ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

# PREPARATION AND EVALUATION OF POLYHERBAL ANTIFUNGAL CREAM

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**ABSTRACT**

During the latter part of the 20th century herbalism has become main stream worldwide. This is due in part to the recognition of the value of traditional and indigenous pharmacopeias, the incorporation of some derived from these sources into pharmaceuticals, the need to make health care affordable for all and the perception that natural remedies are somehow suffer and more efficacious then remedies that are pharmaceutically derived. Through the world, there has been an increasing incidence of fungal infections, and because of drug resistance and toxicity associated with long term treatment with antifungal drugs, search for new drugs to treat fungal infection is ongoing. The aim of the present study was to formulate poly herbal anti-dermatophytic antifungal cream containing extracts of *Sapindus trifoliatus & Manilkara zapota*. One general best cream formulation was selected from the standard books and prepared formulation was evaluated for its efficacy, uniformity, stability and appearance. The final product was a w/o emulsion cream with suitable appearance and desirable physiochemical stability. Due to the stability of the extract in the cream formulation, it can be used for treatment of fungal skin infections.

Keywords: *S.Trifoliatus, M.Zapota,* Polyherbal cream, Anti dermatophyte cream

## Introduction

*Sapindus trifoliatus* is belonging to the family Sapindaceae, are rich in saponins [1,2]. Is one of the oldest cultivated medicinal plants in the world. Infact Botanist traced it to the period of the Vedas about 5000 years ago. It is a medium sized deciduous tree growing wild in South India. Sapindus is the genus includes both deciduous and ever green species. Members of the genus are commonly known as soapberries or Soap nuts, because the fruit pulp is used to make soap. The generic name is derived from the two latin words saponins, meaning " Soap '', and Indicus meaning "of India". Soap nut powder is a very good anti bacterial and anti fungal agent. It is mostly used in the cosmetic and contraceptive creams. Powdered seeds are used for the treatment of Arthritis, Common cold, constipation, nausea and dental caries [3]. The poultice of soap nut is prepared and it is applied on the affected portions of joints for the relief from joint pains [4]. *Sapindus trifoliatus* have historically been used in folk remedies as a mucolytic agent, emetic, contraceptive and for the treatment of excessive salivation, Epilepsy and to treat chlorosis. The pericarp of the fruit of this plant reported for its various medicinal properties like tonic, stomachic, spermicidal and also used in the treatment of hemicranias, Migraine, Hysteria etc [5]. The thick watery solutions of the pericarp is used for the relief of Hemi- crania, Hysteria or Epilepsy [6]. Saponins obtained from the fruit of Soap nut have shown Spermicidal activity [7]

*Manilkara zapota* [ Sapotaceae] commonly known as the sapodilla and is a long – lived , evergreen tree native to southern Mexico, Central America and the Caribbean, grown in huge quantities in India Pakistan and Mexico. The fruit is a large ellipsoid beery, 4-8 cm in diameter, very much resembling a smooth - skinned potato and containing two to five seeds. The seeds are black and resemble beans, with a hook at one and that can catch in the throat swallowed. The fruit has a high latex content and does not ripen until picked. The fruit has an exceptionally sweet, malty flavor. Many believe the flavor bears striking resemblance to caramel or a pear candied with brown sugar. The unripe fruit is hard to touch and contains high amounts of saponin, which has astringent properties similar to tannin, drying out the mouth . The sapodilla trees yield fruit twice a year, through flowering may continue year round. Seeds have been to have [8], antibacterial [9] and anthelminthic activity [10]. Bark extract has been evaluated for antimicrobial and anticancer activities [11]. It is also traditionally used for the treatment of fever and pain. Leaves of the plant possess analgesic, anti – inflammatory [12], antioxidant, antihyperglycemic and hypocholesterolemic activities [13]. Roots are found to have hypoglycemic activity [14]

Materials and methods:

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Plant Resource

*Manilkara zapota* and *Sapindus trifolitus* seeds were collected from the nearby market of Vijayawada, fresh seeds were washed with distilled water thoroughly to remove trace of contaminants. These processed seeds were then shade dried for one month. After complete drying both the seed coats were broken and embryo was powdered mechanically and were subjected to Soxhlation using methanol as the solvent system for about 72 h. Both the obtained extracts were concentrated in vacuum under reduced pressure and allowed for complete evaporation of the solvent on water bath and finally vacuum dried and stored in desiccators until use. The yield obtained from the extract of *Sapindus trifolitus* is 12% and *Manilkara zapota* is 16% .

