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Formulation and Evaluation of Anti-Fungal activity of Aegle marmelos Ointment

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Abstract
This study evaluate efficacy of an herbal ointment formulated from extracts of Aegle marmelos leaves in case of fungal infections. Aegle marmelos commonly known as Beal, The beal leaves are obtained from the genus Aegle marmelos belonging to family Rutaceae. In this research study of formulation and evaluation Herbal ointment of beal leaves due to their anti Fungal property has been Traditionally used for its medicinal properties. The Aegle marmelos leaves were extracted using ethanol as solvent. The antifungal activity against common fungal infections such as candida albicans and Aspergillus fumigatus. The ointment base was ready Following formulation completion, the product was assessed using the levigation method for assessment characteristics such as colour, smell, pH, consistency, spreadability, solubility, and washability. The beal leaves have been use effectively in Anti-bacterial, Anti-acne, Anti-inflammatory activities As per given activity with aim we focus on creation and assessment of a herbal ointment with antifungal properties.

Keywords: Aegle marmelos, levigation**,** Anti-fungal, herbal ointment

Introduction

Skin is major part of the human body. Due to their antifungal property the beal leaves uses in preparation of ointment for skin. The formulation of beal leaves Antifungal ointment is very effective on foot ringworm and jock itch. The main advantage of beal leaves ointment is to treat fungal infection of skin. Aegle marmelos is a member of the Rutaceae family and is used medicinally. Hindus worship the Eagle Marmelos tree, also known as Shiva duma, and offer it as an offering to the gods Lord Shiva and Parvati.[1] The Eastern Ghats and central India are the origins of the beal tree. It is grown and planted in the shape of a temple tree. It is often referred to as "Beal" or wood apple. The fruit of the tree is used to treat intestinal parasites, dry eyes, and diarrhoea. A traditional cure for fever is to combine leaf juice with honey. [2]
Herbal remedies have long served as the foundation for traditional Indian medical practices like Ayurveda, Unani, and Siddha, which treat a wide range of physiological ailments and disorders. Therapeutic goals can include anything from curing wounds to treating skin lesions, leprosy, dysentery, scabies, venereal illnesses, ulcers, and snake bites. [2]
Beal is regarded as one among India's most significant medicinal plants. From different plant components, more than 100 phytochemical substances, including phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoid, steroids, and tannins, have been identified. It is commonly known that these substances have both biological and pharmacological action against a range of chronic illnesses, including cancer, heart disease, and gastrointestinal problems. Crude extract from this plant has been shown to have

antioxidant, antiulcer, antidiabetic, anticancer, anti-inflammatory, and antimicrobial properties in a variety of animal models.

Every portion of the Aegle marmelos plant, including the fruit, stem, bark, and leaves, has therapeutic value and is used to treat a variety of skin and eye problems. On the other hand, not much is known about Beal's applications in external medicine for products like ointments. [1]

Figure 1- Aegle marmelos

1. **Synonyms :** wood apple , Bengal quince
2. **Kingdom :** Plantae
3. **Order :** Sapinadales
4. **Family :** Rutaceae
5. **Subfamily :** Aurantioideae
6. **Genus :** Aegle
7. **Species :** Aegle marmelos
8. **Botanical name :** Aegle marmelos
9. **Chemical constituents :** linalool, cocumin, xanthotixol,
10. **Uses :** to treat infection, constipation [3]

Benefits of Beal :

1. Anti-inflammatory;
2. Anti-fungal;
3. Anti-acne
4. Anti-oxidant Activity;
5. Anti-ulcer Activity;
6. Anti-diabetic Activity;
7. Anti-malarial Activity;
8. Anti-cancer Activity;
9. Anti-bacterial Activity;
10. Anti-microbial Activity;
11. Anti-viral Activity; [4]

Table 1- Significant application of different Beal tree parts:

Part	**Application in medicine**
Leaf	Diarrhea, asthma, skin condition
Bark	Digestive health, respiratory issues, fever reduction
Flower	Gastrointestinal issue, treating skin disorders
Seed	Help in fighting bacterial and fungal infections

Material and Method

Collection of Plant material :

Aegle marmelos leaves that were identified and collected from the Nashik neighborhood .The beal leaves was cleaned, dried in room temperature, transfer into moderately coarse powder and stored in well closed container before the extraction.

