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Formulation and Evaluation of Polyherbal Ointment for Its Wound Healing Activity

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I. INTRODUCTION

The human skin is the largest organ in the body and represents its first line of defense. Beside protection, the skin has two other main functions; regulation and sensation. More specifically, it provides protection from mechanical impacts and pressure, limits the influence of variations in the temperature, fights microorganism infections, restricts radiations effects and prevents entrance of chemicals [3].

A wound is a disruption of living tissue‘s cellular, anatomical, and functional integrity caused by physical, chemical, electrical, or microbial threats to the tissue.[1] It may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue. When skin is torn, cut, or punctured it is termed as an open wound and when blunt force trauma causes a contusion, it is called closed wound, whereas the burn wounds are caused by fire, heat, radiation, chemicals, electricity, or sunlight [18].

CLASSIFICATION OF WOUNDS [1]

Wounds are generally classified according to the underlying cause of the development of wounds.

- ACUTE WOUNDS

Acute wounds, there is tissue damage/injury that generally occurs through an orderly and time-reparative phase that results in the anatomical and functional integrity being restored sustainably. Acute wounds are typically caused by the cuts or surgical incisions.

- CLOSED WOUNDS

The blood escapes from the circulatory system in closed wounds but stays inside the body. It becomes evident in the form of bruises.

- OPEN WOUNDS

Blood leaks from the body through an open wound and bleeding is clearly noticeable. The open wound may be divided further into categories according to the source causing the wound.

- INCISED WOUNDS

This is a wound with no loss of tissue and minor damage to tissue. It is caused primarily by sharp objects like a scalpel or knife.

- TEAR OR LACERATION WOUNDS

This is the non-chirurgical injury in conjunction with other types of traumas which results in tissue loss and damage.

- PUNCTURE WOUNDS

These are caused by an object which, like a nail or a needle, which punctures the skin. Since dirt may penetrate deep into the wound, chances of infection are common in them.

- ABRASIVE OR SUPERFICIAL WOUNDS

Sliding slip onto a rough surface induces abrasion. During this time, abrasion is scraped off the top layer of the skin, i.e., epidermis which exposes nerve endings resulting in a painful injury.

- PENETRATION WOUNDS

Penetration wounds are chiefly caused by an object like a knife going in and out of the skin.

- GUNSHOT WOUNDS

They are typically produced by bullet or similar projectile which drives through or into the body.

- CHRONIC WOUNDS

Chronic wounds are wounds that have not gone through the usual healing stages and hence reach a state of pathologic inflammation. They need extended healing time [12].

WOUND HEALING

Wound healing is defined as a complex process occurring by regeneration or reconstruction of damaged tissue. The normal response to wound healing is a concerted sequence of events that begins with an injury. When platelets come into contact with exposed collagen, the healing cascade

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is initiated, causing the accumulation of platelets as well as the release of coagulating factors which in turn result in the formation of a fibrin clot at the injury site. The fibrin clot functions as a temporary matrix which sets the tone for activities that accompany healing. Inflammatory cells, along with the platelets provide essential signals known as cytokines or growth factors; also arrive at the injury site. The fibro blast is the connective tissue responsible for collagen deposition that is needed to fix tissue damage. Collagen provides strength, integrity, and structure in normal tissues. Collagen is required to repair the defect and restore anatomical structure and function when tissues are damaged after injury. If healing does not progress stepwise in the usual way, then it can lead to chronic growth of wounds.

Infections are known as one of the most important factors influencing efficiency of wound healing. It is reported that a high percentage of wound related complications and hence costs in wound care, can be directly linked to infected wounds. Reducing the bacterial load may be one of the most important necessary requirements for better wound healing, since wound infection (either secondary or primary by opportunistic microorganisms) can lead to reduction of local inflammation and consequentially avoid tissue destruction. An ideal medicine for prevention of wound infection should act antimicrobial and at the same time stimulate the body's natural immune activity without damage to surrounding healthy tissue [3].

Reported patents and articles state that various herbal formulations help accelerate the wound healing process and are useful in its treatment. Medicinal plants such as Curcuma longa (L.) and Gum acacia have confirmed wound healing activity and are found to be effective in the treatment of wounds. Natural medicinal products and plant extracts have been widely used as topical applications for wound healing. Use of plants as a source of drug was the backbone of traditional medicine [9].

A poly herbal formulation consists of more than one herb. It is known that plants have different phytoconstituents which are responsible for the various activities that are attributed to them and when a combination of plants with these constituents are combined together it may show better activity when compared to the individual extract. But at the same time presence of many constituents may lead to chemical incompatibility which may result in instability. Hence it is a challenging task to formulate a stable polyherbal formulation [9].

