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Research Article

Preparation And Evaluation of Polyherbal Wound Healing Ointment Containing *Annona Squamosa* Linn and *Phyllanthus Emblica*

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ABSTRACT

This study focuses on the formulation and evaluation of a polyherbal wound healing ointment using extracts from *Annona squamosa* Linn and *Phyllanthus emblica*. Both plants are traditionally recognized for their antimicrobial, antioxidant, and wound healing properties. The herbal extracts were prepared using the Soxhlet extraction method and subjected to various physicochemical tests including loss on drying, ash value, and water-soluble extractive value. Preliminary phytochemical screening confirmed the presence of flavonoids, phenols, alkaloids, and tannins. The formulated ointment, prepared using standard excipients like wool fat, paraffin, and cetostearyl alcohol, was evaluated for organoleptic properties, pH, spreadability, solubility, washability, and non-irritancy. Antioxidant activity was confirmed using reducing power assays, and antimicrobial activity was assessed through zone of inhibition tests. The results demonstrated that the formulation possesses strong antioxidant and antimicrobial properties, supporting its potential effectiveness as a natural wound healing agent. Herbal remedies were chosen due to their biocompatibility, minimal side effects, cost-effectiveness, and the presence of multiple phytoconstituents that promote synergistic therapeutic action. This polyherbal ointment presents a promising alternative to synthetic wound care products.

INTRODUCTION

Wound:

It defines as an injury to the body from violence, accident or surgery that typically involve breaking of membrane. Acute wounds occur when

the soft-tissue envelope that envelops any part of the body breaks down. The extent, depth, and anatomical structures implicated define an acute wound. The length of time it takes to heal and distinguish between acute and chronic wounds is very ambiguous and depends on the patient's age, physical state, and the location and source of the

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wound. Four to six weeks separate an acute wound from a chronic one. If an acute wound has not healed on its own within this period, it is likely to turn chronic.^[1]

Wound Healing:

Following an injury, the tissue repairs itself through a complex process known as wound healing. It is a process that includes homeostasis, tissue integrity coordination, and the activation of intercellular pathways. Wound healing can be categorized based on the type and extent of the injury. The hemostasis phase, the inflammatory phase, the proliferation phase, and the remodeling phase are the four stages of wound healing.^[1]

Phases of Wound Healing

- Phase 1-hemostasis/coagulation
- Phase 2- inflammation
- Phase 3-Granulation/proliferation
- Phase 4- Remodeling

OINTMENT:

An ointment is a homogenous, viscous semisolid preparation, most commonly a greasy, oily (Oil-80%, Water-20%) with high viscosity that is intended for external application to skin or mucous membranes. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a degree of occlusion is desired. Ointments are used topically on a variety of body surfaces. These include the skin and the mucous membrane of the eye (an eye ointment), chest, vulva, anus and nose. Ointment have very moisturizing characteristic and are effective for dry skin. They have very low risk of sensitization due to having few ingredients beyond the base oil or fat and also low irritation risk. They have more greasiness so mostly disliked by patients.

Types of Ointment

Ointment may be medicated or non-medicated.

- a) Medicated ointment: For the application of API to skin for protective, therapeutic, or prophylactic purpose.
- b) Non-medicated ointment: These are used for physical effect. They are used as protectant, emollients, or lubricants.

Characteristics of an ideal ointment

- 1) It should be physically and chemically stable.
- 2) In ointment base, finely divided active ingredients should be uniformly distributed.
- 3) The base of ointment should not possess any therapeutic action.
- 4) The ointment should be smooth and free from grittiness.

Advantages of an ointment

- 1) They have site specific application of drug on affected area, which avoids unnecessary non target exposure of drug thereby avoiding side effect i.e. site specific action with less side effect.
- 2) They avoid first pass metabolism of drug.
- 3) Convenient for unconscious patients having difficulty in oral administration.
- 4) Comparatively they are chemically more stable and easy to handle than liquid dosage forms.
- 5) They are suitable dosage forms for bitter taste drugs.^[2]

MATERIAL AND METHOD:

1. Plant Material

Herbal Treatment for Wound Healing:

1] *Annona Squamosa* Linn:





Fig. no 1 :Annona Squamosa leaves

Annona squamosa is the second biggest genus of flowering plant in the annonaceae family. It is medium-sized, tiny, and evergreen. It is cultivated in many areas of India and is native to the West Indies and America. Annona Squamosa Linn is a tiny evergreen tree that is planted throughout India for its various fruits. Parts of Annona squamosa Linn. are used in traditional healthcare to cure a variety of diseases. This plant is often known as custard apple in English, sharifa in Hindi, and sitaphalam in Telugu in India. Annona squamosa Linn. is a shrub or small tree 7 m tall and is cultivated throughout India.