Experimental part

Phytochemical screening

Obtained extracts were screened for its phtyochemical constituents by performing qualitative phytochemical tests. Results were given in Table no : 1.1

Determination of MIC value

The Minimum Inhibitory Concentration (MIC) Assay is widely used to measure the susceptability of yeasts to antifungal agents. In serial two-fold dilutions, the lowest concentration of antifungal drug that is sufficient to inhibit fungal growth is the MIC. Typically, 50% inhibitory ($MIC_{50}$) or 80% inhibitory ($MIC_{80}$) values are reported. To facilitate visualization of antifungal susceptibility data, heat maps are generated whereby optical density values are represented quantitatively with colour.

A culture of the test fungi was grown on Potato Dextrose Agar (PDA) medium for certain period (generally 7 days) at optimum temperature (25±1 °C) for growth. The solvent used for dissolving extract was taken on the basis of polarity. PDA supplemented with different plant extracts at four concentrations (0.5, 1.0, 1.5, and 2.0 %) was poured in the Petri plates under aseptic conditions. After solidification, small dish (0.5 cm dia.) of the fungus culture was cut with a sterile cork borer and transferred aseptically upside down at the centre of Petridish. Suitable checks were maintained, where the culture discs were grown under same conditions on PDA without extract. Solvent checks were maintained to check out the inhibitory effect of solvent on fungi in which PDA was mixed with solvent (a solvent, which is used for dissolving extracts). Petri plates were incubated at 25 ±1 C. The radial two fungi were selected for bioassay viz. *Aspergillus niger* and *Candida albicans*. Growth of fungus colony was measured after 24h. Three replications were maintained for each treatment.

Formulation Design

Water in oil emulsion base was chosen for its emollient and detergency properties. The base was mainly composed of bees wax, mineral oil, paraffin wax, spermaceti wax, borax, preservative and perfume. The required amount of herbal extract was added to make a proper formula having the best antifungal activity. The composition and amounts of the ingredients were shown in [Table 1.2]

**Physicochemical evaluations:**

1 Homogeneity Test

Five hundred mg of each sample was spread on a clean slide and observed using an optical microscope [x10 and x40].

2. Centrifugation

A 10 g portion of each formulation was placed in a centrifuge tube [1 cm diameter] and centrifuged at 2000 rpm for 5, 15, 30, and 60 min. then the phase separation and solid sedimentation of the samples were evaluated.

3. Thermal Cycle Test

The portion were stored at $5^0$C for 48 h and then at 25 $^0$C for 48h. The procedure was repeated 6 times and then their stability and appearance were evaluated.

4 Freezing and Thawing

Twenty gram portion of each formula were stored periodically at 45 to **50**$^0$C and $4^0$ C for 48 h each. The procedure was repeated six times the samples were screened for their appearance and stability.

5. Determination of $p^H$

A suspension of each portion in 1% potassium nitrate solution was prepared and its pH was determined. A magnetic stirrer was used to produce homogeneity. The pH was determined at 48 h, one and three after preparation.

6. Viscosity Determination

Using a Brookfield viscometer [model DV-I with No. 6 spindle] the rheological behavior of the portion were studied. Each sample was placed in a container and spindle velocity was raised gradually to maximum extent. Then the viscosity was determined at 0.3, 0.6, and 3, 6 and 60 rpm. If needed, student t- test [Microsoft excel software] were performed to compare test results with the control $P< 0.05$ was assumed as significant difference.

7.Antimicrobial preservative effectiveness determination

To evaluate the effectiveness of the formulation preservation, the single microbial challenge test was employed. The was performed by adding 0.2ml of $10^8$ cfu/ml *Staphylococcus aureus* [PTCC No. 1189] and *Pseudomonas*

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

*aeruginosa* [PTCC No. 1074] to each of the 20ml pre-diluted sample of the formulation . Innoculated containers were kept at room temperature for 4 weeks. At appropriate time intervals [1,7, 14,21,and 28 days], aseptically , 1 ml portions from each sample was withdrawn and were subjected to the pour plate count procedure; and changes in microbial numbers at various intervals were recorded.