PHYTOCHEMICALS

Phytochemicals, naturally occurring substances found in plants, react with oxygen groups or other biologic macro-molecules to instigate biological effects and combat human diseases. Several research groups across the world have shown that phytochemicals may play a critical role in preventing and/or treating a number of deadly diseases such as cancer and inflammatory conditions and may also provide medicinal benefits for wound healing and skin regeneration as shown in table no. 1 [2].

Phytochemicals are well known for their chemo preventive properties and are found to be beneficial in treating various disorders, including skin diseases. Phytochemicals protect the skin by quenching free radicals and reducing inflammation through the inhibition of NF-kB. Phytochemicals also affect other signaling pathways, including transforming growth factor-bet and mitogen activated protein kinase pathway. Extensive research has increased the disease portfolio for which phytochemicals may be beneficial, and the identification of molecular targets will help future clinical trials in the development of phytochemicals as an important therapeutic agent [2].

Table no. 1 Comprehensive details on role of phytocompounds /naturally derived substances in wound healing studies [22].

Name of Phyto-compound	Type of formulation	Uses /applications in wound healing	Possible mechanism of action	Typeof study or wound model used	Outcome

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Vitamin A	Topical and systemic	Anti-inflammatory property; Necessary for growth, differentiation, and maintenance of epithelial tissues	Influencesmorphogenesis, epithelial cell proliferation and differentiation in time and dose dependent manner	Preclinical and clinical	Prevents and treats infectious as well as inflammatory skin diseases
Vitamin E	Oral and topical	Used for resurfacing of the skin	Modulate cellular signaling, gene expression	Diabetic rats	Increased wound contraction
Vitamin C	Plant extract	Modulators of angiogenesis & collagen production; critically important for tensile strength of a wound	Act through induction of protein-kinase-C-dependent pathway that activates protein-1 DNA binding activity; hydroxylation of lysine and proline during the synthesis of collagen	In vitro	Stimulated growth of keratinocytes
Alkaloids	Topical	Anti-inflammatory effects	Stimulate the growth of colonies from fibroblast precursors	In vitro and in vivo	Promotes early phases of wound healing (≤7 days) in a dose-dependent manner
Silymarin (polyphenol)	Topical ointments	Antioxidant properties	Help to prevent oxidative damage, increase epithelialization of wounds	Streptozotocin-induced experimental diabetic rats	Reduced inflammation in the wound that promoted the healing process
Flavonoids	Pure Phytocompound/extract	Antioxidant, anti-allergic, anticarcinogenic, anti-viral and anti-inflammatory agents	Involve hydrogen bonding and hydrophobic interactions	In vitro	Collagen fibers treated with catechin are stable
Tannins (phenolic compound)	Topical ointments	Act as astringents	Astringent property is responsible for wound contraction and increased rate of epithelialization at the granulation formation and scar	In vitro and preclinical	Significant effect in wound closure and wound healing rate

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			remolding phases		
Terpenoids	Topical	Modulators of cytokines and growth factors	Increase in cell migration; increased collagen synthesis and tensile strength of wound tissues	Diabetic animals and clinical	Enhanced rate of wound healing
β-sitosterol	Extract	Plant-derived angiogenic factor	Stimulates neovascularization and motility of human umbilical vein endothelial cells	In vitro (chick embryo	Showed potent angiogenic activity
Kaempferol and quercetin (flavonoid)	Extract	Promising compounds for scar reduction; Regulators of extracellular matrix	Inhibition of fibroblast activities	In vitro and in vivo	Reduced scar formation

DRUG PROFILE
VACHELLIA NILOTICA

Many studies reported that Acacia nilotica is a great source of many active secondary metabolites, which may serve as potential candidates for drug business in future. All parts of the Acacia nilotica have medicinal properties as described in Table 1. Apart from that, Acacia nilotica presents a great source of gum production which may serve also as potential candidates for gum industry [4].

