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Formulation and Evaluation of Herbal Ointment Containing *Moringa oleifera* Extract

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ABSTRACT

This research developed and assessed a herbal ointment containing Moringa oleifera leaf extract for topical therapeutic applications. Leaves were collected from Kudwa, Gondia, shade-dried, and powdered, followed by ethanolic extraction using a Soxhlet apparatus to obtain bioactive compounds. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, terpenoids, glycosides, and reducing sugars, validating the extract's medicinal potential. Three ointment formulations (F1, F2, F3) were prepared using a base of petroleum jelly, stearyl alcohol, propylene glycol, and sodium lauryl sulphate, with varying concentrations of Moringa extract (0.1g, 0.5g, 0.7g) and eucalyptus oil. The formulations underwent comprehensive evaluation: physical characteristics revealed pale green, homogeneous preparations with an oily, greasy consistency and skin-compatible pH (5.3–5.7). Rheological properties indicated optimal spreadability (F3: 14.66 g·cm/sec) and viscosity (1075–1446 cP at 20 rpm), ensuring ease of application and adhesion. Antibacterial testing against E. coli and S. aureus via agar well diffusion demonstrated dose-dependent activity, with crude extract showing zones of inhibition of 5–6 mm, while formulations achieved 2–4 mm. F3 exhibited superior efficacy against S. aureus (4 mm), attributed to enhanced extract concentration. Although excipients in formulations slightly reduced antibacterial potency compared to pure extract, F3 emerged as optimal due to its balanced performance in spreadability, antimicrobial action, near-neutral pH, and stability under accelerated conditions (45°C for 20 days). These results underscore Moringa oleifera's promise as a natural ingredient in topical formulations for wound healing and skin infections, with F3 identified as the lead candidate. Further studies to refine extract concentration and excipient synergy are recommended to maximize therapeutic outcomes.

Keywords: Herbal Ointment, Moringa Oleifera, Soxhlet Extraction, Spreadability, Viscosity, pH, E. Coli, S. Aureus.

1. INTRODUCTION[1]

Ointment:-

An ointment is a semisolid, greasy substance applied topically to the skin or mucous membranes to soothe, heal, or treat various skin conditions.

Herbal ointments are topical preparations made from plant extracts or other natural ingredients. They are used to treat a variety of skin conditions, such as dryness, irritation, and inflammation. Herbal ointments are typically made with a base of oil, wax, or other emollient, which helps to deliver the active ingredients to the skin.

Moringa oleifera Leaf Extract:-

Moringa oleifera Lam (M. oleifera), belonging to the family Moringaceae, is an important plant that is used as food and medicine in different parts of the world. It is called a "miracle tree" or "Tree of Life" because of its medicinal properties that include antioxidant, antimicrobial, antidiabetic and anticancer properties, to name a few. Though all parts of the plant are active, the leaves are considered to be the most active part, and they are consumed as both food and medicine.

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Fig.no.1

M. oleifera effectively promoted wound healing and was devoid of any skin irritation. Each of the parameters studied indicate a stage of healing. Wound contraction shows the progress of the healing process, while the period of epithelization indicates complete healing of the wounds [23]. M. oleifera extract formulation increased the healing of wounds in both MRSA and P. aeruginosa infected wounds. The effect was more prominent in the former compared to the latter, implying that it may be less effective in the treatment of P. aeruginosa-infected wounds. Earlier studies suggest that Gram-negative bacteria are tolerant to chemicals and natural compounds due to their inherent resistance because of their cell wall structure.

Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess anti-tumour, antipyretic, anti-epileptic, antiinflammatory, anti-ulcer. Other important medicinal properties of the plant include anti-spasmodic, diuretic, anti-hypertensive ,cholesterol lowering , antioxidant, anti-diabetic, hepatoprotective , anti - bacterial and anti-fungal activities . The aqueous extracts of roots and barks were found to be effective in preventing implantation, aqueous extracts of fruits have shown significant antiinflammatory activity, methanolic extracts of leaves have shown anti-ulcer activity and ethanolic extracts of seeds exhibited anti-tumour activity . The leaves of Moringa contains bioactive compounds called β-sitosterol which are highly involved in the stabilization of the cholesterol level in the serum of the high fat diet fed rats . Moringa leaves are highly rich in β–carotene and leutin which supplies the vitamin A that is highly responsible to prevent the night blindness and also the eye problems in the children.

2. MATERIAL AND METHOD -

2.1 Chemical and reagents

The various chemicals used throughout experimental work are summarized.

