



AYUR-TRIDEX PROCUMBENS POWDER FOR WOUND HEALING

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Abstract:

India is consisting of large economy of medicinal plant having number of medicinal uses. The ayurvedic plant having the medicinal effects also less side effects produce. The project based on the nature plant which having the chemical constituents which shows the action of wound healing, by using tridex procumbens as main ingredient for herbal formulations and other expipients are turmeric, merigold, and rose which having action of wound healing. The natural products cause less side effects as compared to other chemical products. This wound healing powder shows the wound healing activity on lactobacillus bacteria. The main motivation of this project as compare to other products for wound healing the natural herbal preparation is affordable and more effective also less side effects.

INTRODUCTION

Injuries are the crucial case of physical conditions. [1] Medicinal plants are globally spread all over the country in India. Herbal remedies support the treatment of several physiological conditions and disorders. The plants containing active constituent performs a significant role in traditional as well as western medicines. Nowadays considerable counts of drugs are derived from plants that in action opposed to number of disabilities. The majority of these include the extraction of the active constituents found in specific plant. As per the survey reports of WHO 80% population still preferred conventional medicines for various traumatic conditions. There are about 25% of plant based medical drugs in the developed countries and these plants are still being used by people in rural areas of many developing countries. [2-6]

A wound may be described as a disruption in the epithelial layer of the skin. Wound are the damage leads to opening of the skin that creates disruption in regular function and anatomy of the skin. [7,8] Present approximation shows that about 6 million people go through incurable wounds. At the site of injury, the unhealed wounds persistently induce inflammatory mediators that give rise to pain and swelling.

Wounds contribute for infection and in turn extend the recovery of injured patients. It is necessary to heal the wounds for the repairing of damaged anatomical progression and interrupted functioning state of

skin. [9-11] There are many factors which extend the process of wound healing together with lymphatic blockage, bacterial infection, diabetes mellitus, necrotic tissue and interference with blood supply, if the alteration of these factors is possible with the help of any agent, then healing is attained. [12] In the wound healing process, medicinal plants possess some chemical constituents having nature of repairing tissue. [13] The skin is the greatest organ in the human body and it act as first line of defense. [14] Skin has two key functions in addition to protection that is regulation and sensation. More particularly it protects against microbial contamination, it restricts the effect of change in temperature, defend against mechanical impacts and pressure, restrict the effect of radiation and hold back pollutants from entering. Skin plays a major role in regulation of body temperature through sweat and hair as it is the largest body organ. It also takes part in synthesis of vitamin D, for which it serves as reservoir. The skin can sense and send changes occur in the environment due to its closed network of nerve cells.

Neuropathy is a condition occurs due to damage of these nerve cells that leads to loss of sensation on affected areas. Because of all the above-mentioned functions, maintaining the quality of skin is necessary to keep the body healthy. [15,16] The disturbance of the tissue or topical layer of the skin due to any physical, chemical, mechanical, immunological, and thermal injury is defined as wound. [17] Patient's wellbeing, self-image, working capacity and independence is bargained because of wound. And hence it is necessary to successfully treat the wound at both individual and community level. [18]

The process of wound healing: Wound healing is a complex and dynamic process of repairing the damaged tissue and replacing the cellular structures and tissue layers. [19]

The wound healing process is divided into 4 phases:

1. Hemostasis
2. Inflammation
3. Proliferative
4. Maturation

1.Hemostasis:

Hemostasis is a first stage in wound healing that can last for 2 days. The skin provides a life-protective barrier between the body and the external environment against physical damage, pathogens, fluid loss, and has immune neuroendocrine functions that contribute to the maintenance of body homeostasis. [20] Stem cells (SCs) responsible tissue for homeostasis and wound healing. Stem cell regenerate the tissue and these results known as SC plasticity, typically resolves as wounds heal. [21,22]

2. Inflammation:

Inflammation is second stage in wound healing that can last for 2 weeks. This phase starts straight away after injury to stop bleeding. It is marked by vasoconstriction and platelet aggregation to induce vasodilatation and phagocytosis, which results in the inflammation of the wound site. [23] The skin is the crucial first-line barrier against foreign pathogens. Compromise of this barrier presents in the context of inflammatory skin conditions and in chronic wounds. [24] Macrophages are important cells in wounds. They do many things in wounds,

like protecting the body, helping with inflammation, getting rid of dead cells, and helping cells grow back and tissues heal after an injury. [25-27]

3. Proliferative:

The second phase happens between day 8 and day 21 after the injury. This is when the skin starts to grow back. During this time, new tissue forms, the skin starts to cover the wound again, and new blood vessels grow. This phase can take a few weeks [28,29] The proliferation phase is really important for healing well. A lot of important things happen during this time, like cells called fibroblasts moving into the wound, the skin growing back over the wound, new blood vessels forming, and the wound getting smaller as it starts to close up. [30] The time it takes for the proliferation phase to naturally complete guides us in treating wounds until they're fully closed. As this phase goes on, the main cell present at the wound site is called a fibroblast. These cells, which come from a type of tissue called mesenchyme, are responsible for making a new support structure needed to repair the injured tissue. Fibroblasts stick to the temporary fibrin scaffold and start making collagen. [31-33] During the proliferation phase, it's crucial to rebuild the network of blood vessels. This process, called angiogenesis, involves forming new blood vessels. The substances that make this happen mostly come from platelets and active macrophages, which release growth factors and cytokines to help cells multiply [34,35]

4. Maturation:

The last phase of wound healing is called the maturation phase, also known as the remodelling phase. This phase can last from 3 weeks to 2 years. Maturation involves laying down collagen in the wound, which helps to strengthen it. During this phase, new, healthy tissue is formed. [36,37] Fibroblasts organize and cross-link the collagen, from collagen type 3 to type 1 (Type 3 collagen fibrils are thinner than type 1 fibrils) strength gradually increases, wound contraction occurs because collagen is high concentration in skin and the wound loses its pink or purple colour as capillary and fibroblast density decrease. tissue matured into scar and tissue tensile strength increases (80%). [38]

