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Formulation And Evaluation of Anti-Inflammatory Ointment Containing Moringa Oleifera Leaves Extract

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ABSTRACT
It is common knowledge that the body uses inflammation as a natural defense against infection and to heal tissue damage. It is possible to link the anti-inflammatory properties of flavonoids to their suppression of the enzyme's lipoxygenase and cyclooxygenase. The primary goal of the research was to create and assess an anti-inflammatory ointment with flavonoids of Moringa oleifera leaf extract. With valuable pharmacological actions like as anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-fertility, anti-cancer, anti-microbial, and anti-oxidant properties, this plant is widely utilized as a nutritional herb. Chemicals such as cetostearyl alcohol, hard paraffin, liquid paraffin, and wool fat were utilized in addition to Moringa oleifera leaf extract. The formulation is made with the appropriate technique and evaluated for spreadability, extrudability, washability, pH, color, smell, texture, and stability. Smooth, non-irritating, and skin-compatible prepared ointment. The suitability of the approach for the production of ointments was demonstrated by the physical evaluation of ointment preparations using extract of Moringa oleifera leaves. According to the study's findings, it serves as a substitute for anti-inflammatory medications that are just synthetic or chemical. These results confirm the M. oleifera plant's historic use as a successful treatment for illnesses and conditions linked to inflammation.

KEYWORDS: Inflammation, Flavonoids, Moringa oleifera, Ointment, Evaluation.

1. INTRODUCTION

Numerous complex biological reactions that cause cellular and tissue damage are triggered by different hazardous substances, and these reactions collectively are called inflammation. Its processes provide a protective role by attempting to eliminate the source of cell injury and start the healing process of damaged tissue [1]. The inflammatory cascade begins when tissue homeostasis is upset, and the immune system responds by generating a number of pro-inflammatory mediators, including as interleukins, tumor necrosis factor alpha (TNF-α), reactive oxygen species, nitric oxide, and prostaglandins [2,3]. This alters the local microcirculation, causing vasodilation and increased vascular permeability to draw various

blood proteins and blood cells that mediate the inflammatory response to the location of damaged tissue [4].

Many of the skin's functions are disrupted by dermal inflammation, most notably its protective function. Therefore, addressing the underlying cause or symptoms of cutaneous inflammation should be the goal of treating it without endangering the skin further [5].

Nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, metformin, and statins are among the medications used to treat inflammation today. Nutraceuticals include ginger root, turmeric, hyssop, devil's claw (Harpagophytum procumbens) [7], capsaicin [8], and hyssop.

When NSAIDs are applied topically, they can cause rashes on the skin [9], and a 2016 Cochrane review discovered that their effectiveness in treating musculoskeletal pain is limited. Of patients treated with topical NSAIDs, ketoprofen or diclofenac, only 10% reported better pain relief when compared to a placebo [10]. Another meta-analysis found that while topical NSAIDs may significantly lessen the traditional GI or renal side effects, a more thorough clinical examination of the drug's impact on the cardiovascular system is still warranted [11].

A different approach that involves using medicinal plants seems to have certain benefits over traditional medications, including a better safety record and reduced expenses [12,13]. The bioactive phytochemicals that are abundant in natural medicines, such as phytosterols, carotenoids, flavonoids, lignans, omega-3 fatty acids, stillbenoids, and polyphenols, have anti-inflammatory and antioxidant properties [14,16,17].

In India, one of the most significant plants that is commonly grown is Moringa oleifera Lam. It is a member of the Moringaceae family. This plant has important pharmacological properties such as anti-inflammatory, anti-diabetic, hepatoprotective, anti-asthmatic, anti-fertility, anti-cancer, antibacterial, and anti-oxidant properties. It is commonly used as a nutritional herb [18]. The naturally occurring substances known as bioflavonoids are secondary metabolites of plants with a wide range of medicinal uses [19].