Sr. No.	Chemicals
1	Ethanol
2	Chloroform
3	Acetic acid
4	Methanol
5	Sodium Lauryl Sulphate
6	Propylene Glycol
7	Stearyl Alcohol
8	Petrolium Jelly
9	Distilled Water
10	Nutrient Agar medium
11	Silica gel G
12	Mayer's reagent
13	Hydrochloric acid
14	Feric Chloride
15	Feheling's reagent
16	Dilute Sulphuric acid

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2.2 Instruments

Sr.No.	Name of Instrument	Brand Name
1	Analytical Balance	Contech
2	Digital Balance	Shimadzu, AUx 220
3	Hot Air Oven	Tempo
4	Digital Autoclave	ASI-254
5	B.O.D. Incubator	HMG

❖ Calibrated Glassware used During Experimentation.

2.3 Soxhlet Extraction Method

The Soxhlet extraction method is highly effective for isolating bioactive compounds like antioxidants and oils from dried, ground Moringa oleifera leaves or seeds. The plant material is placed in a porous thimble within the Soxhlet apparatus. A suitable solvent (e.g., ethanol, hexane, or methanol) is heated in a flask below. The solvent vapor rises, condenses, and drips onto the sample, dissolving soluble compounds. Once the thimble chamber fills, the solvent, now enriched with extracted compounds, siphons back to the boiling flask. This cyclic process of continuous washing and refluxing occurs repeatedly over several hours, efficiently concentrating the target compounds into the solvent. The method is particularly valued for its thoroughness and efficiency in extracting thermostable components.

Fig.no. 2 : Ethanolic Extract of Moringa oleifera

2.4 Phytochemical Screening of Moringa oleifera extract

The different qualitative phytochemical tests were carried out as per the standard tests for the phyto-constituents such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, glycosides, reducing sugars, fats and oils, etc., present in the leaf extracts. The positive tests were noted as present (+) and absent (-).

1.Saponins

Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

2. Tannins

- **Feric Chloride Test**

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue colour is observed for gallic tannins and green colour indicates for catecholic tannins.

3. Reducing sugars

- **Fehling's Test**

To 0.5 ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

4. Alkaloids

- **Wagner's test :**

Wagner's test was carried out to confirm alkaloids in M. oleifera leaf extract. Reddish brown precipitates developed when 2 ml extract was mixed thoroughly in 1% HCl solution with 0.5 ml of Wagner's reagent.

- **Mayer's test :**

Extracts were dissolved individually in dilute hydrochloric acid (HCl) and filtered. Mayer's test: Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow-colored precipitate indicates the presence of alkaloids.

5. Flavonoids

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoid's and orange colour for flavones.

6. Phenols

To 2 ml of extract, a few drops of ferric chloride solution was added. The appearance of a greenish yellow colour, confirms the presence of phenol.

7. Glycosides

25 ml of dilute sulphuric acid was added to 5 ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, then 5 ml of Fehling's solution added. Glycosides are indicated by a brick red precipitate.

8. Terpenoids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoids.

9.Solubility Test :

- **Water :** 2ml of extract dissolved in 4ml distilled water.

- **Ethanol :** 2ml of extract dissolved in 4ml distilled water.

2.5 TLC of Moringa oleifera Extract[9]

The principle of Thin Layer Chromatography (TLC) is based on the separation of mixtures according to differences in polarity, adsorption, and partitioning. The process begins with the application of a sample to a TLC plate, which is then developed by allowing a mobile phase (solvent) to move up the plate. As the mobile phase travels, it separates the compounds in the sample based on their interactions with the stationary phase (silica gel) and the mobile phase. The separated compounds are then visualized using various detection methods, such as ultraviolet (UV) light, spray reagents, or fluorescence. The degree of separation is determined by the Rf value (Retention Factor) of each compound, which is the distance traveled by the compound relative to the solvent front.

