

Preparation And Evaluation Of Anti-Inflammatory Ointment From Aeglemarmelos Leaves: An Observational Study.

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ABSTRACT

Background: Aegle marmelos commonly known as Bael belongs to family Rutaceae, which is widely used in indigenous systems of Indian medicine due to its medicinal properties. This study aims to Prepare and evaluate anti-inflammatory ointment from aeglemarmelos leaves.

Materials and Methods: The leaves of the plant were properly washed with tap water and rinsed with distilled water. The rinsed leaves were hot air dried for 3 days. The dried leaves of aeglemarmelos plant were crushed into powder using a mortar and pestle and stored in an airtight glass container at 4 ° C until use. A 10 g powder sample was separately immersed in distilled water and ethanol (100 mL) at room temperature for 1.5 hour for three times. The extract was then filtered, concentrated to a final volume of 50 mL concentrated extract was dried using hot plate. The anti-inflammatory properties were studied via different means.

Results: The extracts Ph was reported to be 6.8-7 which is considered as neutral. It was light green in colour and aromatic in odour. The ointment was non-irritant in nature and smooth in consistency

Conclusion: Aeglemarmelos showed significant anti-inflammatory activity in the models studies, it can be a promising anti-inflammatory agent.

Keywords: Aeglemarmelos, Anti-inflamatory, ointment.

INTRODUCTION:

Nature has a complete depository of remedies to cure ailment of mankind ⁽¹⁾. About 80% of the world's population depends wholly or partially on traditional medicine for its primary health care needs. World health organization states that about 80% of patients in India, 85% in Burma and 90% in Bangladesh patients are treated with traditional system of medicine. Herbal medicine play an indispensable role in traditional medical system and these are used for thousand years and made a great contribution to maintain human health⁽²⁾. The medicinal plants are potential sources of drugs (rich in secondary metabolites) and essential oils of therapeutic importance. They most important therapeutic use is that they are safe, economical, effective and their easy availability.

Aegle marmelos (L.) Correa (*A. marmelos*), commonly known as Bael belonging to the family Rutaceae, has been widely used in indigenous systems of Indian medicine due to its various medicinal properties. *A. marmelos* is native to Northern India, but widely found throughout the Indian Peninsula and in Ceylon, Burma, Bangladesh, Thailand and Indo-China. It is a medium to large sized deciduous glabrous, armed tree with the axillary and 2.5 cm long alternate trifoliate leaves, short flower and globular fruit ^(3, 4).

Table 1 depicts the Taxonomical classification of aeglemarmelos.

Table 1

Kingdom	Plantae
Subkingdom	Tracheobonata
Super division	Spermatophyte
Division	Magnoliophyta
Family	Rutaceae
Class	Magnoliospida
Subclass	Rosedale
Order	Sapinales

This is generally considered a sacred tree by Hindus because its leaves are used to worship Lord Shiva. According to Hindu mythology, the tree is another form of Lord Kairashnas. Leaves, fruits, stems and roots of this tree are used as folk medicine for various things at all stages of Human illness. This study aims to Prepare and evaluate anti-inflammatory ointment from aeglemarmelos leaves.

MATERIALS AND METHODOLOGY

Table: 2 List of equipment

Sr.No.	Name of Equipment	Manufacturer
1	Weighing Balance	Contech Instruments
2	Mortar And Pestle	Borosil Limited
3	Measuring cylinder	Borosil Limited
4	Spatula	Borosil Limited
5	Pipette	Borosil Limited
6	Beakers	Borosil Limited
7	Stirrer	Borosil Limited
8	Petri Plate	
9	Round bottom flask	Garg process glass private limited
10	Reflux condenser	Biohall life sciences private limited
11	Heating mantle	Garg process glass India privates

Extraction

The leaves of the plant were properly washed with tap water and rinsed with distilled water. The rinsed leaves were hot air dried for 3 days. The dried leaves of aeglemarmelos plant were crushed into powder using a mortar and pestle and stored in an airtight glass container at 4 ° C until use. A 10 g powder sample was separately immersed in distilled water and ethanol (100 mL) at room temperature for 1.5 hour for three times. The extract was then filtered, concentrated to a final volume of 50 mL concentrated extract was dried using hot plate.

