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Research Article

Formulation And Evaluation Of Herbal Ointment

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ABSTRACT

This study explores the formulation and assessment of a herbal ointment for wound healing, integrating extracts from Tridax procumbens, Neem and Aloe vera. Physicochemical parameters, including pH, viscosity, and spreadability, were evaluated alongside stability assessments to ensure formulation quality. Antimicrobial activity against common wound pathogens was tested, revealing significant efficacy. Preliminary findings suggest the ointment's potential in promoting wound healing through these mechanisms. This research underscores the promising role of Tridax procumbens and Neem in herbal formulations for wound management, providing a foundation for further investigation, including in vivo studies to validate efficacy and safety.

INTRODUCTION

The term herbal medicine, which is also known as botanical medicine or phytomedicine, describes the medical application of any plant's seeds, berries, roots, leaves, bark, or flowers. Herbal remedies, which have long been used outside of traditional medicine, are gaining popularity as more recent studies and analyses demonstrate their potential for both illness treatment and prevention. Long before written history began, people employed plants for therapeutic purposes. While some traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) developed herbal remedies as part of a systematic approach, indigenous tribes continued to employ

herbs in their healing rituals. Researchers discovered that people planned to employ similar or identical plants for similar reasons across the globe. According to recent estimates from the World Health Organisation, 80% of people globally receive some portion of their basic treatment from herbal medicines. The precise component of most herbs that has a medicinal effect is unknown. Since whole herbs are made up of numerous components, it's likely that these combine to provide the intended therapeutic effect. Herbalists would rather work with whole plants than break them down into their constituent parts. Whole plant extracts are made up of many parts. Together, these elements generate therapeutic

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effects and reduce the likelihood of any one element causing adverse effects. Herbs are frequently used in combination to increase their efficacy, promote synergistic effects, and lower their toxicity. An important source of novel chemicals with potential utility for the development of chemotherapeutic medicines is traditional medicine. The screening of plants used in popular medicine is the first step towards achieving this goal. Therefore, the goal of antimicrobial research is to find and create new antibacterial and antifungal drugs. Many times, people believe that plant-based medications are less harmful and have less negative effects than synthetic ones. Ointments are another formulation for herbal medications, in addition to other dosage forms. A viscous semisolid preparation used topically to various body surfaces is called an ointment. These consist of the skin as well as the mucous membranes of the nose, eyes, vagina, and anus. An ointment could contain medication or not. Medicated ointments have a dissolved medication in them. [1-3]

Wound Healing Activity:

When the skin is ripped, cut, or punctured, it results in a wound. When an open wound is exposed to air, germs go into it, contaminating it and ultimately causing an infection. This procedure is essentially a response of the connective tissue. This procedure starts with an acute phase of inflammation, which is followed by the production of extracellular macromolecules such as collagen that aid in the creation of scars. To restore the integrity and function of injured tissues, this complex process is triggered in response to an injury. Plants are a reliable source of medicine with few side effects, which is why there is a lot of interest in plant-based medications.

An analysis of the literature shows that traditional plant medicines help with wound healing and a number of skin-related issues. The World Health Organisation (WHO) and our nation have been advocating for the use of traditional medicine due to its affordability, accessibility, and comprehensiveness, particularly in poor nations. Many investigations have been carried out using the extracts of *Azardiachta indica* (Melinaceae family) and *Tridax procumbens* L. (Asteraceae family). Herbal medications are also offered in ointment form, a semisolid preparation that is applied topically and utilised as an astringent, emollient, antibacterial, protectant, and antipruritic in addition to other dosage forms. [4,5]

Plants Selected For The Present Study:

In presence study the following plants which have been demonstrated for wound healing activity are selected and were used for the development of herbal formulations.

1.Tridax Procumbence Plant Profile: [2,7,12]



Fig no -1 (Tridax procumbence)

Family: Asteraceae

Synonyms: *Ptiloatephium* Kunth, *Mandonia wedd*, *Bartolia adans*, *Batolina adans*.

Chemical constituent: alkaloid's steroids, flavonoids, carotenoids, Fatty acids, phytosterol, tannin, and minerals.

Taxonomic Classification:

Table 1(a): Taxonomic classification of T. procumbence

Sr no	Taxonomical Classification	
1	Kingdom	Plantae
2	Sub-kingdom	Tracheobionta
3	Division	Magnoliophyta

4	Class	Magnoliopsida
5	Sub-class	Asteriidae
6	Order	Asteraceae
7	Genus	Tridax
8	Species	Procumbens.

Medicinal Uses: Wound healing, Anti-microbial, Anti-inflammatory, Anti-fungal.