Fig no. 1. Vachellia nilotica

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TAXANOMICAL CLASSIFICATION

- Kingdom: Plantae
- Subkingdom: Tracheobionta
- Super division: Spermatophyta
- Division: Magnoliopsida
- Subclass: Rosidae
- Order: Fabales
- Family: Fabaceae
- Genus: Acacia
- Species: nilotica
- Synonyms: Gum Arabic tree, Gum acacia, Babool tree

DESCRIPTION

Babul (Acacia nilotica (L.) Wild. ex-Delille) is a medium sized, thorny, nearly evergreen tree that can reach a height of 20-25 m but may remain a shrub in poor growing conditions. The trunk is short, thick (1 m in diameter) and cylindrical, covered with grey bark. The crown may be flattened or rounded. The root system depends on the growing conditions and subspecies: a deep taproot in dry conditions and extensive lateral roots in flooded conditions. The leaves are 5-15 cm long, alternate and compound with 7 to 36 pairs of elliptical, 1.5-7 mm long x 0.5-2 mm broad, grey-green, hairy leaflets. Flowers are sweetly scented and bright to golden yellow in colour. The fruits are linear, flattened, narrow indehiscent pods, 4-22 cm long and 1-2 cm broad, dark-brown to grey in colour and glabrous or velvety. The pods contain 8 to 15 elliptical, flattened bean-shaped dark seeds. There are two groups of Acacia nilotica subspecies. The first group (nilotica, tomentosa, cupressiformis, indica) consists of tall riverine trees that grow in seasonally flooded areas. Their pods have a characteristic "necklace" shape with constrictions between the seeds. The second group (adstringens, kraussiana, leiocarpa, subalata) grows in drier areas and has straight-edged pods [19].

CHEMICAL CONSTITUENT

Earlier traditional description confirmed that A. nilotica has a rich amount of nutrients and contains a high therapeutic value which is capable of prevention, mitigation, and treatment of various infectious diseases and deleterious conditions. The studies based on the animal model established that A. nilotica and its chief phytoconstituents play a pivotal role in anti-bacterial, anti-inflammatory, anti-diabetic, anticancer, and anti-hypertensive management. It is considered a safe medicinal plant and modulates the numerous therapeutic actions without any adverse effect [8].

Acacia species contains secondary metabolites including amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, nonprotein amino acids, terpenes (including essential oils, diterpenes, phytosterol and triterpene genins and saponins), hydrolysable tannins, flavonoids and condensed tannins (Siegler, 2003) [6].

Table no. 2. Medicinal properties and chemical compounds extracted from Acacia nilotica [4].

Part of Acacia nilotica used medicinal purpose	**Medicinal properties and chemical compound**
Stem bark	Sexually transmitted diseases, bark is reported to have antibacterial, antioxidant, anti-mutagenic, and cytotoxic activity.
Fruits	The fruits are sold for medicinal purposes some West African countries such as Niger, Nigeria and Ghana.
Leaves	The leaves are used as antibacterial, chemo preventive, astringents, anti-inflammatory and as anti- Alzheimer.

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Flower	53 phytoconstituents present in petroleum ether extract of Acacia nilotica flower, flowers are used in gastrointestinal disorders.
Roots	The root is used used against tuberculosis and tumors of ear, eye, and testicles.
All parts of the plant	All parts of the Acacia nilotica have medicinal properties.

MEDICINAL ACTIVITY OF ACACIA

ANTI-MICROBIAL POTENTIAL

A study was conducted to investigate the in vitro antibacterial activity of Acacia nilotica methanolic fruits extract against clinical isolates performed by cup-plate agar diffusion method against five gram-negative bacteria (E. coli, S. flexneri, Salmonella typhi, Pseudomonas aeruginosa, and Klebsiella pneumonia) and 2 gram-positive bacteria i.e., Listeria monocytogenes and Bacillus cereus. Out of 7 cultures tested, it showed good activity against Salmonella typhi and Bacillus cereus. The authors concluded that the methanolic fruit extract of A. nilotica showed significant inhibition against grampositive and gram-negative species. One of the studies found that the methanolic extracts of A. nilotica pods were most active against different bacterial and fungal strains. The methanolic extract of pods showed the highest activity against E. coli, S. aureus and A. Niger [8].

The antimicrobial property of 50 percent aqueous ethanolic leaf extract of A. nilotica (L.) exhibited antifungal property against Rhizoctonia solani. A. nilotica demonstrated the highest activity against three bacterial strains (E. coli, S. aureus and Salmonella typhi) and two fungal strains (Candida albicans and Aspergillus niger). Pods and leaf extracts exhibited the anti-viral effect. Pods of A. nilotica were reported to inhibit HIV-1 induced cytopathogenicity [8].

ANTI-INFLAMMATORY ACTIVITY

Traditionally, A. nilotica is used in various inflammatory conditions like bronchitis, pharyngitis, vaginitis, and conjunctivitis as it possesses Muhallil al waram (anti-inflammatory) property. The decoction of the bark is locally useful in cystitis, and vaginitis. The juice of bark mixed with breast milk is dropped into the eye in conjunctivitis. Theointment of the young leaf around the eyes is beneficial in Ashob-ichashm harr (Acute conjunctivitis). It is used in ophthalmia, tender leaf fried in ghee and wrapped around the eyes in chronic ophthalmia and subconjunctival haemorrhage. The bruised leaves are applied to sore eyes in children. The tender leaves growing tops rubbed into a paste with sugar and water and given two times a day are useful in cough. The bark is also used in asthma and bronchitis [8].