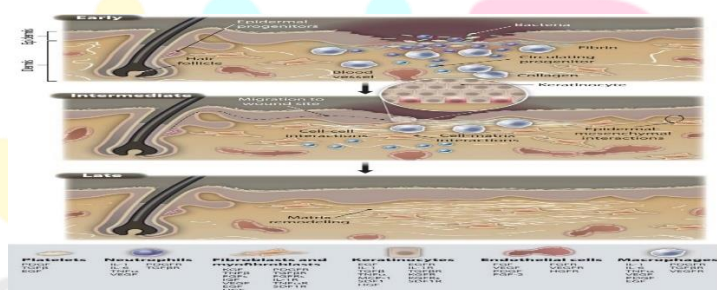


Figure no. 1 Mechanism of Wound Healing

Important activities involved in the management of wound healing:

Numerous physiological occurrences can have a big impact on how quickly a wound heals. Regardless of the precise underlying mechanism, the anti-inflammatory, antibacterial, analgesic, and antioxidant actions are among the most crucial. Early on in the healing process after injury, the acute inflammatory response produces cytokines and chemokines that are crucial for healthy tissue growth. [39] The wound may not be able to enter the remodelling phase or begin the process of matrix formation if there is persistent inflammation. Delays in wound closure and an increase in pain near and inside the wound frequently arise as a result, further impeding healing. [40] In light of the aforementioned, substantial research has been done on anti-inflammatory

therapeutic approaches for wound care, particularly in connection to increased patient comfort and wound healing. [41] One of the most crucial factors that impact the wound healing is infection. It has been reported that the costs of wound care are straight away associated with infected wounds. [42] One of the most crucial prerequisites for better wound healing may be reducing the bacterial burden because wound infection (either secondary or primary by opportunistic microorganisms) might reduce local inflammation and therefore prevent tissue loss. The best prescription for wound infection prevention should be antibacterial and also activate the body's natural immune response without causing harm to the surrounding healthy tissue. [43,44] Most wounds cause pain to be felt. Pain is associated with pain in the patient, the release of stress hormones, and frequently lowers the patient's quality of life. restricted mobility, psychological concerns related to stress brought on by pain, and of course the financial load brought on by prolonged treatment all play a role in the ineffectiveness of wound healing. [45] Chronic pain impairs a patient's ability to heal, delaying their overall recovery. [45,46] An earlier time for ambulation, adequate oxygenation, nourishment, and stress reduction can all be attributed to appropriate and successful pain treatment. All of the aforementioned contribute to faster wound healing, reduced likelihood of developing chronic pain, and finally decreased treatment costs. [47]

Medicinal plants traditionally used in wound healing:

In around 80% of the world's less developed and developing countries, people still rely heavily on plant-based medicine for their basic healthcare needs. Nature has long provided remedies for healing, and many plants and their extracts have been traditionally used because of their potential to manage and treat wounds. Natural substances found in plants help tissues to regenerate and heal through various interconnected pathways. These plant-based medicines are often affordable and have minimal negative side effects. However, before they can be effectively used in wound care, it's important to scientifically standardize, validate, and systematically evaluate their safety due to increasing awareness of their potential activities and safety concerns [48] In recent years, there has been a significant increase in research focused on using medicinal plants for treating and healing wounds. [48-51]

REVIEW OF LITERATURE

- **D. Kumarasamyraja;** A Review on Medicinal Plants with Potential Wound Healing Activity - Ayurveda, the traditional medical system of India, attributes anti-aging and wound-healing powers to a variety of herbs, fats, oils, and minerals. Injuries to the skin that damage the soft tissue led to wounds. Anatomic continuity and function are restored throughout the complex dynamic process of wound healing. Herbal extracts that speed up wound healing also aid in blood clotting, infection control, and wound healing. Therefore, a list of the plants used in traditional medicine for the healing of wounds was examined for the current review. It is helpful for researchers to provide extensive information regarding the development of safe and efficient and globally accepted herbal drugs for cuts and wounds. [52]
- **Roodabeh Bahramsoltani;** Burn wound healing is a complex process involving various factors like tissue growth, blood vessel formation, and skin repair. Certain medicinal plants like garlic, aloe vera,

gotu kola, and sea buckthorn have shown promising results in healing burn wounds when used individually. These plants contain beneficial compounds such as flavonoids, alkaloids, saponins, and phenolic compounds that help in wound closure. Additionally, active ingredients like proteolytic enzymes and glycosides play a significant role in promoting healing. Overall, herbal medications have proven effective in managing burn wounds and can serve as an alternative treatment option. [53]