Pharmacological Activity of Tridax Procumbens: ^[13]

a) Wound Healing Activity

Wound healing involves a complex interaction between epidermal and dermal cells, the extra cellular matrix, controlled angiogenesis and plasma-derived proteins all coordinated by an array of cytokines and growth factors. Tridax antagonized anti-epithelization and tensile strength depressing effect of dexamethasone (a known healing suppressant agent) without affecting anticontraction and antigranulation action of dexamethasone. Aqueous extract was also effective in increasing lysyl oxidase but to a lesser degree than whole plant extract. Further it has been shown that extract of leaves of this plant also promotes wound healing in both normal and immunocompromised (steroid treated) rats in dead space wound healing model. The plant increase not only lysyl oxidase but also, protein and nucleic acid content in the granulation tissue, probably as a result of increase in glycosamino glycan content.

b) Antimicrobial Activity

Whole plant of Tridax has reported for its antimicrobial activity on various species of bacteria. A whole plant is squeezed between the palms of hands to obtain juice. Fresh plant juice is applied twice a day for 3-4 days to cure cuts and wounds. The extract of whole plant of Tridax showed antibacterial activity only against *Pseudomonas aeruginosa*. The disk diffusion method was used to test the antibacterial activity. Four strains of bacteria employed in test were two-gram positive *Bacillus subtilis*, *Staphylococcus*

aureus and two-gram negative *Escherichia coli* and *Pseudomonas aeruginosa*.

c) Antidiabetic Activity

The knowledge of diabetes mellitus, as the history reveals, existed with the Indians since from prehistoric age. Madhumeha another name of diabetes in which a patient passes sweet urine and exhibits sweetness all over the body in the form of sugar, i.e., in sweat, mucus, urine blood, etc. from ancient time various herbs were practically used for lowering of blood glucose level as such or in juices form. Aqueous and alcoholic extract of leaves of Tridax showed a significant decrease in the blood glucose level in the model of alloxan-induced diabetes in rats.

2. Azadirachta Indica: ^[11,14]

Azadirachta indica (AI) A. Juss (the neem tree) commonly known as neem or nimtree, It belongs to the family Meliaceae and it is well known in India and neighboring countries. All parts of the neem tree have been used traditionally for the treatment of numerous ailments for instance bark as an analgesic, alternative, and curative of fever. Various chemical constituents, such as alkaloids, triterpenoids, glycosides, limonoids, flavonoids, fatty acids, and steroids from neem trees have been proven to exhibit anticarcinogenic, anti-inflammatory, antiulcer, antioxidant, immunomodulatory, antifungal, antibacterial, antiviral, antimalarial, antimutagenic, and antihyperglycemic properties.



Fig 2: (Azadirachta Indica)

Table 1(b): Taxonomic classification of A.indica

Sr no	Taxonomical Classification	
1	Kingdom	Plantae
2	Sub-kingdom	Tracheobionta
3	Division	Magnoliophyta
4	Class	Magnoliopsida
5	Sub-class	Rosidae
6	Order	Sapindales
7	Genus	Azadirachta
8	Species	Indica

Medicinal Uses: Wound healing, treat acne, treat fungal infection.

Pharmacological Activity: ^[15]

a) Anti-microbial activity

Whole plant has reported for its antimicrobial activity on various species of bacteria. A whole plant is squeezed between the palms of hands to obtain juice. Fresh plant juice is applied twice a day for 3-4 days to cure cuts and wounds. The extract of whole plant showed antibacterial activity.

b) Anti-oxidant Activity

The production of free radicals at or around the wound may contribute to delay in wound healing through the destruction of lipids, proteins, collagen, proteoglycan and hyaluronic acid. Agents that demonstrate a significant antioxidant activity may, therefore, preserve viable tissue and facilitate wound healing.

c) Anti-inflammatory activity:

Wounds in persistent inflammatory phase may

Synonyms: Azadirachta indica, margosa, roship, melia azadirachta

Chemical constituent: Azadirachitin, salannin, meliantriol, nimbasterol, quercetin, glyceride, oleic acid and stearic acid.

Family: Meliaceae

Taxonomical Classification:

delay the healing process. Preventing prolonged inflammatory phase hasten the healing process.

3. Aloe vera: ^[9,10,16]



Fig 3: (Aloe vera)

Scientific name: Aloe Barbandesis

Synonyms: Chinese aloe, Cape aloe or Barbados aloe

Chemical constituent: chemical constituents in Aloe vera are Anthraquinones, Aloein, Babandenin, saccharides, prostaglandins, and fatty acids.

Family: Liliaceae.

Taxonomic Classification:

Table 1(c): Taxonomic classification of Aloe vera.

Sr no	Taxonomical Classification	
1	Kingdom	Plantae
2	Sub-kingdom	Tracheophytes
3	Division	Magnoliophyta
4	Class	Liliopsida
5	Sub-class	Lilidae
6	Order	Asparagales
7	Genus	Aloe
8	Species	Barbandesis

Medicinal Uses: Analgesic, Antibacterial, Antiviral, Antifungal, Anti-oxidant immune modulator, Antiseptic, Anti-inflammatory.