Toxonomy^[3]:

Tab. No 1: Toxonomy of Annona squamosa

Kingdom	Plantae
Order	Magnoliales
Family	Annonaceae
Genus	Annona
Species	squamosa

Synonyms^[3]:

Tab. No 2: Synonyms of Annona squamosa

English	Custard apple, Sugar apple, Sweetsop
Hindi	Sitafal
Bengali	ata
Malayalam	Aathappazham, seetha, pazham
Telugu	Seetha phalam

Traditional Uses:

Communities commonly employ all parts of A. squamosa to treat a variety of acute and chronic ailments, including bug bites, cancer, and skin issues. A. Squamosa leaves have antibacterial and

wound healing activities. In America, India, and Thailand, A. squamosa leaf is used to treat urinary tract infections and diarrhea. In India, the leaf is used as traditional medicine, crushed and applied to wounds. Furthermore, in traditional American medicine, decoctions of A. squamosa leaves or combinations of other plants can be absorbed by the body and used as a febrifuge, cold treatment, and bath to alleviate rheumatic pain.^[4]

2]Phyllanthus emblica



Fig. no 2 : Phyllanthus Emblica leaves

Phyllanthus emblica L (Phyllanthaceae), also known as amla or Indian gooseberry, is an extensively utilized herb in Indian Ayurvedic traditions. According to Mirunalini et al. (2013) and Singh et al. (2012), this plant is referred to as the "King of Rasyana" due to its remarkable ability to rejuvenate and rebuild. Charak Smitha is a term used in Ayurvedic medical literature dating back to 500 BC. In addition to Indian Ayurveda, it is employed in the Unani medical system. P. emblica contains high levels of amino acids, vitamin C, and minerals, making it a valuable nutritional source. The structure resembles a tree and is 8-18 meters tall, with unevenly growing branches. The plant has elliptical leaves measuring 7-10 cm in length and yellowish-green blooms. The fruit is spherical, with six vertical bands, and contains high levels of ascorbic acid.^[5]

Toxonomy^[6]:

Tab. No 3: Toxonomy of Phyllanthus emblica

Kingdom	Plantae
Division	Angiospermae

Class	Dicotyledone
Order	Geraniales
Family	Euphorbiaceae
Genus	Emblica
Species	Officinalis Gearth

Synonyms^[6]:

Tab. No 4: Synonyms of Phyllanthus emblica

English	Indian Gooseberry
Hindi	Amla
Bengali	Amloki
Marathi	Avala
Tamil	Nelli
Gujrati	Amla

Traditional Uses:

These herbs can help with diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, diuretic, anemia, biliousness, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematogenesis, inflammations, anemia, emaciation, hepatopathy, jaundice, strangury, dysentery, hemorrhages, leucorrhoea, menorrhagia, cardiac disorders, fevers, and greyness of hairs. In addition, the fruit pulp is applied to the head to relieve headaches and dizziness, as well as snakebite and scorpion stings. The fruits are recognized for their high vitamin C content and are widely used in pickles, preserves, and jellies. The leaves are used as an aphrodisiac and antipyretic, as well as to treat biliousness, asthma, bronchitis, and vomiting. The roots, bark, and ripe fruit are astringent, although the blooms are cool and refreshing. The unripe fruit contains cooling, diuretic, and laxative properties. The exudation from the fruit's incisions is utilized as an external application in ocular inflammation. Fresh bark juice mixed with honey and turmeric is used to treat gonorrhoea. Decoction of the roots has resulted in myalgia after a feverish illness.^[7]

2. Excipients

i) Wool fat:

Wool fat is also known as Lanolin. Lanolin is a yellow fat obtained from sheep's wool. It has traditionally been used topically to treat sore, cracked nipples during breastfeeding. Highly purified lanolin products (e.g., HPA lanolin, Lansinoh) have the pesticide and detergent residues removed and the natural free alcohols reduced to below 1.5% to improve safety and reduce the allergic potential. However, even highly-purified lanolin should be avoided in patients with a known allergy to wool.^[8]

ii) Hard Paraffin:

Hard paraffin is a mixture of solid hydrocarbons, also known as paraffin wax. It is used to stiffen ointments and creams and to coat capsules and tablets. At one time it was used for cosmetic enhancement.^[9]

iii) Cetostearyl alcohol

Cetostearyl alcohol is a chemical found in cosmetic products. It's a white, waxy mixture of cetyl alcohol and stearyl alcohol, both fatty alcohols. They're found in animals and plants, like coconut and palm oil. They can also be made in a laboratory. They're used in personal care products, mainly skin lotions, hair products, and creams. They help create smoother creams, thicker lotions, and more stable foam products.^[10]

iv) Yellow soft paraffin:

Yellow soft paraffin are mixtures of semi-solid hydrocarbons. They are used as bases for ointments, as emollients in skin diseases, and as lubricants in treating dry eyes. Soft paraffin is also known as petroleum jelly, petrolatum, and Vaseline.^[9]

METHOD

1. Plant Material



Fresh leaves of *Annona squamosa* linn and *Phyllanthus emblica* were collected from the local garden, Pune

2. Preparation of plant extract:

The collected leaves of *Annona squamosa* and *Phyllanthus emblica* were shade-dried, then triturated into a fine powder, and subjected to Soxhlet extraction as follows;

Soxhlet extraction method :

A soxhlet extraction is a form of continuous solid-liquid extraction where a desired compound is extracted from solid material (containing unwanted products) using a solvent. Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. Normally a solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor. The soxhlet extractor is placed on to a flask containing the extraction solvent. The soxhlet is then equipped with a condenser.

Procedure for Soxhlet Extraction method :

- a) Soxhlet extraction is a continuous process of extraction with a hot organic solvents like ethanol, ethyl acetate, hexane and water etc.
- b) 25 g of the powdered tridax plant material is taken in a thimble which is placed in the soxhlet extractor.
- c) The extractor, which has a siphoning system, is fitted on the top of a round bottom flask. A condenser is fitted at the top of the extractor.

- d) 300 ml of the extracting solvent is poured into the flask placed on a heating mantle.
- e) On heating, the solvent evaporates, raises to the condenser, where it condenses and drains back to the extractor holding the thimble with the feed.
- f) When the extractor becomes full with the hot solvent. The solvent siphons down to the flask along with the extracted constituents.
- g) The recycling of the evaporated solvent is allowed to continue until the extraction is complete.^[11]

Physicochemical Test:

1.DETERMINATION OF LOSS ON DRYING (LOD)

Gravimetric Method:

- (i) Weigh about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish
- (ii) Dry in the oven at 100° C or 105° C, until two consecutive weighings do not differ by more than 0.5 mg.
- (iii) Cool in a desiccators and weigh. The loss in weight is usually recorded as moisture.^[11]

2.DETERMINATION OF TOTAL ASH VALUE:

Procedure:

- (i) Weigh and ignite thin, porcelain dish or a tared silica crucible
- (ii) Weigh about 2 g of the powdered drug into the dish/crucible.
- (iii) Support the dish on a pipe-clay triangle placed on a ring
- (iv) Heat with a burner, using a flame about 2 cm high and supporting the dish about 7 cm above the flame, heat till vapours almost cease to be evolved; then lower the dish and heat more strongly until all the carbon is burnt off



- (v) Cool in a desiccator.
- (vi) Weigh the ash and calculate the percentage of total ash with reference to the air-dried sample of the crude drug.^[11]

3.DETERMINATION OF WATER-SOLUBLE EXTRACTIVE VALUE

Procedure;

- (i) Weigh about 4 g of the coarsely powdered drug in a weighing bottle and transfer it to a dry 250 ml conical flask.
- (ii) Fill a 100 ml graduated flask to the delivery mark with the solvent (Water), Wash out the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask
- (iii) Cork the flask and set aside for 24 hours, shaking frequently.(Maceration).
- (iv) Filter into a 50 ml cylinder. When sufficient filtrate has collected, transfer 25 ml. of the filtrate to a weighed, thin porcelain dish, as used for the ash values determinations.
- (v) Evaporate to dryness on a water-bath and complete the drying in an oven at 105° C for 6 hrs
- (vi) Cool in a desiccator for 30 minutes and weigh immediately.
- (vii) Calculate the percentage w/w of extractive with reference to the air-dried drug.^[11]

Tab. No 5:Result of Physicochemical Test:

Sr.no	Name of Parameter	Annona Squamosa Value %w/w	Phyllanthus emblica Value %w/w
1	Loss on drying	6%	2%
2	Ash Value	9%	4%
3	Water soluble extractive value	1.96%	2.48%

Preliminary Qualitative Phytochemical Analysis

This study was carried out to identify the presence of secondary metabolites in plant. The aqueous extracts of Annona squamosa and Phyllanthus emblica was prepared and preliminary phytochemical analysis were performed by using the following standard method.