- **Kiran Kumar Chereddy;** The wound healing process involves several interconnected steps. Applying exogenous lactate from a polymer called poly (lactic-co-glycolic acid) (PLGA) helps speed up the formation of new blood vessels (angiogenesis) and wound healing. Curcumin, a compound found in turmeric, is a popular topical treatment for promoting wound healing, especially for both healthy and diabetic wounds. When curcumin is encapsulated in PLGA nanoparticles, it offers several advantages including protection from light degradation, improved water solubility, and a sustained release of curcumin over 8 days. Overall, combining lactate from PLGA with encapsulated curcumin enhances wound healing. [54]
- **Gulzar Alam;** Wound Healing Potential of Some Medicinal Plants - In life, injuries are an inevitable occurrence. Physical, chemical, or microbiological factors can all cause wounds. Healing is a coping technique that entails an effort to preserve typical anatomical structure and function. The management and treatment of wounds can greatly benefit from the use of plants and plant extracts. The use of phyto-medicines for wound healing is not only inexpensive and convenient, but also ostensibly safe as hypersensitive responses are hardly reported. [55]
- **Jadhav V. D.;** The use of herbal medicine has become increasingly important worldwide, both in terms of health and economics. This study aimed to develop and evaluate a herbal gel containing extract from the *Tridax procumbens* leaf. The gel formulation included ingredients such as Carbopol 940, *Tridax procumbens* leaf extract, propylene glycol, methyl paraben, propyl paraben, and distilled water. Physicochemical characteristics like pH, spreadability, and viscosity of the formulations were assessed. Herbal remedies are often considered safer than conventional medicines due to fewer side effects. One way to enhance their effectiveness is by preparing extracts and formulations to improve absorption and penetration into the body. [56]
- **Abhishek Niranjana;** Chemical constituents and biological activities of turmeric (*Curcuma longa* L.) - A review - The root of the turmeric plant, known as *Curcuma longa*, serves various purposes such as a spice, coloring agent, and for medical use. It's effective in scavenging different reactive oxygen species (ROS) like superoxide anion, hydroxyl radical, and others. Turmeric inhibits enzymes like lipoxygenase and cyclooxygenase, which produce ROS, and helps suppress inflammatory responses by targeting COX-I and COX-II enzymes. It also protects against lipid peroxidation caused by hydrogen peroxide in red blood cells, haemoglobin, and lipids. Safety studies indicate that turmeric is well-tolerated even at high doses (0.5 to 1.5 g/day/person) without toxic effects. Turmeric contains 3-6% polyphenolic compounds, called curcuminoids, which include curcumin, demethoxycurcumin, and bisdemethoxycurcumin. These curcuminoids are responsible for many of turmeric's biological

effects. Curcumin, one of the main components, acts as a pro-oxidant in the presence of certain metal ions (Cu and Fe) and has potential therapeutic applications as a powerful bioprotectant. [57]

- **Ratree Maenthaisong**; The efficacy of aloe vera used for burn wound healing: A systematic review - Although there is some clinical proof, aloe vera has long been used to treat burns. To ascertain the effectiveness of topical aloe vera for the treatment of burn wounds, we carried out a systematic study. Aloe vera may be an effective treatment for first- to second-degree burn wound healing, according to accumulating research. To ascertain the efficacy of aloe vera, well-designed studies with adequate information on the components of aloe vera products should be conducted. [58]
- **Nader Pazyar**; Skin Wound Healing and Phytomedicine: A Review - A physiological process aimed at healing the injured tissues restores the integrity of the skin. Hemostasis, inflammation, proliferation, and remodelling are the four stages of the healing process. The treatments offered by phytomedicine have considerable pharmacological effects. Aloe vera, mimosa, grape vine, chamomile, ginseng, jojoba, tea tree oil, rosemary, lemon, soybean, comfrey, papaya, oat, garlic, ginkgo, olive oil, and Ocimum are a few of these. This article reviews the most often used medicinal herbs that are effective in treating skin wounds and makes an effort to explain how they work. [59]
- **Suresh Kumar Dev**; Antimicrobial, anti-inflammatory and wound healing activity of polyherbal formulation - Individual herbs are not enough to have the intended therapeutic effect, according to Ayurveda. When it is optimised as a combination of several herbs in a certain ratio, it will have a greater medicinal impact with less toxicity. The study also sought to determine how polyherbalism affected the antibacterial and antioxidant effects, and how best to treat a wound by adjusting the mix of several plant extracts. The 2% and 5% polyherbal carbopol-940 gels were tested in preclinical studies for their ability to reduce inflammation and speed up the healing of wounds. The ability to combat microbes was assessed by agar well diffusion and broth dilution method while wound healing activity was evaluated by excision and incision wound models. Polyherbal Carbopol- 940 gels (2% and 5% w/w) promoted the wound healing and anti-inflammatory effect. The high rate of wound contraction (< 0.0001), early epithelialization period (< 0.0001) and increased wound breaking strength (< 0.0001) were observed in 2% and 5% polyherbal gel treated group when compared to the normal control and negative control group. The antimicrobial and anti-inflammatory effect of Polyherbal drug provoked and promoted the wound healing process through accelerated remodelling of damaged tissue. [60]
- **Arash Moeinia**; Wound healing and antimicrobial effect of active secondary metabolites in chitosan-based wound dressings: A review - Improving wound healing is crucial due to its potential complications. Chitosan, a biopolymer known for its biocompatibility, biodegradability, non-toxicity, and antibacterial properties, has been extensively studied for wound healing applications. Its versatility allows it to be easily processed into various forms such as beads, gels, foams, and membranes. This review focuses on chitosan-based wound dressings containing active secondary metabolites and covers research on natural compounds' effects on wound healing. The review thoroughly examines

how bioactive substances enhance the wound-healing properties of different chitosan formulations. [61]

- **Yang Woo Kim;** Wound Healing Effects of Rose Placenta in a Mouse Model of Full-Thickness Wounds - A plant called *Rosa damascena* has been used traditionally in Eastern medicine to treat wounds. In a full-thickness wound model in mice, the objective of this study was to assess the efficacy of rose placenta from *R. damascena*. [62]
- **Dixit Priyanka;** A BRIEF STUDY ON MARIGOLD (*TAGETES SPECIES*): A REVIEW - The Asteraceae family contains the most prevalent species of *Tagetes*, which are utilised for decorative purposes as well as for various medical and aesthetic purposes. A kind of oxy-carotenoid known as xanthophyll, lutein has the fundamental C-40 isoprenoid structure shared by all carotenoids as well as two cyclic end groups (beta and alpha-ionone rings). It is the primary pigment and one of *Tagetes erecta*'s key components. It contains a potent essential oil (*Tagetes* oil), quercetin, a glucoside of quercetin, phenolic compounds, syringic acid, methyl-3, 5-dihydroxy-4-methoxy benzoate, quercetin, thienyl and ethyl gallate, terpenes, and other significant phytochemical components from various plant parts. The leaves are reportedly beneficial in treating wounds, ulcers, piles, renal problems, and muscle discomfort. The flower is used to treat illnesses of the eyes as well as fevers, epileptic fits, scabies, liver problems, astringent, carminative, stomachic, and liver ailments in Ayurveda. It demonstrates a variety of pharmacological effects, including antibacterial, anti-microbial, hepatoprotective, insecticidal, mosquitocidal, nematocidal, wound-healing, anti-oxidant, and analgesic effects. larvacidal behaviour, For the detailed management of nematodes, sub-acute toxicity studies also examine *Tagetes* species. [63]