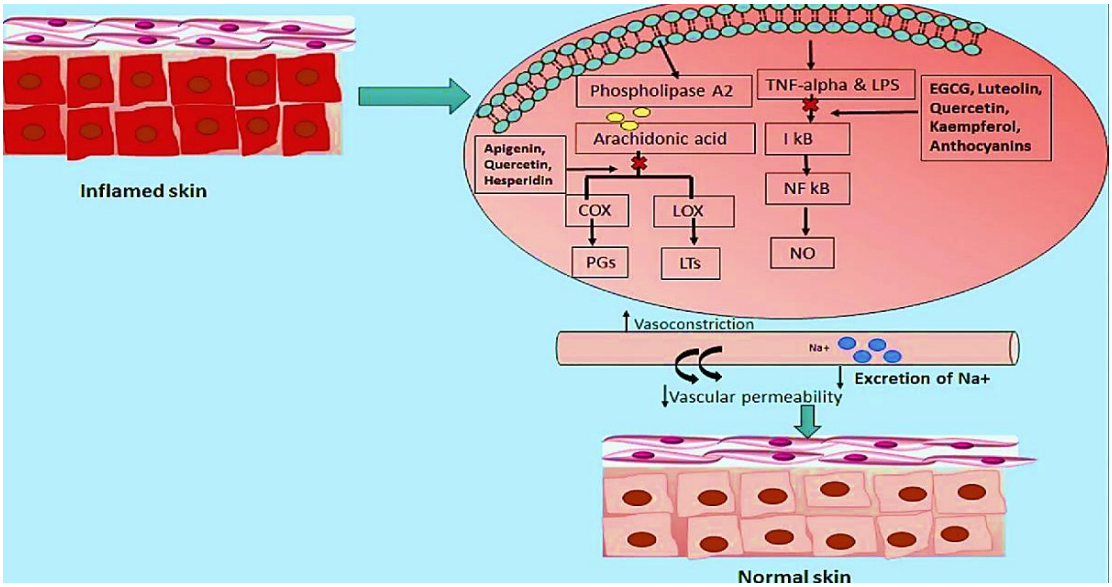

Fig 1: anti-inflammatory effect of Flavonoids [16]

Because flavonoids are very lipophilic substances, they are challenging to absorb orally. When taken orally, they experience increased first-pass metabolism, which reduces their oral bioavailability. As a

result, a different topical delivery method for several flavonoids has been investigated [20]. The preparation of an anti-inflammatory ointment with extract from Moringa oleifera leaves that contains flavonoids is the goal of this work.

2. MATERIAL AND METHODS

Materials:

2.1 Drugs and chemicals:

Ethyl acetate (High Purity Lab. Chemical Pvt. Ltd.), Wool fat (S D Fine Chem Ltd.), Ceto stearyl alcohol (Fine Chemical Co. Ltd.), Hard paraffin & yellow soft paraffin (S D Fine Chem Ltd) and all other ingredients utilized in the preparation of the formulation were of the analytical grade and procured from store of RP College of Pharmacy, Dharashiv.

2.2 Collection of plant material:

Fresh leaves of Moringa oleifera was collected from Dharashiv (Osmanabad), Maharashtra, India and transported to laboratory and shade dry.

Fig 2: Plant of *Moringa oleifera*

3. EXTRACTION

Removal of Plant Matter After collecting leaves, the dirt is removed by washing them under running tap water. Microbes are eliminated from these leaves by immersing them in 1% saline solution (NaCl) for five minutes. After that, leaves are given two more washes in distilled water and 70% ethanol. This stage is crucial in getting rid of the germs, viruses, and dust that are on the surface of the leaves. One way to get rid of extra water from leaves is to spread them out in the shade for a little while until any remaining water on the leaf surface evaporates. The leaves are stored in an airtight container once they have dried and been roughly powdered using a mixer grinder.[21]

3.1 Extraction Method (Percolation):

a) Before percolation the coarsely powdered leaves were soaked with Ethyl acetate (Menstrum) for 4 hrs.
b) For packing a piece of cotton wool or a filter paper is placed on the false bottom of percolator:

c) Then the moistened drug is introduced into the percolator.
d) Again, a filter paper or cotton wool is placed over the top of drug on which small quantity of washed sand is placed to prevent the disturbance of packed material.
e) After 24 hours the lower orifice is opened and menstrum is collected with a controlled speed until 3% of menstrum is collected.
f) Then more menstrum is added and collected from the lower orifice so that the marc does not become dry.
g) Then marc is pressed to extract which is combined with previous liquid.
h) Then it is allowed to stand and then it is filtered.