Preparation of Beal Extract :

The ethanolic extract of Aegle marmelos leaves were prepared by using Soxhlet appratus. The ethanolic extraction has performed until the colour of extract does not change to colourless by passing through syphon tube . After cycle has been completed, alcohol extract was gathered and Condensed to produce residue that is greenish-black in colour. The beal leaf extract was kept in a cool, dark place in an airtight, tightly closed container.

Figure 2-Powder of Aegle marmelos leaves

Figure 3- Soxhlet Apparatus

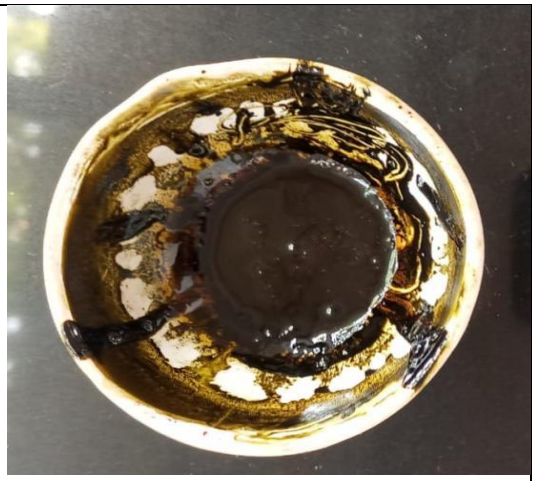

Figure 4- methanolic Aegle marmelos leaves extract

Method of Preparation :

The formulation of herbal ointment using beal extract. formulation of the herbal ointment. Making and Evaluation of herbal ointment utilizing herbal extract. Formulation and evaluation of herbal ointment by using natural ingredient.[6]

Formulation Table :

Sr.No.	Ingredients	F 1	F 2	F 3	Uses
1	Beal extract	1%	2%	3%	Anti-fungal
2	Wool fat	0.5gm	0.5gm	0.5gm	Emollient
3	Hard paraffin	0.5gm	0.5gm	0.5gm	Emollient
4	Cetostearyl alcohol	0.5gm	0.5gm	0.5gm	Emulsifying agent
5	Yellow soft paraffin	7.5gm	7.5gm	7.5gm	Ointment base

Table 2- Formulation Table

The process for making herbal ointment :

Weighing each item precisely allowed us to produce the ointment base. Ointment base was prepare in porcelain dish on water bath. Melt the cetostearyl alcohol and hard paraffin in a porcelain dish submerged in water. To melt above mixtures then add wool fat and soft parrafin and stir it well. After melting all ingredients, remove porcelain dish from water bath. Mix homogeneously until the semisolid base is obtained. Transfer In a suitable container.[7]

Evaluation :

The herbal ointment was evaluated using the following criteria: color, odour, pH, consistency, spreadability, solubility, and washability of the formulation.

Color: Through visual inspection, the ointment's color was determined.

Odour : An observation was made regarding the aroma of herbal ointment.

Consistency : Smooth and without any signs of greediness was the consistency.

pH : A digital pH meter was used to prepare the herbal ointment's pH. 50 milliliters of distilled water were used to dissolve one gram of herbal ointment. For the ointment, the pH was measured four times.

Spreadability:

The spreadability was measured by sandwiching an excess of sample between two slides that had been uniformly thickened by applying a given weight for a given amount of time. The spreadability was defined as the amount of time needed to separate the two slides. The formula used to calculate spreadability was as follows.

Where S is spreadability and $S= M \times L/T$

M= The upper slide's weight

L= Glass slide length

T= Duration of slide separation

Solubility:

Water and ether are both soluble in this substance.

Washability:

After applying the formulation to the skin, it was determined whether it was simple to wash with water.

Non-irritancy Test:

- A human being's skin was treated with the prepared herbal ointment, and the results were monitored. A little amount of the sample was applied to the hand, and the effects—such as redness, inflammation,

etc.—were monitored for a full day. then no such impact was noticed, indicating that it doesn't irritate the skin.

Stability: At 37°C, the produced ointment is the subject of a stability investigation. –[8]

Antifungal Activity :

Fungal species -

Candida albicans, the test organism, was further subcultured for a full day at 37°C. Throughout the investigation, the fungus cultures were kept on their suitable agar slant at 4°C and utilized as stock cultures.