1.TESTS FOR AMINO ACIDS

Ninhydrin test (General test): Heat 3 ml TS. and 3 drops 5% Ninhydrin solution in boiling water bath for 10 min. Purple or bluish colour appears.^[11]

2.TEST FOR GLYCOSIDE

Test for deazysugars (Keller-Killiani test): To 2 ml extract, add glacial acetic acid, one drop 5% FeCl₃ and conc. H₂SO₄, Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green.^[11]

3.TEST FOR ALKALOIDS

Dragendorff's test: To 2-3 ml filtrate, add few drops Dragendorff's reagent. Orange brown ppt. is formed.^[11]

4.TEST FOR TANNIS AND PHENOLIC COMPOUND

Lead acetate solution: white ppt.^[11]

5.TEST FOR FLAVONOIDS

Sulphuric Acid Test: On addition of sulphuric acid (66% or 80%) flavones and flavono dissolve into it and give a deep yellow solution. Chalcones and aurones give red or rec bluish solutions. Flavanes give orange to red colours.^[11]

6.TEST FOR PHENOLS

Ferric Chloride Test, where a solution of ferric chloride (FeCl₃) is added to the extract, and a blue,

green, black, or purple color indicates the presence of phenolic compounds.^[11]

Molisch's test: To 2-3 ml aqueous extract, add few drops of alpha-naphthol solution in alcohol, shake and add conc. H₂SO₄ from sides of the test tube. Violet ring is formed at the junction of two liquids.^[11]

7. TEST FOR CARBOHYDRATE



Fig no 3: Phytochemical Screening of Annona Squamosa and Phyllanthus Emblica

Tab no 6: Result of Phytochemical screening

Sr no	Test	Annona Squamosa	Phyllanthus emblica
1	Test for Amino acid	-	-
2	Test for Glycoside	-	+
3	Test for Alkaloids	+	+
4	Test for Tannis	+	+
5	Test for flavanoids	+	+
6	Test for Phenol	+	+
7	Test for Carbohydrate	+	+

Antioxidant Activity Test:

❖ Reducing Power Ability Assay:

Reagents: Phosphate buffer, potassium ferricyanide, Sulphosalicylic acid

Procedure:

1. Prepare the different concentrations of samples results of this method are based on the fact of increased absorbance values of different concentrationis indicaltes A higher reducing power.
2. Mix 1.0ml of sample with 2.5ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. Incubate at 50°C for 20 min.
3. Then add 2.5 ml of Sulphosalicylic acid (10%) to the mixture, centrifuge at 3000 rpm for 10 min. Finally, mix 1.25 ml from the supernatant with 1.25 ml of disilled water and 0.25 ml FeCl₃ solution (0.1%, w/v). Measure the absorbance at 700 nm.
4. Carry out the assays. Increased absorbance values indicate a higher reducing power.^[12]

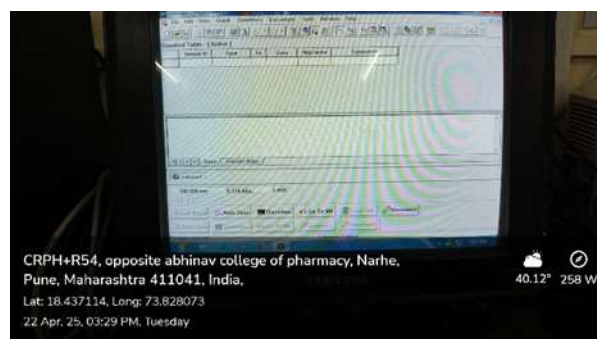
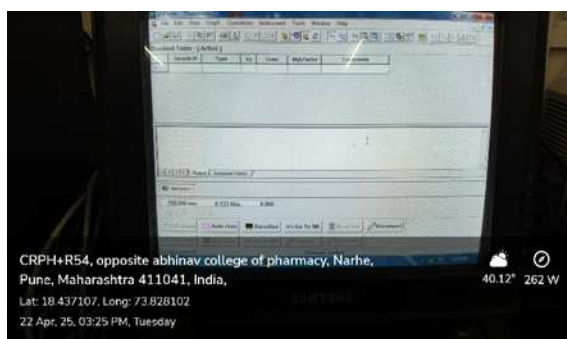