NEED OF WORK

1. According to a survey by the WHO, over 80% of people worldwide still rely on traditional medicines for treating different illnesses.
2. Powder sprays used for wound healing primarily act as a protective barrier against the environment.
3. While multi-herbal natural spray has shown promise as an alternative treatment option for wound healing of the skin, there is still a need for further research in several areas.

A] Safety: This multi-herbal natural spray is safe for use. It has a long-term safety and very few side effects as it is a natural spray. Additionally, more research is needed to evaluate its safety in new born infants.

B] Mechanism of action: As *Tridax procumbens* is the active pharmaceutical ingredient. The leaves parts of this plant are used. It shows anti-microbial activity in this formulation.

C] Cost effectiveness: As compared to other formulations this is the cost effective as it is a natural multi-herbal spray which can be extracted easily from the plants.

AIM: To prepare formulation and evaluation of multi-herbal natural powder spray for wound healing.

OBJECTIVES

More and more people are seeking natural wound healing products.

Our goal was to create a spray formulation using extracts from *Tridax procumbens*, *Curcuma longa*, *Tagetes erecta*, and *Rosa rubiginosa*.

Sprays and dusts are commonly used to control pests, diseases, and weeds in plants and animals using chemicals.

Herbal remedies are gaining popularity among patients due to their lack of common side effects associated with conventional medicines.

PLAN OF WORK

- a. Collection and authentication of plants
- b. Preparation of powder
- c. Pharmacognostic & Phytochemical evaluation of plant powder

1. FOM
2. Moisture content
3. Ash Value
4. Swelling index
5. Foaming index

- d. Development of Powder spray container

- e. Evaluation parameter of ingredients

Particle size determination

1. Colour consistency
2. Weight variation
3. Moisture content
4. pH
5. Angle of repose
6. Bulk density
7. Tapped density
8. Hausner's ratio
9. Carr's index
10. Power of spray
11. Angel of spray
12. Antimicrobial activity

MATERIALS AND METHODS

The present study pertains to an herbal formulation with highly potent wound healing properties, in humans and animals. The composition consists of *Tridax procumbens*, *Curcuma longa*, *Tagetes erecta*, *Rosa rubiginosa* in well-defined ratios. The invention also includes a process for preparing dry powder form of crude drug with fine particles of each ingredient.

I. DRUG AND EXCIPIENT PROFILE

1. *Tridax procumbens*:



Figure no. 2 Leaves of *Tridax procumbens*

Tridax procumbens, commonly known as coat buttons or Tridax daisy, is a species of flowering plant in the family Asteraceae. [64]

Chemical constituents: This is rich in alkaloids, terpenoids, steroids, carotenoids, flavonoids (such as catechins, centaurein and bergenins), fatty acids, phytosterols, tannins and minerals. [65] The matured leaves are crushed to make a paste/ powder and applied on the surface of the Wound. [66,67]

2. *Curcuma longa*:



Figure no. 3 Rhizomes of *Curcuma longa*

Turmeric is a rhizome containing plant and its scientific name is *Curcuma longa*, of the ginger family, Zingiberaceae.

Chemical constituents: Turmeric contains 3-6% polyphenolic compounds, collectively known as curcuminoids, which is a mixture of curcumin, desmethoxycurcumin and bisdemethoxycurcumin. [68,69]

Turmeric powder: With its high antiseptic properties, dabbing a generous amount of turmeric powder on a fresh cut will help stop the oozing of blood and also prevent infections. [70,71]

3. *Tagetes erecta* (Marigold):



Figure no. 4 Petals of *Tagetes erecta*

(Genus *Tagetes*), genus of about 50 species of annual herbs of the aster family (Asteraceae).

Chemical constituents: Marigold plants contain various compounds including carotenes, flavonoids, resin, saponin, sterols, triterpenes, bitter glycosides, volatile oils, calcium, and alkaloids. Commercial extracts of marigold flowers typically have trans-lutein as the primary carotenoid, along with several minor cis-lutein isomers. [72,73] Marigold petals have been made into ointments, extracts, and infusions. [74]

4. *Rosa rubiginosa*:



Figure no. 5 Petals of *Rosa Rubiginosa*

A Rose is either a woody perennial flowering plant of the genus *Rosa*, in the family Rosaceae.

Chemical constituents: *Rose Damascena* contain anthocyanins, terpenes, flavonoids and glycosides. It contains myrcene, carboxylic acids, kaempferol, quercetin and vitamin C. Flower contains fatty oil, tanning matter and organic acids. [75,76]

5. Talc:



Figure no. 6 Talc powder

Talc is made up of hydrated magnesium silicate with the chemical formula $Mg_3Si_4O_{10}(OH)_2$. [77]. Glidants like talc enhance the flow properties of powder by reducing friction between particles, minimizing van der Waals forces and electrostatic charges, altering particle size distribution, and shielding host particle surfaces from humidity by creating a mechanical barrier. However, it's important to note that a glidant works effectively only within a specific concentration range. If the concentration exceeds this range, the glidant may actually impede the flowability of the powder. [78,79]

6. Standard sample -Cipladine powder:



Figure no. 7 Cipladine powder

Cipladine is an iodophore known for its potent broad-spectrum germicidal activity against various microorganisms including bacteria, viruses, fungi, protozoa, and spores. It works by oxidizing cell constituents and iodinating proteins, rendering them inactive. Iodine is the active component responsible for its microbicidal actions. It inhibits crucial bacterial cellular processes and structures, while also oxidizing nucleotides, fatty acids, and amino acids in bacterial cell membranes.

