing starts instantly after any injury or cut to the skin's epidermal layer. Wounds exist as an important global growing medical problem requiring dedicated care services. Wounds have various pathophysiology, with sorting grouped according to periods, which is divided