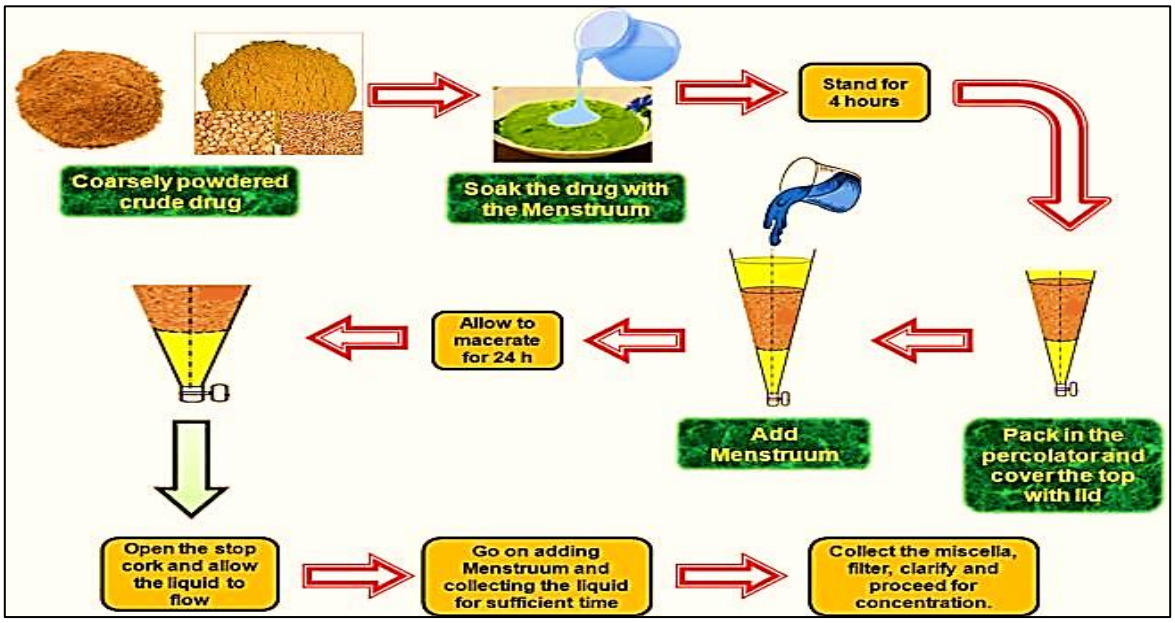

Fig 3: Percolation Method [22]

Fig 4: Soaking of powdered drug

Fig 5: Powdered drug in percolator

Fig. 6: Extract of *Moringa oleifera* leaves

3.2 Phytochemical screening:

To find the presence of phytoconstituents, the Extract of Moringa oleifera underwent a qualitative analysis. The standard procedures and tests provided by Khandelwal K.R., 2010 were used to conduct qualitative tests.[23]

4. FORMULATION OF OINTMENT

4.1 Formulation of ointment base:

Sr. No.	Name of Ingredient	Role	Quantity taken
1	Wool fat	Emollient	0.5 gm
2	Ceto stearyl alcohol	Stabilizer	0.5 gm
3	Hard Paraffin	Stiffening agent	0.5 gm
4	Yellow Soft Paraffin	Lubricant	8.5 gm

Table 1: Formula for ointment base

4.2 Formulation of ointment:

Sr. No.	Name of Ingredient	Quantity taken
1.	Prepared *Moringa oleifera* Extract	0.12 gm
2.	Ointment base	10 gm

Table 2: Formula for ointment

4.3 Formulation Method:

1. Firstly, hard paraffin was finely ground and weighed to create the ointment basis. This was then put in an evaporating dish over a water bath.
2. After the hard paraffin melted, the additional ingredients were added, and the ointment base was cooled after gently stirring to promote homogenous melting and mixing.
3. To create a smooth paste that was twice or three times the weight of the base, precisely weighed Moringa oleifera extract was added to the ointment base using the levigation method. The base was then gradually added until the ointment was homogenous.
4. Moved at last into an appropriate container

Fig 7: Melting all the ingredient

Fig 8: Moringa oleifera ointment

5. EVALUATION OF FORMULATION

5.1 Physical evaluation: Moringa oleifera ointment formulations were evaluated based on their color, odour, appearance, texture and consistency. Texture was determined on the basis of grittiness/ smoothness.