Detail of label

Cipladine powder 5% w/w

Microbicidal powder

composition

Povidone-iodine IP 5% w/w

(Available iodine 0.50% w/w)

Base..... q.s

FOR EXTERNAL USE ONLY

II. METHODS OF PREPARATION AND EVALUATION OF HERBAL POWDER SPRAY:

A) Collection and authentication of plant material:

Plant material was collected from different region of bopdev ghat Pune and prepare herbarium of each plant and authentication of Plant Specimen from botanical survey of India Pune western region Pune Maharashtra. The specimen was authenticated by D.L. Shirodkar Botanist.

B) Pharmacognostic & Phytochemical evaluation of plant powder:

1. **FOM:** The parts of the organ or organs other than those parts of drugs mentioned in the definition and description of the drug are known as foreign organic matters. They may be insect, moulds, earthy material, animal excreta, etc.
2. **Moisture content:** The moisture content of a drug will be responsible for decomposition of crude drugs either producing chemical change or microbial growth. So, the moisture content of a drug should be determined and controlled. The moisture content is determined by heating a drug at 105°C in an oven to a constant weight.

E.g., The moisture content of digitalis and ergot should not be more than 5% W/W, respectively.

3. **Ash Value:** The determination of ash is useful for detecting low grade products, exhausted drugs, and excess of sandy or earthy matter. Different types of ash values are used in detection of crude drugs like, total ash, acid- insoluble ash, water- soluble ash and sulphated ash.
4. **Swelling index:** Transfer 1gm of substance to a measuring cylinder. Fill the cylinder up to 50 ml mark with water. Gently agitate the mixture occasionally over a period of 24 hours. Measure the volume occupied by the swollen. The observe the occupies a volume.
5. **Foaming index:** Weigh approximately 1 g of coarsely powdered drug and transfer it to a 500 ml conical flask containing 100 ml of boiling water. Maintain moderate boiling at 80-90°C for about 30 minutes. Allow the mixture to cool, then filter it into a volumetric flask and add enough water through the filter to make the volume up to 100 ml (V1).

Clean and label 10 test tubes with numbers 1 to 10. Take successive portions of 1 ml, 2 ml, up to 10 ml of the drug in separate tubes, and adjust the remaining volume with liquid up to 10 ml in each test tube. After closing the tubes with stoppers, shake them for 15 seconds and allow them to stand for 15 minutes. Then measure the height of the foam.

If the height of the foam in each tube is less than 1 cm, the foaming index is less than 100 (considered not significant). If the foam is more than 1 cm in height after the dilution of plant material in the sixth tube, then the corresponding test tube number is the foaming index.

If the height of the foam in every tube is more than 1 cm, the foaming index is considered more than 1000. In this case, measure 10 ml of the first decoction of the plant material and transfer it to a 100 ml volumetric flask (V2). Adjust the volume up to 100 ml and follow the same procedure as above.

Foaming index is calculated using the following formulas:

- Foaming index = $1000/a_n$ in case of V1
- Foaming index = $1000 \times 10/a_n$ in case of V2

Where 'a' represents the volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam is observed.

C] Procedure for preparation of powder spray –

The herbal powder of each ingredient is prepared by five steps

1. Drying
2. Size reduction
3. Sieving
4. Weighing
5. Mixing
6. Sieving
7. Filling of powder in a container

1. Drying-

- i. Leaves of *Tridax procumbens*: The *Tridax procumbens* leaves are collected from the surrounding and kept for drying in cool and shady place protected from sunlight at room temperature for 3-4 days.
- ii. Rhizomes of *Curcuma longa*: The turmeric is collected and kept for drying in cool and shady place protected from sunlight at room temperature for 3-4 days.
- iii. Petals of *Tagetes erecta*: The petals of Merigold are collected from the surrounding and kept for drying in cool and shady place protected from sunlight at room temperature for 3-4 days.
- iv. Petals of *Rosa rubiginosa*: The petals of rose are collected from the surrounding and kept for drying in cool and shady place protected from sunlight at room temperature for 3-4 days.



Figure No. 8 Drying of leaves and petals

2. Size reduction –

- i. *Tridax procumbens*: After drying for 3-4 days the size of the dried leaves is reduced to fine powder by using mortar and pestle in a laboratory.
- ii. *Curcuma longa*: After drying for 3-4 days the size of the dried rhizomes of turmeric is reduced to fine powder by using traditional method at home as the turmeric is very hard.
- iii. *Tagetes erecta*: After drying for 3-4 days the size of the dried petals is reduced to fine powder by using mortar and pestle in a laboratory.
- iv. *Rosa rubiginosa*: After drying for 3-4 days the size of the dried petals is reduced to fine powder by using mortar and pestle in a laboratory.



Figure No. 9 Size reduction of ingredients

3. Sieving –

- i. *Tridax procumbens*: The powder of *Tridax procumbens* is then passed from sieve no. 85 to get uniform particle size of the powder.
- ii. *Curcuma longa*: The turmeric powder is then passed from sieve no. 85 to get uniform particle size of the powder.
- iii. *Tagetes erecta*: The obtained powder of Merigold is then passed from sieve no. 85 to get uniform particle size of the powder.
- iv. *Rosa rubiginosa*: The rose powder is then passed from sieve no. 85 to get uniform particle size of the powder.



Figure No. 10 Sieving of ingredients

4. Weighing –

After sieving, each ingredient is weighed according to the formula with the help of weighing balance.



Figure No. 11 Weighing of ingredients

5. Mixing –

The weighed powder is then mixed in mortar and pestle.