RESULTS AND DISCUSSION

Dermatophytes live in the dead, top layer of skin cells in moist areas of the body, such as between the toes, the groin, and under the breasts. The fungal infections of dermatophytes may cause only a minor irritation, could be more serious. They can penetrate into the cells and cause itching, swelling, blistering and scaling. The dermatophytes ,*Trichophyton, Epidermophyton* and *Microsporum canis* are commonly involved in such infections. However, their clinical differention is difficult [15].

Form the literature review it was found that *S.Trifoliatus* and *M.Zapota* both are seed are proved for its antifungal activity hence in our present research we aimed to prepare a best polyherbal forumulation from the seed extracts of both the plants.

From the results of zone of inhibition it was found that $F_1$ exhibits significant action when compare to the other two formulations. $F_1$ contains high concentration of *S.Trifoliatus* extract and less concentration of *M.Zapota* extract, exhibits significant action.

From the results of physicochemical tests all the formulations exhibited the similar physicochemical properties

**Conclusion**: From this research work F1 was found to possess significant antifungal action and is having antidermatopytic activity hence it could be used in the treatment of fungal skin infections.

**Acknowledgments**: Authors are thankful to the management of Nirmala college of pharmacy for constant encouragement and providing necessary facilities. We are also thankful to Dr.G.surendra, for providing necessary assistance during the preparation of manuscript.

Table1.1: Results of Qualitative phytochemical tests

| Phyto constituents | *S.Trifoliatus* | *M.Zapota* |
|---|---|---|
| alkaloids | - | + |
| amino acid | - | + |
| Tannins | - | + |
| Flavonoids | - | - |
| glycosides | + | - |
| steroids | + | - |
| carbohydrates | + | + |

+ = positive, - = Negative

Table No. 1.2 Amounts of ingredients per 100gm

| Ingredients | Blank | F1 | F2 | F3 |
|---|---|---|---|---|
| Beeswax | 0.5gm | 0.5gm | 0.5gm | 0.5gm |
| Mineral oil | 25gm | 25gm | 25gm | 25gm |
| Paraffin wax | 2.5gm | 2.5gm | 2.5gm | 2.5gm |
| Spermaceti | 1.5gm | 1.5gm | 1.5gm | 1.5gm |
| Water | 14.2gm | 14.2gm | 14.2gm | 14.2gm |
| Borax | 0.3gm | 0.3gm | 0.3gm | 0.3gm |
| Preservative | q.s | q.s | q.s | q.s |
| Perfume | q.s | q.s | q.s | q.s |
| *S.Trifoliatus* extract | ---- | 250mg | 500mg | 750mg |
| *M.Zapota* extract | ---- | 750mg | 500mg | 250mg |

Table No. 1.3 Results of Physicochemical tests property

| Property | Base | F 1 | F 2 | F 3 |
|---|---|---|---|---|

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| | | | | |
|---|---|---|---|---|
| Homogeneity test | Homogeneous | Homogeneous | Homogeneous | Homogenous |
| Appearance test | Very softy &fatty | Clear , good flow | Clear, good flow | Clear, good flow |
| Centrifugation test | Stable | Stable | Stable | Stable |
| Thermal cycle test | Stable | Stable | Stable | Stable |
| Freezing & thawing | Stable | Stable | Stable | Stable |
| pH [average] | Neutral | Neutral | Neutral | Neutral |
| Long term stability | Stable | Stable | Stable | Stable |
| Fluidity | * | * | * | * |

[*-Not determined]

Table No 1.4 Zone of Inhibition exhibited by the formulations

| S. no | Formulation | Zone of inhibition in cm |
|---|---|---|
| 1 | Standard | 4 ± 0.5 |
| 2 | Blank | 0.6±0.02 |
| 3 | F1 | 3.8±0.6 |
| 4 | F2 | 2.2±0.1 |
| 5 | F3 | 1.2±0.02 |

Results are average of triplicate

Acknowledgement:
Authors are declaring there is no conflicts of interest regarding any issue towards publishing this research article.
Authors are thankful to Nirmala college of pharmacy for funding.

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