5.2 pH: pH of 1% aqueous solution of the formulation was measured by using pH meter at constant temperature.

5.3 Spreadability: The Spreadability is expressed in terms of time in seconds taken by two slides to slip off from ointment, placed in between two slides under the direction of certain load. Lesser the time taken for separation of two slides, better the Spreadability of ointment Spreadability of Moringa oleifera ointment formulations was determined by using the formula-

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

Where S =Spreadability, M =Weight tied to upper slide,
L =Length of glass slides and T =Time taken to separate the slides.

5.4 Washability: Moringa oleifera ointment formulations were applied on the skin and then ease extend of washing with water was checked. Washability was checked by keeping applied skin area under the tap water for about 10 min.

5.5 Extrudability test: it is the measure of the force required to extrude the material from a collapsible tube when certain amount of force has been applied on it in the form of weight. In the present study the quantity in percentage of ointment extruded from the tube on application of certain load was determined. The extrudability of prepared Moringa oleifera ointment formulations was calculated by using following formula.

Ext = Amount of Ointment extruaded from the tube. 100 / Total amount of ointment filled in the tube

5.6 Loss on drying: The loss in weight, in the sample so tested, principally is due to loss of water and small amount of other volatile material from it. Loss on drying was determined by placing the 1gm of Moringa oleifera ointment formulations of different batches in a petri dish on a water bath and dried until constant weight was obtained.

5.7 Centrifugation: It is believed to be a unique tool for the evaluation of accelerated deterioration of ointments. It was determined by using centrifuge in 10 ml graduated cylinder at 10,000 rpm for 10 min.

5.8 Stability study: Moringa oleifera ointment formulations were evaluated for their stability at an ambient condition of pressure and temperature for two weeks. Formulations were observed for phase separation and particle agglomeration.

6. RESULT AND DISCUSSION

Moringa oleifera extract was used to make the ointment. It was discovered that the formulations had a glossy look, were homogenous, free of phase separation, and no grittiness. Formulations met spreadability, pH, and physical stability requirements for physical examination. The prepared ointment is gentle, non-irritating, and skin-compatible.

Sr. No.	Evaluation Test	Observation
1	Colour Odour Appearance Texture	Whitish-yellow Characteristic Non greasy Smooth
2	pH	5.5
3	Spreadability (sec)	Easily spreadable
4	Extrudability (gm)	0.5
5	Loss on drying	20%
6	Centrifugation	No phase separation
7	Washability	Good
8	Stability study	stable

Table 3: Evaluation Test

Given that viscosity and spreadability have an inverse relationship, spreadability research have shown that the spreading area decreases as viscosity increases. There were no skin irritations from the formulation. Clinical practice can safely use the mixtures, according to the findings. Centrifugation produced no evidence of phase separation. Room temperature stability of the formulation was observed.

7. CONCLUSION

Now a days lots of peoples are suffering from many inflammatory disorders. The aim of this study is to prepare a formulation of anti-inflammatory ointment that contains a Moringa oleifera ethyl acetate leaf

extract. In this study suitable extraction method is used for extraction of flavonoids from leaves. From preliminary phytochemical testing's it is observed that flavonoids are present in the ethyl acetate extract. Later on, formulation was prepared using suitable method and other chemicals. The prepared formulation was evaluated using different parameters like colour, odour, appearance, texture, spreadability, washability, loss on drying, extrudability, stability, etc.

From this evaluation parameters it is observed that the prepared ointment is safe to use and stable under room temperature.

8. FUTURE PERSPECTIVES

Further evaluation tests and long-term stability testing at different temperature is required as well as long term application pre-clinical and clinical studies are required.

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