ANTI-BACTERIAL ACTIVITY

The methanol leaf and bark extracts of Acacia nilotica showed antibacterial activity against Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus and Xanthomonas axonopodis pv. Malvacearum.38 Amin et al (2013) has studied Methanol, acetone and water extracts of different parts of Acacia nilotica (L.) Delile, Calotropis procera (Aiton) W.T. Aiton, Adhatoda vasica Nees, Fagoniaar abica L. and Casuarina equisetifolia L. to evaluate the anti-bacterial activity against thirty-four clinical isolates and two reference strains of H. pylori. Minimum inhibitory concentrations (MICs) of the extracts were determined using the agar dilution method and compared with some standard antibiotics like amoxicillin, clarithromycin, tetracycline and metronidazole, used in the triple therapy for H. pylori eradication [21].

A recent study reported a bactericidal effect of the ethanolic extract of Acacia leaves alone or in combination with extracts of some other plants (Psidium guajava, Eucalyptus hybrid, and Murraya koenigii L. Sprengel) versus Porphyromonas gingivalis and Fusobacterium nucleatum using the method of agar well diffusion. The combination of the Acacia extract with the other extracts was more effective than Acacia extract alone, but the inhibition zones remained more with chlorhexidine. The superiority of the

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combination is due to synergism and reduced antibiotic resistance to bacteria [5].

ANTI-FUNGAL ACTIVITY

The extracts, produced by 80% methanol, from leaf, bark and seed of three medicinal plants namely neem (Azadirachta indica A. Juss), kiker (Acacia nilotica L.) and jaman (Eugenia jambolana L.), were assessed for their antifungal activities against two fungal strains viz. Aspergillus flavus and Aspergillus parasiticus using disc diffusion method and micro dilution broth susceptible assay. Result showed all the extracts exhibited inhibitory effect against A. flavus and A. parasiticus. Mohan Lal Saini et al (2008) examined comparative antimicrobial studies of Acacia species and A. nilotica exhibited highest activity against two fungal strains Candida albicans and Aspergillus Niger. The crude methanolic plant extract of A. arabica showed considerable anti-fungal activity against Streptococcus cerevisiae. Dried fruits are active against C. albicans and used to treat oral candidiasis. Its fatty oil and unsaponifiable matter were found to possess antibacterial and antifungal properties [21].

CURCUMA LONGA

Turmeric (Curcuma longa L.) is a popular natural drug, traditionally used for the treatment of a wide range of diseases. Its root, as its most popular part used for medicinal purposes, contains different types of phytochemicals and minerals. In short, curcumin is considered as the fundamental constituent in ground turmeric rhizome. Turmeric possesses several biological activities including anti-inflammatory, antioxidant, anticancer, antimutagenic, antimicrobial, antiobesity, hypolipidemic, cardioprotective, and neuroprotective effects [23].

Fig no. 2. Curcuma longa

TAXANOMICAL CLASSIFICAION

- Kingdom: Plantae
- Subkingdom: Tracheobionta
- Super division: Spermatophyta
- Division: Mangoliophyta
- Order: Zingiberales
- Family: Zingiberaceae
- Genus: Curcuma
- Species: Longa
- Synonyms: Turmeric, Safflower, Yellow ginger, Haldi

DESCRIPTION

Turmeric has been used in Asia for centuries and is a major part of Ayurveda, Siddha medicine, traditional Chinese medicine, Unani, and the animistic rituals of Austronesian peoples. It was first used as a dye, and then later for its supposed properties in folk medicine. Turmeric is a perennial herbaceous plant that reaches up to 1 m (3 ft 3 in) tall. It has highly branched, yellow to orange, cylindrical, aromatic rhizomes. The leaves are alternate and arranged in two rows. They are divided into leaf sheath, petiole, and leaf blade. From the leaf sheaths, a false stem is formed. The petiole is 50 to 115 cm (20–45 in) long. The simple leaf blades are usually 76 to 115 cm (30–45 in) long and rarely up to 230 cm (7 ft 7 in). They have a width of 38 to 45 cm (15 to 17+1⁄2 in) and are oblong to elliptical, narrowing at the tip. The greatest diversity of Curcuma species by number alone is in India, at around 40 to 45

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species. Thailand has a comparable 30 to 40 species. Other countries in tropical Asia also have numerous wild species of Curcuma [20].