Solvent 1 **Solvent 2**

Chloroform : Ethanol : Methanol(1:1:1) Ethanol : Water : Chloroform (4:2:4)

(Fig no.3 :TLC of Moringa Oleifera Extract) **(Fig no. 4: TLC of Moringa Oleifera Extract)**

$$Rf = \frac{\text{Distance of Centre of Spot from Starting point}}{\text{Distance of Solvent front from Starting point}}$$

2.6 Optimized Formula for Ointment

Sr.no.	Ingredients	Quantity		
		F1	F2	F3
1	Sodium lauryl Sulphate	0.15gm	0.15gm	0.15gm
2	Propylene Glycol	5.1ml	4.8ml	5ml
3	Stearyl Alcohol	4.8gm	4.95gm	4.95gm
4	Petrolium jelly	5gm	4.9gm	5gm
5	Moringa Extract	0.1gm	0.5gm	0.7gm
6	Eucalyptus Extract	0.1ml	0.2ml	0.2ml
7	Dist.Water	15ml	15ml	14ml

Method

The ointment preparation begins by accurately weighing the base components (such as petroleum jelly or a mixture like beeswax and stearyl alcohol) according to the formula and melting them using a double boiler or heating mantle, with occasional stirring to ensure even heating. Once the base is fully melted, the precisely weighed active ingredient (e.g., Moringa oleifera extract) and excipients (such as sodium lauryl sulphate, propylene glycol, and distilled water) are added directly into the molten base and stirred thoroughly to achieve initial incorporation. The mixture is then subjected to rigorous mixing and homogenization using an ointment mill or mechanical mixer to ensure a perfectly smooth, uniform consistency. Subsequently, the homogenized ointment is allowed to cool to approximately 30-40°C while being stirred occasionally to prevent crystal formation; once it reaches the appropriate consistency, it is poured into ointment tubes or jars. Finally, the containers are sealed tightly, labeled clearly with the product name, ingredients, and usage instructions, and stored in a cool, dry place to maintain stability.

2.7 Evaluation of Herbal Ointment[3]

1. Physical appearance:

The prepared Ointment formulations containing *Moringa oleifera* were inspected visually for their color, homogeneity, consistency and phase separation.

2. Physical appearance:

The prepared Ointment formulations containing Moringa oleifera were inspected visually for their color, homogeneity, consistency and phase separation.

3. Spreadability:

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

Spreadability was calculated using the following formula:

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

Where.

S- Spreadability,

M- weight in the pan (tied to upper slide),

1. Length moved by the slide,

T-Time (in sec.)

4. Determination of Viscosity:

The Brookfield viscometer was used to test the viscosity, with the proper number of spindles selected. The 50 ml beaker was used to hold 50gm of preparation until the spindle groove was dipped and the rpm was set. Ointment viscosity was measured at 5,10,20,50, and 100 rpm. The viscosity was computed using the factor obtained from the reading.

5. Washability:

This test is carried out by simply washing applied Ointment on the skin surface with water.

6. Homogeneity:

The formulation were tested for the homogeneity by visual appearance and touch.

7. Irritancy Test:

Mark an area (one sq. em) on the left hand dorsal surface. The Ointment was applied to the specified.

area and time was noted. Irritancy erythema, edema was checked if any for regular interval up to 24 hrs and reported.

8. Stability Testing:

Stability testing of formulation was conducted at room temp, studied for 7 days. And then the formulation studied at 45 ± 1°C for 20 days. The formulation was kept both at room and elevated temperature and observed on 0h 5th, 10, 15th and 20th day for all the evaluation parameter.

9.Procedure for antibacterial activity by pour plate method[5]:

The agar well diffusion method was adopted for the antimicrobial sensitivity test. For antibacterial studies, the microbial strains of Escherichia coli and Streptococcus aureus was collect form Manoharbhai Patel Institute of Bachelor of Pharmacy, Kudwa, Gondia.

Prepare nutrient agar Petri plates for the growth of bacterial cultures. Pour the cultures in agar media. The test cultures used such as Streptococcus aureus and Escherichia coli. Prepare well in seeded plates by using cork borer that is sterile by burning with absolute ethanol. Plant extract 1 ml of (0.1 mg/ml) are added in the labeled well and incubated. One well is prepared as control using ampicillin 10mg/ml. Bacterial test culture plates are incubated at 32-37° c for 48 hrs.

3. RESULT

- **Phytochemical Screening[9]:** The phytochemical Screening of herbal Ointmentis done for identification of active chemical ingredients present in the leaves of *Moringa oleifera.*

Sr. No.	Tests	Observation	Result
1.	**Test for solubility :** a. Water b. Ethanol	Partially Soluble in water Highly soluble in Ethanol	+ve
2.	**Test for alkaloid :** a. Wagner's test b. Mayers test	Reddish Brown Precipitate Yellow Color Precipitate	+ve
3.	**Test for flavonoids :** a. Shinoda test	Yellow Hue	+ve
4.	**Test for Tannins :** a. Ferric chloride test	Orange Color	+ve
5.	**Test for Phenol**	Dark Green color	+ve
6.	**Test for Saponins**	Honeycomb not observed	-ve
7.	**Reducing Sugar Test** a.Fehling's Test	Brick red precipitate	+ve
8.	**Terpenoids Test**	red violet color	+ve