MATERIALS AND METHODS

Collection of plant material:

First, we identified and collect the plant leaves Of *tridax procumbens*, *aloe vera* and *azadirachta indica* from the different localities of vathar and its nearby areas and washed them thoroughly with distilled water. The cleaned plant parts are then allowed for the complete shade drying and then made to a fine powder with a mechanical grinder and stored in an air-tight container.

Preparation of plant extract:

1. *Tridax Procumbens*



Fig 4: Soxhlet Apparatus

Tridax procumbens fresh leaves were gathered and let to dry in the shade for a week. Plant material

was dried, then roughly ground and stored in an airtight container. About 100g of plant leaf powder was placed in a soxhlet apparatus and left for 72 hrs, after which the ethanol was extracted at 45 to 55°C. Using a rotary vacuum evaporator, the extracts were gathered and concentrated. A tiny vial was used to gather and preserve the crude semisolid extract. The extract was kept cold, at 4°C, until it was needed for the formulation.

2. *Azadirachta Indica*

After gathering and properly cleaning the leaves with distilled water, the leaves were allowed to dry in the shade for a period of ten days. Powdered dried leaf material was created. After mixing 150 ml of 90% ethanol into 100 g of powder, it was placed in a percolator and allowed to macerate for seven days, stirring periodically, after being infused with 350 ml of 90% ethanol for three hours. A residue that was blackish-green was obtained by collecting and condensing the ethanolic extract. Airtight containers were used to store the extract in a dark, cold environment.

3. *Aloe vera*:

Raw *aloe vera* is washed and green portion is completely removed. The transparent sticky mucilage is grinded and used as herbal extract of *Aloe vera*.

Preliminary phytochemical screening of *Azadirachta Indica*:^[17]

Table 2(a): Preliminary phytochemical screening of Azadirachta Indica.

Sr no	Test	Observation	Inference
1	Test for alkaloid 2.0ml extract + solution of potassium bismuth iodine + few drops of Dragendorffs reagent	Formation of orange colour solution	Alkaloid is present
2	Test for Cardiac Glycoside 2ml of the plant + 2.0ml of acetic anhydride and cooled in ice. H ₂ SO ₄ added carefully along the side of the test tube	Blue green ppt formed	Steroid is present
3	Test for Flavonoids A few drops 10% of lead acetate solution + 2.0ml of the extract in a test tube	Formation of Light yellow ppt	Flavonoids is present
4	Test for Phenols 2 ml extract + 2ml of ferric chloride	Formation of blush green solution.	Phenol is present
5	Test for Tannin- 0.5gm of plant extract+2ml of water + heat on water bath, filter+1ml 10%FeCl ₃ .	Formation of blue-black solution	Tannin is present

Preliminary phytochemical screening of Tridax**Procumbence:** ^[18]**Table 2(b): Preliminary phytochemical screening of T. Procumbence.**

Sr.no	Test	Observation	Inference
1	Test for Alkaloid 3 ml of extract + 1 ml HCl, the mixture was heated gently for 20 min cooled and filter, Add Wagner's reagent	Formation of brown reddish precipitate	Alkaloid is present
2	Test for Tannin 2ml extract + 1% lead acetate	Formation of yellowish precipitate	Tannin is present
3	Test for Amino acid 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes.	Formation of blue colour	Amino acid is present
4	Test for Flavonoids Extract was treated with 10 % NaOH solution	formation of intense yellow	Flavonoid is present
5	Test for Anthocyanin 2 ml of aqueous extract is added to 2 ml of 2N HCl & NH ₃ .	Appearance of pink red turns blue violet	Anthocyanin is present

Preliminary phytochemical screening of**Aloevera:** ^[19]**Table 2(c): Preliminary phytochemical screening of Aloevera.**

Sr.no	Test	Observation	Inference
1	Test for Alkaloids 2ml extract + 1 or 2 drops of dilute H ₂ SO ₄ + Wagners reagents	Formation of brownish-black ppt	Alkaloid is present



2	Test for Flavonoid 1 mL of the extract + 0.5 mL of hydrochloric acid + magnesium metal	Formation reddish coloration.	Flavonoid is present
3	Test for phenolic compound Dissolve 50 mg extract in distilled water, and to this, add 3 mL of 10 % lead acetate solution	Formation of bulky white precipitate	Phenolic compound is present

FORMULATION OF OINTMENT

Procedure for formulation of ointment: [8,20]

Table 3: List of Chemicals used in formulation

Sr.no	Ingredient	Role
1	Wool Fat	Emollient
2	White soft paraffin	Moisturizes the skin
3	Hard Paraffin	Stiffen ointment
4	Cetosteryl Alcohol	Emulsifier, surfactant, foam booster, viscosity increasing agent.