HISTORY OF TURMERIC

Marco polo in 1280 named turmeric as an Indian saffron which is used as a colorant. In India, at the time of Lord Ram Chandra turmeric was used for face protection during sun worship. Turmeric has a history of 6000 years for its use in medicine. Turmeric is mostly cultivated in India, China, Thailand, Bangladesh, Malaysia and Indonesia. It is also cultivated in tropical areas of America and Africa. Turmeric has been proved helpful in number of health complications and in different pathological conditions [7].

CHEMICAL CONSTITUENT:

Curcumin, a polyphenol derived from Indian dietary spice turmeric (the common name for Curcuma longa L., Zingiberaceae family), has been widely used asherbal remedy for centuries in indigenous medicine to treat a variety of inflammatory conditions and other diseases. The active medicinal ingredient of turmeric has been identified as curcuminoids, which includes an active components curcumin (diferuloylmethane)-(1,7bis(4-hydroxy-3-methoxyphenyl)-1,6hepadine3,5-dione) and is found to be beneficial in treating various disorders, including skin diseases. It has also been reported that curcumin possesses anti-inflammatory, antioxidant, and antiproliferative properties that are mediated through the regulation of several inflammatory cytokines, growth factors, protein kinases, transcription factors, and other enzymes.9 In addition, it has been shown that curcumin also induces apoptosis through mitochondrial and receptor-mediated pathways, as well as through activation of caspase cascades [2].

Turmeric is a prompt source of bioactive compounds like antioxidants, polyphenols and flavonoids, which may be the substitute of antibiotics used in food and food products. Turmerone, zingiberene, ar-turmerone and curlone are included in volatile constituents of turmeric while the nonvolatile volatile constituents of turmeric while the nonvolatile [7].

MEDICINAL ACTIVITYOF CURCUMA LONGA

The reviews by Thangapazham et al. and Maheshwari et al. described the various biological activities of curcumin. It has already been shown that curcumin protects the skin by quenching free radicals and reducing inflammation through NF-jB inhibition. Curcumin reduces not only the wound-healing time but also improves the collagen deposition, increases fibroblast number and vascular density. Curcumin also acts as a pro-angiogenic agent during wound healing and wound repair by regulating TGF-beta [2].

It has been used for various dermal infections and is a part of the South Asian diet. Curcumin, its major bioactive component, promotes wound healing by increasing fibroblast production, granulation tissue formation, collagen deposition, tissue remodeling, and increased vascular density and contraction of wound. Oral administration of turmeric has lesser bioavailability. However, topical administration is much effective as it is in direct contact with wound site [11].

ANTIMICROBIAL EFFECTS

Turmeric may be an alternative antimicrobial agent against fatal bacterial infections. The utilization of essential oil of turmeric leaves significantly inhibits fungal growth, as well as aflatoxins B1 and G1 production. Although curcumin is a very active agent, its reduced aqueous solubility hinders its applications. The nanocurcumin actually performs its antibacterial action by completely breaking the cell wall, leading to cell death. Curcumin antibacterial activity against multidrug-resistantAcinetobacter baumanniinoticeably increases in the presence of epigallocatechin gallate (EGCG). The combination of EGCG and curcumin can be used in medicine to avoid or control Acinetobacter baumannii infections [23].

ANTI-INFLAMMATORY

Oxidative stress has been implicated in many chronic diseases, and its pathological processes are closely related to those of inflammation, in that one can be easily induced by another. In fact, it is known that inflammatory cells liberate a number of reactive species at the site of inflammation leading to oxidative stress, which demonstrates the relationship between oxidative stress and inflammation. In addition, a number of reactive oxygen/nitrogen species can initiate an intracellular signaling cascade that enhances pro-inflammatory gene expression. Inflammation has been identified in the development of many chronic diseases and conditions [25].

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Tumor necrosis factor α (TNF-α) is a major mediator of inflammation in most diseases, and this effect is regulated by the activation of a transcription factor, nuclear factor (NF)-κB. Whereas TNF-α is said to be the most potent NF-κB activator, the expression of TNF-α is also regulated by NF-κB. In addition to TNF-α, NF-κB is also activated by most inflammatory cytokines; gram-negative bacteria; various disease-causing viruses; environmental pollutants; chemical, physical, mechanical, and psychological stress; high glucose; fatty acids; ultraviolet radiation; cigarette smoke; and other disease-causing factors. Therefore, agents that downregulate NF-κB and NF-κB–regulated gene products have potential efficacy against several of these diseases. Curcumin has been shown to block NF-κB activation increased by several different inflammatory stimuli. Curcumin has also been shown to suppress inflammation through many different mechanisms beyond the scope of this review, thereby supporting its mechanism of action as a potential anti-inflammatory agent [25].