Fig no 4: Ascorbic acid Absorbance at 700 nm

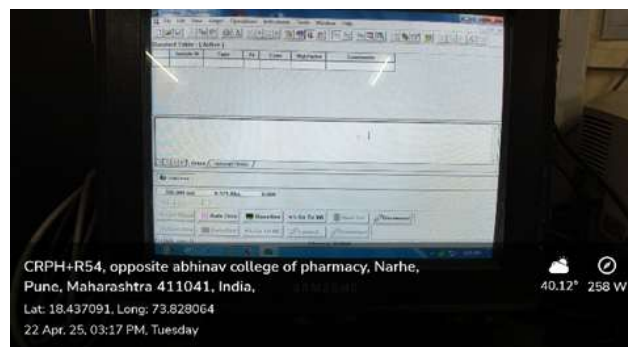
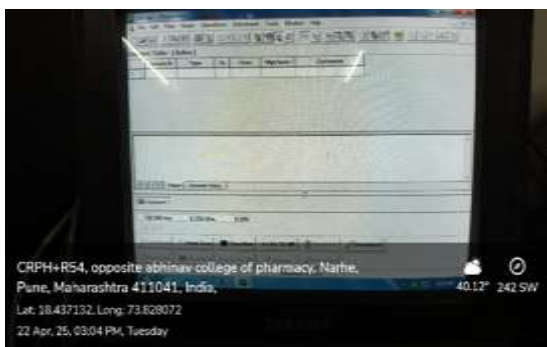


Fig no 5: Annona Squamosa Absorbance at 700 nm

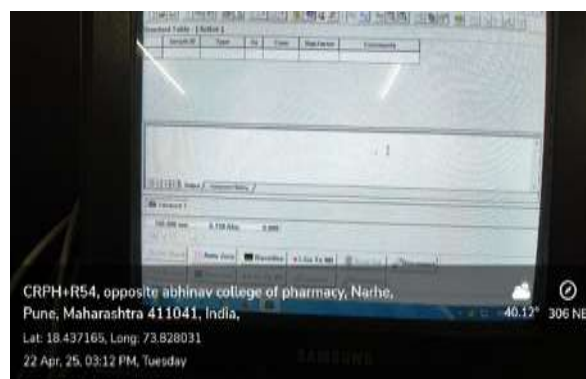


Fig no 6: Phyllanthus Emblica Absorbance at 700 nm

Tab.no 7: Antioxidant Activity Observation Table:

Concentration	Ascorbic acid (Standard) Absorbance at 700nm	Annona squamosa (Sample 1) Absorbance at 700nm	Phyllanthus emblica (Sample2) Absorbance at 700nm
0.5	133	0.237	0.158
1.0	134	0.175	0.159

Conclusion:

The absorbance of the sample solutions is greater than that of the standard (ascorbic acid), hence “The sample exhibit higher reducing power than standard ascorbic acid, indicating a stronger

electron donating capacity and higher antioxidant activity.”

❖ **Antimicrobial Activity Test:**

Requirement:

Culture: Microbial culture

Media: Nutrient agar plate

Reagents: Beef extract, Peptone, Nacl, Distilled water

Apparatus: Test tube, Flask, pipette, Petri dish, Cork borer

Equipment: Hot air oven, incubator, soxhlet extractor, water bath.

Procedure:

Prepare nutrient agar Petri plates for the growth of bacterial culture. Spread the test cultures on the plates by spread plate method. Prepare wells in seeded plates by using cork borer that is sterilised by burning with absolute ethanol. Plant extract (0.1ml) are added in the labelled wells and incubated. One well is prepared as control having 0.1 ml of pure solvent. Bacterial test culture are incubated at 32- 37°C for 48 hours. The sensitivity of test organism to each of extracts is indicated by clear zone of inhibition around the well.^[13]