Ointment formulation

Ointment: ointment is preparation for external use, intended for application to the skin. Typically, they have an oily or greasy consistency and can appear stiff as they are applied to the skin.

Anti-inflammatory ointment- a drug or substance that reduces inflammation (redness, swelling, and pains) in the body. Anti-inflammatory agents block certain substance in the body and cause inflammation. They are used to treat many different conditions

Method of preparation-

When the ointment base contains a number of solid ingredients of different melting Points, such as white beeswax, stearic acid, hard paraffin and cetyl alcohol it is necessary to melt them in decreasing order to their melting point. This means, that the substance with highest melting point .The medicament is incorporated slowly to the melted mass, stirred thoroughly until the mass cools down and homogeneous product is formed. In case any liquid ingredients or aqueous substance is also to be incorporated, that should heat to almost to the same temperature as the melted bases. In case, this precaution is not observed, then upon mixing the two portions, the waxes or solid will cool down quickly and get separated this will prevent the uniform mixing of all the ingredients after mixing the two portions the stirring should be done uniformly and thoroughly to make a homogeneous mass. The rapid cooling should be avoided to get uniform product. The vigorous stirring should be avoided, when the ointment has just begun to thicken it is necessary otherwise the air will gate entrapped in the ointment.

Sometimes, due to rapid cooling of the melted mass, the waxy solids separates out from the ointment and uniform product is not obtained .In order to produce a uniform product, it can be re-melted over a water bath and again stirred until cold.

Excipients profile:

Stearic acid: Stearic acid, another name for octadecanoic acid $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$, is one of the most common fatty acids. It exists as a glycerol ester in most animal and plant fats (Beare-Rogers, Dieffenbacher, & Holm, 2001). Stearic acid is more abundant in animal fat (up to 30%) than vegetable fat (typically <5%). The important exceptions are cocoa butter and shea butter, in which the stearic acid content (as a triglyceride) is 28–45% unlike the other long-chain saturated fatty acids, stearic acid has no effect on lipoprotein cholesterol concentrations in men or women. With a polar head group that can bind with metal cations and a nonpolar chain that confers solubility in organic solvents, stearic acid is commonly used in the production of detergents, soaps, and cosmetics, such as shampoos and shaving cream products

White wax: White wax (white beeswax, ceraalba, bleached wax) occurs as a yellowish-white solid that is somewhat translucent in thin layers. It has a faint, characteristic odor and is free from rancidity. White wax is a chemically bleached form of yellow wax and is used in similar applications. It has an SG of about 0.95 to 0.96 and melts at about 61°C to 65°C. It is insoluble in water and sparingly soluble in cold alcohol. White wax is completely soluble in ether and in fixed and volatile oils. It is used as a stiffening agent, emulsion stabilizer, and controlled-release vehicle

Yellow Vaseline: Yellow Petroleum Jelly is semi solid mixture of hydrocarbons with specially selected waxes, forming ointment like gels, which are nearly odorless with excellent

hydrating characteristics. The resulting gels have excellent emollient properties and consistency for formulating with other active ingredients.

Triethanolamine: It is a common ingredient in formulations used for both industrial and consumer products. The triethanolamine neutralizes fatty acids, adjusts and buffers the pH, and solubilizes oils and other ingredients that are not completely soluble in water.

Propylene glycol: Propylene glycol is used as an emollient because it forms an oily layer on the skin, and prevents water loss. Viscosity control: Propylene glycol is used to reduce the thickness of cosmetic formulations and products. This helps them spread across the skin better, and it also improves how well the products are absorbed.

PRESERVATIVES

Propyl paraben

- Propyl paraben functions as a preservative in cosmetics and personal care products.
- Antimicrobial.
- Further, by limiting the growth of bacteria and fungi, preservatives like isobutylparaben help keep us safe from infection and other disease.