ANTICANCEROUS EFFECTS

Cancer is the main leading cause of the death in many developing countries. Several epidemiological studies had shown that the incidence of cancer is less in the people who depend more on the vegetables and fruits. This outcome is owed to bioactive composites that are present in plant foods recognized as flavonoids. While Immense body evidences has revealed chemo-preventive potency of flavonoids. Oxidative stress in the body cause harm to DNA which leads to the genetic mutation. The mutation deviates normal cell division cycle and as a outcome unwanted cell aggregates forms that are known as tumor. Flavonoids prevent genetic mutation of DNA from oxidative stress via scavenging free radicals that are produced in the vicinity of DNA which cooperate with carcinoma formed by detoxification process [7].

SAFETY AND TOXICITY

Turmeric and its constituents were examined in many researches for their safety through in vitro studies, animal studies, and clinical trials. According to a comprehensive review on this subject, the administration of standardized powder/extract of turmeric and curcumin via oral route revealed no significant side effects or toxicities to animals. In addition, cell culture studies showed that ”curcumin has antiproliferative effect in normal cells and can reduce cell viability.” However, 001curcumin in human is safe, even at extraordinary doses. Itching, tongue redness, tachycardia, and gastrointestinal complaints (e.g., flatulence, diarrhea, nausea, and constipation) were reported in a small proportion of cases. It should be noted that there are several problems regarding bioavailability of oral curcumin. However, its intravenous formulations have a greater absorption. Therefore, intravenous curcumin should be administered at lower doses than oral use [23].

INGRIDEINT USED IN PREPARATION

1. METHANOL

Manufacturers of SHEs often favor the use of methanol for extraction of medicinal plants due to its lower boiling point, higher volatility, and higher extraction efficiency compared to ethanol (depending on the desired secondary metabolite composition).1-3 Methanol also is used as a co-solvent to enhance extraction efficiency in supercritical fluid extractions [17].

2. EMULSIFYING AGENT

An emulsifying agent is a compound that concentrates at the interface of two immiscible phases, usually an oil and water. It lowers the interfacial free energy, reduces the interfacial tension between the phases, and forms a film or barrier around the droplets of the immiscible, discontinuous phase as they are formed, preventing the coalescence of the droplets [14].

3. WHITE SOFT PARAFFIN

White Soft Paraffin is a soft unctuous mass and is poorly soluble in water and alcohols but is soluble in methylene chloride. As such it is a useful product to use in liquids, creams, emulsions and ointments, of which, ointments will only be considered within this body of work. WSP is an excellent carrier material (base) for active dermatological ingredients as well as acting as a barrier to the environment, providing moisturizing trapping, oily characteristics preventing evaporation of water to the environment. WSP is a mixture of hydrocarbons derived from petroleum [15].

4. LIQUID PARAFFIN

Liquid paraffin is a light fraction of kerosene and gas oil obtained from crude petroleum distillation and is a mixture of

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hydrocarbon alkane molecules ranging from C11-C24 (Freund et al., 1982). Liquid paraffin is added to white soft paraffin and is trapped within a three-dimensional crystal network made by crystalline waxes. When more liquid paraffin is added, greater slip qualities occur within the white soft paraffin allowing for a less viscous, more fluid, white soft paraffin to be produced [15].

5. AGAR-AGAR

The agar-agar is one of the most commonly used elements due to its suspensive, emulsifying, stabilizing and gelling properties that add no oil to the product, which is a veryimportant feature when developing skin products. In addition, it is non-toxic and non-irritating, essential for people with skin conditions [16].

AIM:

Formulation and evaluation of Polyherbal Ointment for Its Wound Healing Activity

OBJECTIVE:

The study has revealed that the polyherbal ointment has shown the wound healing effect due to the synergistic activity of the phytoconstituents present in the extract of Turmeric and Gum Arabic tree used as a potential herbal formulation for wound healing.

II. MATERIALS AND METHODS

COLLECTION OF PLANTS

The plants were collected from local area. The leaves were washed and allowed to dry under the shade. Plants were wrapped and stored until further use.The dry leaves were ground to coarse powder using mortar and pestle.

CHEMICALS AND REAGENT

- Emulsifying agent
- White soft paraffine
- Liquid paraffine
- Methanol
- Agar

Which were obtained from laboratory of Maharashtra Institute of Pharmacy Betala, Bramhapuri.