Preparation of Niutrient Agar

3 gram of agar nutrient dissolved in 100 ml of water and it heated until it get dissolved and show slightly transparency

Sterilization –

After preparation nutrient agar it has been keep for sterilization in autoclave along with other equipment at pressure 121°C for 1 hour

Preparation of petri plates

Before preparing the petri plate the sterilization has been done of area by using Dettol . After sterilization . The equipment and agar media has been sterilized in autoclave has been removed. After few minute through nutrient agar the petri plate has been prepared media for the well diffusion study , while the other two were used for other purposes. The zone of inhibition (in mm) was used to assess the antifungal activity.

Bacteria Inoculation-

Through inoculating loop the subcultured Fungai has been inoculated in prepared petri plate . After that 4 cavities has made [9, 10]

Observation:-

Evaluation Table :

Evaluation parameters	**F 1**	**F 2**	**F 3**
Color	Light white	Light white	Light white
Odour	Characteristics	Characteristic	Characteristics
pH	5.8	6	6.4
Consistency	Smooth	Smooth	Smooth
Spreadability	9 sec	7 sec	8 sec
Solubility	Water and ether soluble	Water and ether soluble	Water and ether soluble
Washability	washable with ease	washable with ease	washable with ease
Non irritancy	Non irrritant	Non irrritant	Non irrritant
Stability	Stable	Stable	Stable

Table 3- Evaluation Table

Antifungal testing :

The formulation including ethanolic extract of Aegle marmelos leaves had noteworthy antifungal activity, according to the antifungal activity results. When the antifungal testing results for the standard sample and test sample were compared, it was discovered that the F3 formulation which (contains 3% extract of aegle

marmelos) had better antifungal action against candida albicans than the F2 formulation, which (contains 2% extract of aegle marmelos) and F1 formulation, which (contains 1% extract of aegle marmelos)

Antifungal activity:

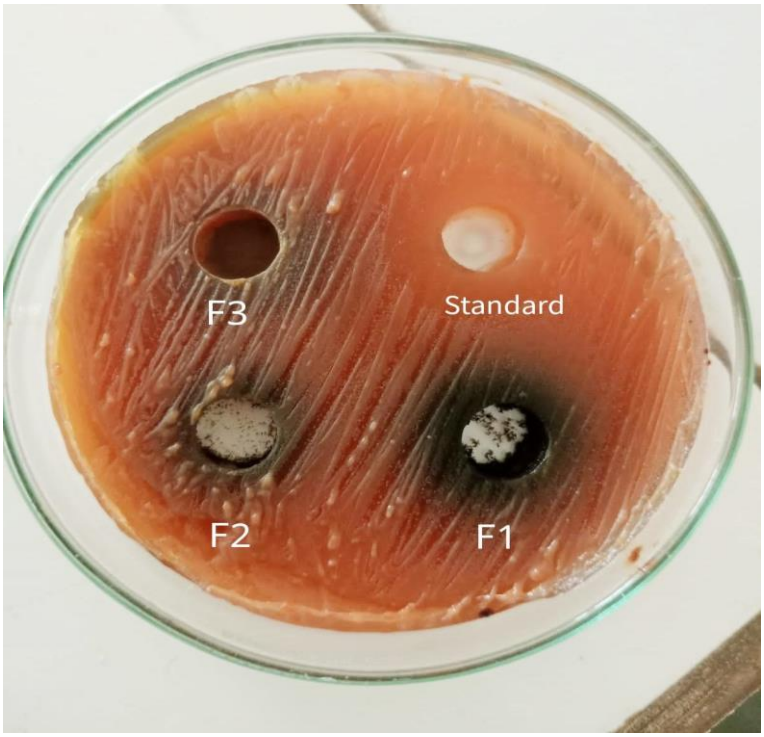

Fig. F1 formulation

Name of Fungi	Zone of Inhibition (mm)			
	Standard (Flucanazole)	F1	F2	F3
Candida albicans	19mm	15mm	17mm	14mm

Table 4- Anti Fungal Activity Evaluation table

Result and Discussion :

The herbal ointment of beal leaves was prepared and evaluated . The beal extract was prepared on extraction process and the herbal ointment was prepared in levigation method. I was prepared four formulation F1,F2,F3, from which F2 formulation shows better colour , odour , pH , and consistency as compared to other formulation.

Figure 5- Formulation of Aegle marmelos ointment

Conclusion :

As per above result it conclude that formulation F2 showing more antifungal activity .

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