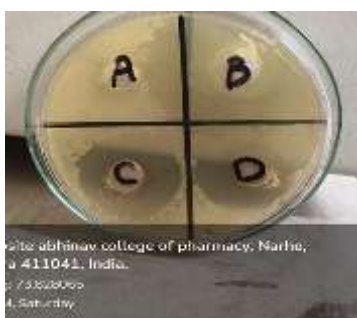


Fig no 7: zone of inhibition of Formulation

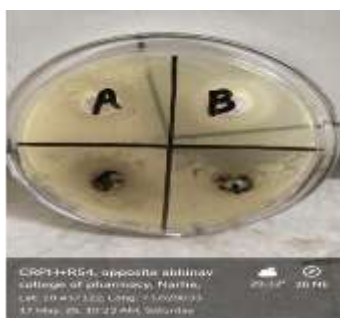


Fig no 8: Zone of inhibition of Annona Squamosa



Fig no 9: Zone of inhibition of Phyllanthus Emblica

Conclusion:

The antimicrobial assay demonstrated that both *Annona squamosa* and *Phyllanthus emblica* leaf extracts possess significant antibacterial activity, as evidenced by the clear and measurable zones of inhibition against tested microorganisms. The polyherbal formulation developed from these extracts also exhibited strong antimicrobial effects, indicating a synergistic action between the plant constituents. These results support the potential of the individual extracts and their combined formulation as effective agents for inhibiting microbial growth, which is a crucial factor in promoting wound healing and preventing infection.

Preparation of Wound healing Ointment:

Following ingredients are used in the formulation of polyherbal wound healing ointment:^[14]

Tab. No 8: Ingredient used to formulate ointment

Sr.no	Ingredients	Quantity	Role
1.	Wool fat	5.0g	Base
2.	Hard paraffin	5.0g	Base
3.	Cetostearyl alcohol	5.0g	Solidifying agent
4.	White soft paraffin or yellow soft paraffin	85.0g	Base
5.	<i>Annona squamosa</i> and <i>phyllanthus emblica</i>	2.5g	API

METHOD OF PREPARATION

1. All ingredients were weighed accurately.
2. Hard paraffin and cetostearyl alcohol were melted over a water bath
3. White soft paraffin and wool fat were added to this mixture.
4. All ingredients were stirred until they melted.
5. The mixture was stirred until it reached room temperature.

Incorporation of powdered active ingredients:

The necessary quantity of herbal extract was measured and blended thoroughly. The combined extract was ground together with the prepared base until a uniform ointment was achieved.^[14]



Fig no10: Formulation of Ointment

EVALUATION PARAMETER

1) Organoleptic Character:^[14]

Tab. No 9: Organoleptic character of formulation

Colour	Greenish Brown colour
Odour	Heena like
Apperance	Smooth

2) Consistency:

Smooth / grittiness are observed. ^[14]

Result: Smooth and no grittiness observed

3)pH:

The pH of the ointment was determined using a digital pH meter. To prepare the ointment solution, 10 ml of distilled water was utilized and allowed to sit for 2 hours.^[14]

Result:4.97



Fig no 11: pH of Formulation

4) Spreadability:

The spreadability is measured by placing the excess sample between two slides that have been compressed to a consistent thickness using a specific weight for a set duration. The time taken to separate the two slides is recorded as the measure of spreadability. A shorter duration needed to separate the slides indicates superior spreadability.^[14] Spreadability was calculated by the following formula:

$$S = M \times L / T$$

$$S = 28 \times 7 / 9$$

$$S = 21.7 \text{ g.cm/sec}$$

Where,

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides.



Fig no 12: Spreadibility of Formulation

5)Solubility :

Soluble in boiling water and miscible with ethanol, ether and chloroform.^[14]

Result: Soluble in hot water and ethanol

6)Washability :

The ointment was applied to the skin and then ease the extent of washing with water was checked.^[14]

Result: easily washable with water

7)Non irritancy test:

The ointment has been smeared onto the skin of a person and monitored for its effects.^[14]

Result: Non irritating

RESULT:

The formulated polyherbal wound healing ointment, prepared using *Annona squamosa* and *Phyllanthus emblica* leaf extracts, was evaluated for its antimicrobial and antioxidant properties to support its wound healing potential.

CONCLUSION:

The polyherbal wound healing ointment formulated with *Annona squamosa* and *Phyllanthus emblica* leaf extracts demonstrated effective antibacterial activity against common wound-infecting microbes and exhibited significant antioxidant properties as shown by reducing power ability assays. These properties contribute to the enhancement of the wound healing process by preventing infection and reducing oxidative stress at the wound site. Thus, the formulation holds promise as a natural, effective alternative to synthetic wound healing agents

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