PREPARATION OF EXTRACT

The extracts were prepared by macerating the powders of the leaves in methanol for 48 hours.

Maceration- It is a process of preparation of herbal extract by soaking plant material in water, vegetable oil, or some organic solvent.

1. The whole or coarsely powdered, air-dried, and pulverized plant material is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 48 hours with frequent agitation until the soluble matter has dissolved.
2. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing [18].

FORMULATION OF OINMENT

The required quantity of the chemicals was weighed and the polyherbal ointment wasformulated by fusion method using emulsifying ointment base.

Fusion method

This method is suitable when base is solid.

Small Scale → Porcelain dish is placed on water bath.

Large Scale → Carried out in large steam-jacketed kettles.

Procedure:

The ingredients and base are melted and properly mixed to obtain a uniform product.

Initially the ingredient of highest melting point is melted then remaining are added in decreasing order of M.P.

i. Mixture is removed from water bath and stir to cool it.
ii. Insoluble drugs in base – added in powdered form
iii. Liquids or semisolids – added at a temp. of 40°C
iv. Volatile or heat-labile ingredients – added at last

Table no.3 Formulation for the ointment

DRUGS AND CHEMICALS	F1 (30gm)	F2 (30gm)
Gum Arabic tree extract	12ml	12ml
Turmeric	8.2ml	10ml

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Emulsifying agent	2.5gm	1gm
White soft paraffin	2.7gm	4gm
Liquid paraffin	1.8ml	3.5ml
Agar	2.8gm	1.5gm

EVALUATION OF THE POLYHERBAL FORMULATION

The polyherbal formulation was evaluated by the following physicochemical parameters [9].

1. COLOR, ODOUR, TEXTURE AND STATE:

This testwas examined by visual examination

2. LOSS AND DRYING:

Loss on drying was determined by placing the ointment in a Petri dish on a water bath and dried until constant weight was obtained.

3. pH:

The pH of the formulation was recorded using a digital pH meter. Weighed quantity of the sample was dissolved in distilled water and stored for two hours. The measurement of pH was done in triplicate and average values were considered.

4. SPREADABILITY:

The spread ability was expressed in terms of times in seconds taken by two slides to slip off from ointment placed in between the slides under the direction of certain load. Spread ability was calculated by using the formula.

S = (M.L/T)

Where, S = Spreadability, M = Weight tied to upper slide, L =

Length of glass slides and T = Time taken to separate the slides

5. DIFFUSION STUDY:

The diffusion study was carried out by preparing agar nutrient medium of known concentration. It was poured into a Petri dish and allowed to set. A hole was bored at the center of the Petri dish and the prepared formulation was placed in it. The time taken for the ointment to get diffused was noted.

III. RESULT AND DISCUSSION

A wound may be defined as the loss or rupture of the cellular, anatomical or functional continuity of living tissue.Wound healing is a complicated process. It involves stages like inflammation, wound contraction and epithelization.The aim of wound care is to promote wound healing in the shortest time possible. The process of wound healing is promoted by several natural products which have been reported and used in Ayurveda, Siddha and Unani systems of medicines. Any extract that posse's activities like analgesic, anti-inflammatory, anti-microbial, antioxidant etc may also exhibit wound healing activity. Several drugs obtained from plant sources are known to increase the healing of wounds by their synergistic activity. Literature survey has revealed that phenolic compounds promote wound healing effect by several mechanisms that include antioxidant, antimicrobial chelation of free radicals and astringent property. We have already reported the number of phenolic compounds, condensed tannins and flavonoids of the plant extract. The wound healing activity of the methanolic extract can be attributed to the presence of these phytoconstituents which may be active individually or due to synergistic activity of these constituents [12].

Table no. 4 physiochemical properties of formulation

PHYSIOCHEMICAL PARAMETERS	**BATCH NO. 1**	**BATCH NO. 2**
Colour	Lime green	Lime green
Odour	Characteristic	Characteristic
Texture	Smooth	Smooth

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State	Semisolid	Semisolid
Loss on drying	12.34%	7.65%
Skin irritation study	No skin irritation was observed	No skin irritation was observed
pH	6.21	6.55
Spreadability (seconds)	12.8	14
Diffusion study	0.1cm	0.3cm

Figure 3. Prepared formulation of ointment (batch no. 1)

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Figure 4. Prepared formulation of ointment (batch no. 2)