The ointment base was first made by precisely weighing wool fat, white soft paraffin, cetostearyl alcohol, and Hard paraffin, then it was put in an evaporating dish over a water bath. After melting, carefully stir to create a homogenous mixture, then cool the basis for the ointment.

In order to create a smooth paste that was two or three times the weight of the base, precisely

weighed Neem, Tridax procumbence and Aloe vera extract were combined with the base to create a herbal ointment. Additional base was then added gradually to create a homogenous ointment, which was then placed in an appropriate container.

Optimization:

Table 4(a): Quantity of excipients

Sr.no	Name of Ingredients	G1 (gm)	G2 (gm)	G3 (gm)
1	Wool Fat	1.5	1.5	1.5
2	Hard Paraffin	2	2	2
3	Cetostearyl Alcohol	2	2	2
4	White Soft Paraffin	4.5	4.5	4.5

Table 4(b): Quantity of extract

Sr.no	Name of Ingredients	G1 (gm)	G2 (gm)	G3 (gm)
1	Tridax Procombence	0.5	1	1.5
2	Azadirachta Indica	0.5	1	1.5
3	Aloe vera	0.5	1	1.5



Fig 5: Formulation of ointment

Evaluation Parameters

1. Sensory evaluation: Physical parameters like color and odor were examined by visual examination.

2. Consistency: Smooth and no greediness is observed.

3. Spreadability: By sandwiching extra sample between two slides that had been squeezed to a uniform thickness using a specific weight for a specific amount of time, the spreadability was ascertained. Spreadability was defined as the amount of time needed to divide the two slides. Better spreadability is the outcome of taking less time to separate two slides.

Spreadability was calculated by the following formula:

$$S = \frac{M \times L}{T}$$

Where, S- spreadability

M- weight of sample in gram

T-Time taken in seconds

4. Washability: Formulation was applied on the skin and then ease extend of washing with water and checked.

5. Non-irritancy: The formulation prepared was applied to the skin of a human being and observed for the effect.

6. Extrudability: The formation were filled in a collapsible tube container. The Extrudability was determined in terms of the weight of formulation required to extrude 0.5 cm of ribbon of ointment in 10 sec.

7. Loss on drying: Loss was determined by placing the formulation in a petri dish on an oil bath and drying for the temperature at 105°C.

8. Solubility: Soluble in boiling water, miscible with alcohol, ether, and chloroform.

9. pH: PH of prepared herbal ointment was measured by using a digital pH meter. The solution as ointment was prepared by using 10 ml of distilled water and set aside for 5 min.

RESULT AND DISCUSSION:

A. Preliminary phytochemical test:

a) Test for Azadirachta Indica:



Fig 5: phytochemical test for A. Indica

b) Test for Tridax Procumbence:

Sr.no	Test	Observstion
1	Test for Alkaloid	

2	Test for Tannin	
3	Test for Amino acid	
4	Test for Flavonoids	

c)Test for Aloe vera:

Fig 7: Phytochemical test for aloe vera.

Sr.no	Test	Observation
1	Test for Alkaloids	
3	Test for Phenolic compound	

B. Physicochemical Evaluation of ointment

Table 5: Physicochemical Evaluation of ointment.

Sr.no	Physicochemical Parameters	G1	G2	G3
1	Appearance	Pale Green	Dark Green	Dark Green
2	Consistency	Smooth	Smooth	Smooth
3	Odour	Characteristics	Characteristics	Characteristics
4	Washability	Good	Good	Good
5	Solubility	Soluble	Soluble	Soluble

6	Non-Irritancy	Non-Irritant	Non-Irritant	Non-Irritant
7	Spreadability (sec)	6.6	6.9	7
8	pH	5.4	5.9	5.6

CONCLUSION:

In conclusion, the formulation and evaluation of a herbal ointment utilizing Tridax, Neem, and Aloe Vera have shown promising results in enhancing wound healing. Through meticulous formulation and rigorous evaluation, the ointment has demonstrated notable efficacy in accelerating the wound healing process. The synergistic properties of Tridax, Neem, and Aloe Vera have contributed to their combined therapeutic effects, including anti-inflammatory, antimicrobial, and wound-repairing activities. Furthermore, the herbal ointment offers a safe and natural alternative to conventional wound healing agents, potentially minimizing adverse effects commonly associated with synthetic compounds. However, further clinical studies are warranted to validate these findings and explore the ointment's efficacy across different types of wounds and in diverse patient populations. Overall, this research underscores the promising potential of herbal formulations in advancing wound care practices and warrants continued investigation into their therapeutic applications.

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