- **Solubility test of Extract :**

Sr.no.	Solvents	Result
1.	Water	Sparingly soluble
2.	Ether	Soluble
3.	Methanol	Soluble
4.	Ethanol	Soluble

- **Thin Layer Chromatography:**

Solvent System	UV Light	No. of Component	*Moringa oleifera* Rf Value
Chloroform : Ethanol : Methanol (1:1:1)	366nm	1	0.81
Ethanol:Water:Chloroform (4:2:4)	366nm	1	0.86

- **Antibacterial Evaluation:**

Sr. No.	Tests Culture	Positive(10mg/ ml)	Plant Extract	Positve Control	Zone of inhibition in (mm)			
					Plant Extract	Formulation		
						F1	F2	F3
1.	E.coli	Ampicillin	Moringa Oleifera	7	05	3	4	4
2.	S.aureas	Ampicillin	Moringa Oleifera	7	06	2	3	4

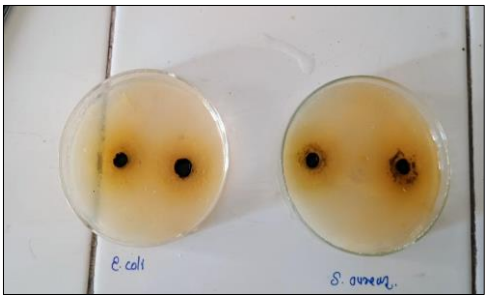

Fig.no.5. Antimicrobial activity of Moringa oleifera Extract

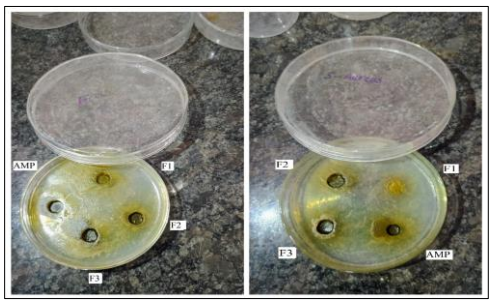

Fig.no.6. Antimicrobial activity of Formulation

1. Color and Appearance

Test	Result
Color	Pale Green
Appearance	Oily, Greasy consistency

2. pH of the formulation:

The of ointment was determined by Digital pH meter 0.5gm of herbal ointment was dissolved in 50ml distilled water and pH was measured.

Sr.no.	Batch	pH
1	F1	5.5
2	F2	5.3
3	F3	5.7

3. Viscosity :

Viscosity of Herbal Ointment is measured by Brookfield viscometer.

Sr.no.	Batch	RPM	CP
1	F1	20 rpm	1075
2	F2	20 rpm	1446
3	F3	20 rpm	1152

4. Spreadability of Formulation :

Sr.no.	Batch	Spreadability
1	F1	21.25
2	F2	21.50
3	F3	14.66

.4. DISCUSSION

Notably, the formulations showed reduced efficacy compared to the crude extract, with F3 performing best (4 mm for both strains). This decline may stem from formulation processes diluting active compounds or introducing excipients that interfere with bioavailability.The smaller inhibition zones overall indicate weak antibacterial potency, though methodological factors (e.g., extract concentration, agar type) could influence these results. Interestingly, S. aureus (Gram-positive) was slightly more inhibited by the crude extract than E. coli (Gram-negative), possibly due to differences in cell wall structure. Further optimization of formulations, including concentration adjustments and stability testing, could enhance activity. These findings highlight Moringa oleifera's potential as a natural antibacterial agent but underscore the need for refined delivery systems to maximize efficacy.

5. CONCLUSION :

From the given data, we can say that Formulation 3 (F3) shows better properties than F1 and F2 and thus is the best herbal ointment.

Here's why:

* Spreadability: F3 has the least spreadability value (14.66). Less spreadability is usually a good sign of greater skin adhesion, which is what is desired in topical ointments.

* Antimicrobial Activity: Though all preparations exhibit activity against E.coli and S.aureus, F3 has the largest zone of inhibition against S.aureus (4mm). This indicates a more effective antimicrobial action against this specific bacteria.

* pH: F3 (5.7) is in a slightly more neutral pH range than F1 and F2. Although all are in a skin-friendly range, a more neutral pH may be desirable for some people.

Thus, taking into account the combined parameters of spreadability, antimicrobial action, and pH, F3 is the best performing and well-balanced product among the three tested.

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