LOSS ON DRYING

For batch 1
Readings
Weight of empty porcelain = 63.42gm
Weight of the ointment taken = 0.55gm
Weight of the empty porcelain + weight of the ointment taken before drying = 63.97 gm
Weight of the empty porcelain + weight of the ointment taken after drying = 57.13gm
Calculation
Moisture content = (63.97- 57.13) gm = 6.84m
Percentage of moisture content:
0.55gm of ointment content = 6.84gm
∴ 30gm of ointment content = 6.84÷0.55×100
Percent of moisture content =12.43%

For batch 2
Readings
Weight of empty porcelain = 63.42gm
Weight of the ointment taken = 0.55gm
Weight of the empty porcelain + weight of the ointment taken before drying = 63.97 gm
Weight of the empty porcelain + weight of the ointment taken after drying = 59.06 gm
Calculation
Moisture content = (63.97- 59.06) gm = 4.91gm
Percentage of moisture content:
0.55gm of ointment content = 4.91gm
∴ 30gm of ointment content = 4.91÷0.55×100
Percent of moisture content = 8.92%

Figure5. pH test of ointment (batch no.1)

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Figure6. pH test of ointment (batch no. 2)

SPREADABILITYCALCULATION

For Batch 1
S = (M.L/T)
Where, S = Spreadability, M = Weight tied to upper slide, L =
Length of glass slides and T = Time taken to separate the slides
M= 100g
L= 20cm
T= 135 sec
S= (100×20÷135) = 2000÷135=**14 seconds**

For Batch 2
M= 100g
L= 20cm
T= 156 sec
S=(100×20÷156) =2000÷156= **12.8 second**

DIFFUSION STUDY

No microbial growth occurred.

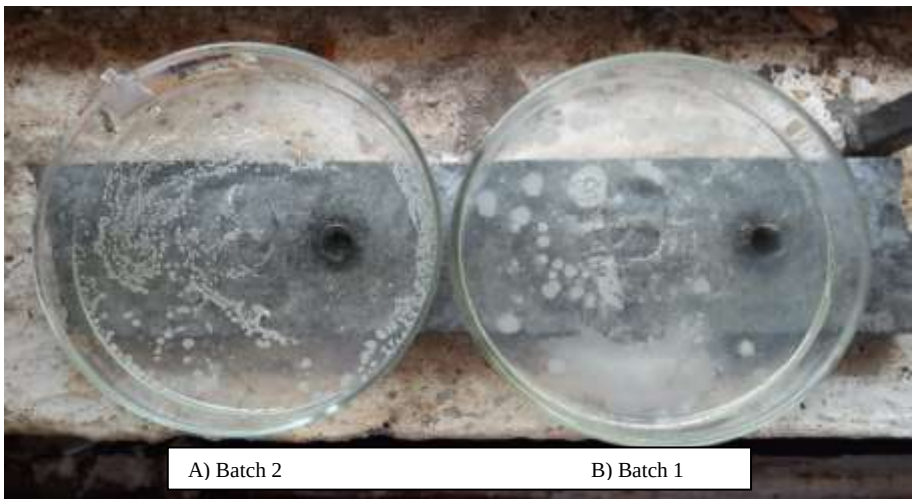

Figure 7. antimicrobial test for ointment

IV. CONCLUSION

Wound healing from ancient times remains a challenging clinical issue for effective wound treatment. Wound healing involves multiple populations of cells, the extracellular matrix and the action of soluble mediators like growth factors and cytokines. Much research has been centered on wound care, with emphasis on new therapeutic methods and the advancement of acute and chronic wound treatment techniques in Ayurveda (herbal).

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New formulas, dressings, and medicinal plant composition are being explored by researchers for developing cost effective, efficient, stable, and sustainable delivery system for the management/treatment of wounds. With the advent of nanotechnology and availability of novel materials, wound management is becoming more effective and patient-centric. Newer technologies like 3D printing are also providing advantageous options for developing different drug delivery systems for managing wounds. Tissue engineering and regenerative medicines are the futuristic view of technologies for developing wound healing systems. Better quality control techniques for identification, screening, and quantification herbal components along with well-designed pre-clinical and clinical studies will open new research gateways in wound care management [1].

The results obtained in this study show that the combination of Gum Arabic tree and Curcumin longa formulated as polyherbal ointment accelerates the healing process by enhancing collagen formation and increasing the breaking strength of the healed wounds [9]. The batchF2 shows the more potent activityas compares to batch F1.The batch F2 shows the best physicochemical properties than batch F1 as shown in table no. 4.This potent activity can be attributed to the Phyto-constituents present in the plants which may be acting synergistically to enhance the wound healing effect [9].

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