6. Sieving-

When the powder seems to be uniformly mixed then, again pass the obtained powder from sieve no. 85

7. Filling of powder in a container –

Procedure:

- i. A method of filling the powder in container as per the composition.
- ii. Powders are fine particles which are as a result of disintegration, crushing or grinding the solid substance.
- iii. The method of filling empty containers with powder which consists in placing a container in position to receive the powder and remove air from within the container.
- iv. The container mouth was kept as close to the funnel as possible and Spatula was used to pour the powder to the funnel.
- v. Then, to achieve a clean fill and a high degree of accuracy the funnel was placed very close to the container top.

vi. Container used:

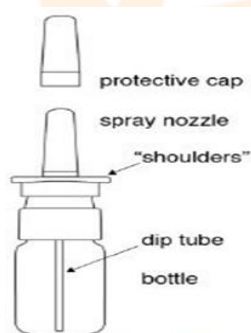


Figure no. 12 Structure of container

- i. We have used a spray bottle for delivering the herbal dosage form.
- ii. Spray bottles are made from polyethylene terephthalate (PET) and comes in sizes ranging from 10 mL to 60 ml and include a white spray nozzle with cap to keep nozzle clean and leak-proof. Polyethylene terephthalate (PET) is lightweight, chemically resistant and has excellent durability.
- iii. The body of the sprayer covers and contains the sprayer's internal mechanisms.
- iv. The nozzle on a sprayer creates the powder stream by forcing it through a small hole. Nozzles have a one-way valve that prevents airflow back into the pump.
- v. Unlike the rubber bulb dispenser which primarily moved air with a small amount of powder, modern spray bottles use a positive displacement pump that acts directly on the powder. The pump draws powder up a dip tube from the bottom of the bottle and forces it through a nozzle.
- vi. Protective cap keeps pump from spraying in luggage.



Figure no. 13 Container for powder spray

How to use:

- i. Remove the bottle cap.
- ii. Hold the spray close to an injury or cuts.
- iii. Press the bottle in the centre once and remove the nozzle before releasing the pressure on the bottle.
- iv. Repeat above process for the covering an injured part.
- v. After using, clean the nozzle and close with cap.
- vi. To avoid other infection, the spray should only be used for external use.

1. Preparation of formulation

Sr. No	Ingredients	Batch-1	Batch-2	Batch-3	Batch-4	Batch-5
1	Tridax procumbens	3gm	3gm	3gm	3gm	3gm
2	Curcuma longa	3gm	2gm	2gm	3gm	3gm
3	Tagetes erecta	3gm	2gm	1gm	1gm	2gm
4	Rosa rubiginosa	1gm	1gm	1gm	1gm	1gm
5	Talc	-	2gm	3gm	2gm	1gm

Table no. 1 Formulation of natural powder spray

III] Evaluation parameter –

1. Particle size determination

To measure particles, the system utilizes a laser to illuminate individual particles and then measures the amount of diffraction produced by the particle. This information is used to calculate the particle size. Laser analysis is highly flexible because it is not restricted by particle size and can be used in both dry and wet measuring systems.

2. Colour consistency

Powder colour consistency to ensure the quality and properties of the materials.

3. Weight variation:

The weight variation statistical quality control test is employed to verify the consistency of dosage units, thereby ensuring product safety, identity, and quality. In the food and beverage industry, examining the weight of packages promptly confirms whether the fill quantities comply with legal standards.

4. Moisture content:

Moisture content of the wound healing powder should be negligible and should be prevented from the moisture because of microbial growth and contamination. It should be completely dry. Merigold used as preservative which prevent growth of microorganisms.

5. pH

The PH of wound healing powder is 7.5

Mean wound surface pH of the healing wounds was 6.91 compared with a mean pH value of 7.42 for the non-healing wounds. It was concluded that low pH and higher wound temperature were conducive to healing. The pH-dependent activity profiles of four proteases important in wound healing were assessed; they showed similar pH profiles, with optimum activity between pH 7 and 8. So the PH of wound healing powder should be alkaline or similar to blood.

6. Angle of repose

The angle of repose is determined by either tilting box, fixed funnel, revolving cylinder, or hollow cylinder methods, in all of which the containers are filled with a sample and gradually lifted up, allowing the sample to accumulate and form a conical heap on the surface.

$$\tan \theta = h / r$$

here,

h=height of conical heap

r=radius of horizontal plane of powder.

7. Bulk density

The ratio of the mass of an untapped powder sample and its void volume.

$$\text{bulk density (g/cm}^3\text{)} = \text{Weight of powder (g)} / \text{Bulk volume (cm}^3\text{)}$$

8. Tapped density

The ratio of the mass of powder to volume occupied by the powder after it has been tapped for defined period of time. The tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing the powder sample.

$$\text{Tapped density (g/cm}^3\text{)} = \text{Weight of powder (g)} / \text{Tapped volume (cm}^3\text{)}$$

9. Hausner's ratio

The ratio of tapped density to bulk density

Hausner's ratio, the basic procedure is to measure (1) the unsettled apparent volume, V_o , and (2) the final tapped volume, V_f , of the powder after tapping the material until no further volume changes occur.

$$Hr = \rho_{tap} / \rho_b$$

10. Carr's index

Based on true density (ρ_T) and bulk density (ρ_B)

Carr Index of any solid is calculated for compressibility of a powder which is based on true density (ρ_T) and bulk density (ρ_B), $CI = 100[(\rho_T - \rho_B) / \rho_B]$.

A Carr index greater than 25 is considered to be an indication of poor flow-ability, and below 15, of good flow-ability.

$$CI = 100[(\rho_T - \rho_B) / \rho_B]$$

11. Power of spray

Spray drying is a pharmaceutical process to create dry powder out of a powder solution, and is used in the production of numerous dry powder products including active pharmaceutical ingredient.