Methyl paraben: Prevents fungus growth: Thanks to its antibacterial properties, Rabach says that methyl paraben is a preservative that is added to many creams and cosmetic products to prevent fungus from growing.

FORMULATION

All ingredients are melted in porcelain dish such as stearic acid is added about 3gm. The white wax and yellow Vaseline are then added about 0.6 gm and 1.6 gm respectively. They all are mixed well. Then extract of aeglemarmelos added in melted bases Yellow Vaseline and white wax are added to increase the spread ability of the ointment. Triethanolamine adjusts the buffer and pH and solubilizes oil and other ingredients that are not soluble in water. About 0.2 ml of triethanolamine was added. Then the preservatives such as methyl paraben and propyl paraben are added. Both added about 0.1gm. To adjust 20gm weight of ointment sufficient quantity of water is added. All ingredients are then mixed well until evenly distributed ointment.

Table: 3 Formulation table:

Ingredients	Quantity
Active ingredients	1.25 gm
Stearic acid	3 gm
White wax	0.6 gm

Yellow Vaseline	1.6 gm
Triethanolamine	0.2 gm
Propylene glycol	1.6 gm
Water	q.s

RESULTS

Evaluation test:

- **Organoleptic Characteristics:** A blank formulation (i.e., formulation without active ingredient) and drug-loaded formulation were tested for physical appearance, color, texture and homogeneity. These characteristics were evaluated by visual observation. Homogeneity and texture were tested by pressing a small quantity of the formulated ointment between the thumb and index finger. The consistency of the formulations and the presence of coarse particles were used to evaluate the texture and homogeneity of the formulations. Immediate skin feel (including stiffness, grittiness, and greasiness) was also evaluated.
- **PH:** 6.8-7 (Neutral)
- **Colour:** light green
- **Odour:** Aromatic odour
- **Spread ability Test:** Spread ability of the formulation was determined by an apparatus suggested by Multi-meter with some modifications. It consists of a wooden block having a pulley at one end with fixed glass slide on block. An excess of ointment (3 g) placed on ground plate. The ointment was sandwiched between this plate and another glass plate having the dimension of fixed ground plate and provided with the hook. A 1 kg weight was placed on the top of the two plates for 5 min to expel air and to provide a uniform film of the ointment between the plates. Excess of ointment was scrapped off from the edges. The top plate was then subjected to pull of 240 g. With the help of spring attached to the hook and time required by the top plate to cover a distance of 10 cm was noted. A shorter interval indicates better spread ability. Spread ability was calculated using the following formula:

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

Where, S = Spread ability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide and

T = Time (in seconds) taken to separate the slide completely each other

- **Homogeneity:** Based on their appearance, all the developed ointments were tested for homogeneity by visual inspection.
- **Consistency:** The ointment prepared is smooth. There are no solid particles present in the ointment. Hence the ointment is consistent.
- **Non-irritancy:** Prepared herbal ointment was applied to the skin of human being and observed for the effect
- **Wash ability** Formulation was applied on the skin and ease extend of washing with water and checked.

In vitro evolution of anti- inflammatory activity:

Inhibition of albumin denaturation:

Denaturation of protein is the main cause of inflammation as part of investigation on mechanism of the anti-inflammatory activity ,ability of the extract to inhibit protein denaturation was studied selected extract were effective in inhibiting heat induced albumin denaturation .

CONCLUSION

1. The purpose of the study was to prepare anti-inflammatory herbal ointment using aeglemarmelos on the basis of anti-inflammatory efficacy leaves of aegle marmelos were taken and there etanolic extract were incorporated inthe most effective ratio in appropriate base the final product readily spread on the skin surface showed on irritant effect diffused well and stable at different temperature.
2. As aeglemarmelos showed significant anti-inflammatory activity in the models studies, it can be a promising anti-inflammatory agent.

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