12. Angel of spray

The spray pattern means the cross-sectional shape of spray. Selecting a suitable spray pattern for each application achieves the most efficient spray performance. Spray distribution varies depending on the spray height and pressure. The spray angle is the angle of spray near the nozzle.

As the spray flies through the air, droplets gradually lose momentum and the area it can cover decreases. In actual spraying, the spray width varies with spray height. Take this into consideration when designing a nozzle layout.

		Calculated spray width (mm)												
		Spray angle	150°	140°	130°	115°	100°	90°	80°	65°	50°	40°	25°	15°
Spray height (mm)	10	74.6	54.9	42.9	31.4	23.8	20	16.8	12.7	9.3	7.3	4.4	2.6	2.1
	20	149	110	85.8	62.8	47.7	40	33.6	25.5	18.7	14.6	8.9	5.3	4.2
	50	373	275	214	157	119	100	83.9	63.7	46.6	36.4	22.2	13.2	10.5
	70	522	385	300	220	167	140	117	89.2	65.3	51.0	31.0	18.4	14.7
	100	746	549	429	314	238	200	168	127	93.3	72.8	44.3	26.3	21.0
	150	1,120	824	643	471	358	300	252	191	140	109	66.5	39.5	31.5
	200	1,492	1,099	858	628	477	400	336	255	187	146	88.7	52.7	42.0
	250	1,866	1,374	1,072	785	596	500	420	319	233	182	111	65.8	52.6

Table No. 2 Angle of spray

E] Antimicrobial activity

An antimicrobial is an agent that kills microorganisms (microbicide) or stops their growth (bacteriostatic agent).

The main classes of antimicrobial agents are disinfectants (non-selective agents, such as bleach), which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics (which are applied to living tissue and help reduce infection during surgery), and antibiotics (which destroy microorganisms within the body).

RESULTS AND DISCUSSIONS

Plant Authentication:

1. *Tridax procumbens*:

Plant Specimen no - KMGTP-1 / No. BSI/WRC/Iden. Cer. /2023/0103230014418 authenticated by D.L. Shirodkar Botanist, BSIWRC Pune-1 on 8/03/2023

2. *Curcuma longa*:

Specimen no - SKCL-1 No. BSI/WRC/Iden. Cer. /2023/2403230041936 authenticated by D.L. Shirodkar Botanist, BSIWRC Pune-1 on 27/03/2023

3. *Tagetes erecta* (Marigold):

Specimen no - TMKTE-1 No. BSI/WRC/100-1/ Tech./ 2023/ authenticated by D.L. Shirodkar Botanist, BSIWRC Pune-1 on 25/04/2023

4. *Rosa rubiginosa*:

Specimen no - VCKRC-1 No. BSI/WRC/Iden. Cer. /2023/2402230027286 authenticated by D.L. Shirodkar Botanist, BSIWRC Pune-1 on 01/03/2023

Pharmacognostic & Phytochemical Evaluation of plant powder

1. FOM

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
1.82%	1.24%	0.12%	1.51%	1.32%	0.01%

Table no. 3 FOM of sample and standard

2. Moisture content

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
2.1	1.9	1.3	2.4	2.6	1.1%

Table no. 4 Moisture content of sample and standard

3. Ash Value

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
0.36	0.44	0.32	0.82	0.61	0.22

Table no. 5 Ash value of sample and standard

4. Swelling index

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
2mm	3 mm	1mm	2mm	2mm	1mm

Table no. 6 Swelling index of sample and standard

5. Foaming index

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
<100	<100	<100	<100	<100	<100

Table no. 7 Foaming index of sample and standard

Evaluation of Natural Powder spray

1. Colour consistency

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
					
Green	Yellow	Light brown	Brownish yellow	Brown	Creamish white

Table no. 8 Colour consistency of sample and standard

2. Weight variation

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
10gm	10gm	10gm	10gm	10gm	10gm

Table no. 9 Weight variation of sample and standard

3. Moisture content

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
1.9 %	1.8 %	1.2 %	1.9 %	2.8 %	0.9 %

Table no. 10 Moisture content of sample and standard**4. pH**

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
7.24	7.30	7.49	7.34	7.26	7.00

Table no. 11 PH of sample and standard**5. Angle of repose**

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
40°	34.99°	34.60°	34.75	37.88	30.21°

Table no. 12 Angle of repose of sample and standard**6. Bulk density**

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
0.40 g/ml	0.42 g/ml	0.52g/ml	0.42 g/ml	0.46 g/ml	0.68g/ml

Table no. 13 Bulk density of sample and standard**7. Tapped density**

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
0.58g/ml	0.5 g/ml	0.58g/ml	0.59g/ml	0.8 g/ml	0.22g/ml

Table no. 14 Tapped density of sample and standard**8. Hausner's ratio**

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
1.48g/ml	1.25g/ml	1.11g/ml	1.35g/ml	1.25g/ml	1.1g/ml

Table no. 15 Hausner's ratio of sample and standard**9. Carr's index**

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
31.03%	20%	10.34%	17.08%	13.06%	5.03%

Table no. 16 Carr's index of sample and standard**10. Power of spray**

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
18cm	24cm	31cm	28cm	28.3cm	35cm

Table no. 17 Power of spray of sample and standard**11. Angel of spray**

Batch-1	Batch-2	Batch-3	Batch-4	Batch-5	Standard
25 ⁰	32 ⁰	35 ⁰	34 ⁰	33 ⁰	30 ⁰

Table no. 18 Angle of spray of sample and standard

12. Antimicrobial activity

Agar plate method (zone of inhibition)

Sample ID	Conc. of Stock solution	Zone of inhibition in mm	
		Staphylococcus aureus NCIM 2079	Escherichia coli NCIM 2065
S1	50mg/ml	18	4
S2		29	7
S3		32	8
S4		24	6
S5		8	4
Std. (Cipladine)	0.5mg/ml	41	21

Table no. 19 antimicrobial activity of sample and standard

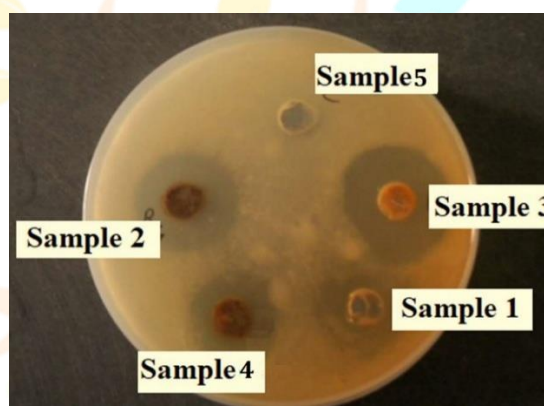


Figure no. 14 Antibacterial activity in terms of Zone of inhibition in mm (Staphylococcus aureus)

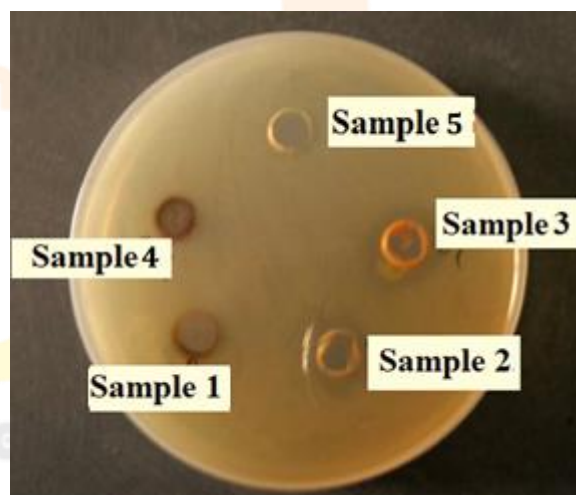


Figure no. 15 Antibacterial activity in terms of Zone of inhibition in mm (Escherichia coli)

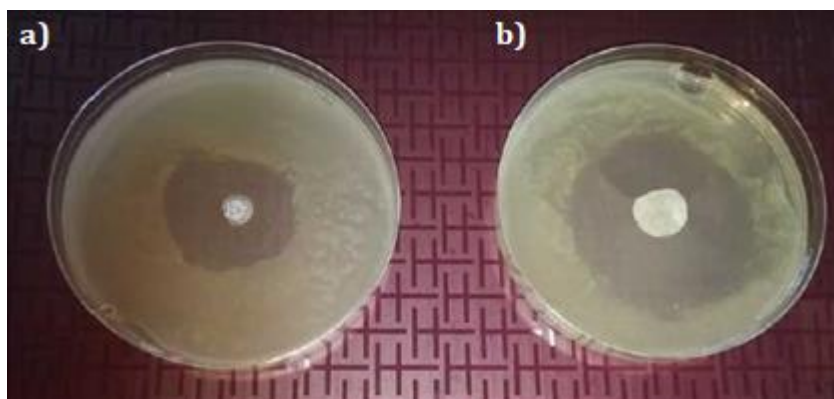


Figure no. 16 Antibacterial activity in terms of Zone of inhibition in mm
a) *Escherichia coli* and b) *Staphylococcus aureus* of standard

SUMMARY

- ✓ We have formulated and evaluated the multi herbal natural powder spray for wound healing.
- ✓ Firstly, we have done the literature survey on plants possessing wound healing activity and selected the plants that shows good wound healing property viz. *Tridax procumbens*, *Curcuma longa*, *Tagetes erecta* and *Rosa rubiginosa* for our herbal powder formulation.
- ✓ Then we have collected the plants from our surroundings, prepared an herbarium for its authentication.
- ✓ We have formulated and evaluated the five sample batches with different formulas of 10g power.
- ✓ After formulating and evaluating the first batch that contains pure crude drugs without adding any excipients the results obtained indicates the poor flow property of the powder.
- ✓ So, to overcome this, we have added the glidant i.e., talc to increase the flow ability (flow property) of the powder and got good results.
- ✓ We have also sent the samples to laboratory to check its antibacterial activity.

CONCLUSION

- ✓ Numerous plants support the skin's natural repair processes, offering great potential for therapeutic use in wound care. The number of herbal remedies for wound therapy is steadily growing as our acquaintance with herbal extracts and isolates grows and as we apply widely accepted scientific methodology to research plants and their extracts from the physiological and pharmacological point of view.
- ✓ Many more herbs have been studied for their therapeutic, either curative or preventative roles as a result of clinical proofs of the therapeutic effects of herbal products.
- ✓ Additional research should focus on isolating and identifying specific active ingredients from plant extracts, which may also reveal substances with higher therapeutic potential.
- ✓ When traditional and modern knowledge are combined, new medications for wound healing can be produced with significantly lowered side effects.
- ✓ All the ingredients that are used in the formulation are suitable and compatible with each other and they all show wound healing activity individually.
- ✓ All these herbs showed anti-oxidant property in modern research. Antioxidants have positive effect on wound healing by protecting the regenerating tissue.
- ✓ Low risk of side effects: mostly natural herbal medications are secured for patients.
- ✓ The major use of herbal medicine is for health promotion and therapy for chronic, as oppose to life threatening conditions.

FUTURE PROSPECTS

- ✓ Developing nanoparticles for wound healing.
- ✓ Studying wound healing in both laboratory (in-vitro) and living organisms (in-vivo), particularly for individuals with type 2 diabetes.

- ✓ Advanced wound care technologies including bioengineered allogeneic cellular therapies, stem cell therapies, xenograft cellular matrices, and growth factors.
- ✓ Utilizing 3D bioprinting techniques to enhance therapeutic outcomes, focusing on skin regeneration while minimizing side effects.
- ✓ Biogenic nanoparticles have demonstrated promising potential as agents for wound healing, as indicated by various scientific reports.

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