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ANTI-DANDRUFF ACTIVITY OF ETHANOLIC EXTRACT OF SAPINDUS MUKOROSI SEED COAT AND FICUS RACEMOSA FRUIT PEEL AND IN SILICO PROTEIN INTERACTION STUDIES

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ABSTRACT

Medicinal plants are the principle source that is useful in treatment of various microbes like *Trichophyton rubrum* that causes dandruff. The ethanolic extract of *Sapindus mukorossi* seed coat and *Ficus racemosa* fruit peel has been tested for Anti-dandruff activity. Both the samples have shown Anti-dandruff activity. *Sapindus mukorossi* has shown zone of inhibition from 20 μ l whereas *Ficus racemosa* has shown inhibition from 30 μ l. This shows that ethanolic extract of *Sapindus mukorossi* seed coat is more effective compared to *Ficus racemosa* fruit peel in inhibiting *Trichophyton rubrum*. Protein interaction studies for a dermatophyte *Candida albicans* and two yeast related species *Pichia stipitis* (or *Scheffersomyces stipitis*) and *Saccharomyces cerevisiae* shows MEK₁ and MEK₂ (also present in *Trichophyton rubrum*) has shown network of interactions with other proteins like GDH₃, GLN₃, HEM₁₂, PYK₁, GLN₁, SAM₂, PGK₁, IFM₁, MSS₁₁, etc.

Key words: *Trichophyton rubrum*, *Sapindus mukorossi*, *Ficus racemosa*

INTRODUCTION

Medicinal plants are having therapeutic qualities which are useful in treating various ailments. The evaluation of medicinal uses of plants and enhance health conditions will also be useful in other purposes like food, care, cleaning, personal and perfumery. Medicinal plants have been identified from ancient times that have been recognized for medical use. Herbal medicine is an oldest form of physical, mental and spiritual medical treatment that is using to treat specific conditions and ailments. Medicinal uses of the plants have utmost importance all around the world provides good health conditions by maintaining human system.

Dandruff is a common scalp disorder affecting half of the pubertal population of any ethnicity in both the genders (most prevalent in male population) between the age group of 20 and 60 years¹. It is generally a major cosmetic problem that causes very great public health concern both in developed and developing countries^{2,3}. In India, from the ancient times people used to take *Sapindus mukorossi* for head bath. Now the usage of this culture is decreased. Hence the disorder is characterized by slight to moderate scaling of the scalp with irritation or erythema associated with the sensations of dryness. The characteristic scaling of the scalp suggest impairment in desquamation process. In most of the dandruff sufferers, the hair fall is a very common problem⁴

In the current scenario, many chemical substances are used for treating dandruff by controlling the abundance of fungi on the scalp. The main active agents used currently for controlling dandruff include imidazole derivatives such as ketoconazole and other compounds such as selenium sulphide, zinc pyrithione, piroctone olamine, ciproxirox olamine and others. A wide variety of antifungal agents are available for the treatment of dandruff but a complete cure is far from reach. Most of the available drugs are also either fungistatic in action or are expensive in nature.

Sapindus mukorossi or soap nut is the oldest cultivated medicinal plants using from vedic period for head bath. There is no much research about the anti-dandruff activity of seed coat and keeping the hair long and healthy. *Sapindus* is mainly used for external applications as herbal powders and sometimes used as internal purpose in minute amounts to remove

accumulated phlegm or mucous from the respiratory tract and promote vomiting (emetic). Care should be taken to avoid contact with the eyes while applying Sapindus for hair or as a body wash. Sapindus is causing miscarriage to pregnant women and hence it is not recommended for usage. It is a good external use herb for a variety of skin problems ranging from removing freckles to eczema and psoriasis. It is mucolytic or capable of attacking fungi. Hence Sapindus (dry fruit/seeds) is the prescribed for treatment of dandruff. Other benefits of Sapindus include its ability to kill sperm, fight common itch, treat chlorosis (a type of anaemia) and control the infection of *Helicobacter pylori*, a gut pathogen. Soapnuts have gentle insecticidal properties and are traditionally used for removing lice from the scalp.

Ficus racemosa is native to South-East Asia, Australia and Indian subcontinent. It is unusual in this plant that its figs grow on or close to the tree trunk. It is one of the herbs mentioned in all ancient scriptures of Ayurveda. The plant can grow over 40 feet tall and 20 to 40 feet wide Gular fig, Cluster fig or Country fig, which is considered sacred, has golden colored exudates and black bark⁵. It is frequently found around the water streams and is also cultivated⁶. The plant is used for various health problems and diseases in the traditional system of medicine⁷⁻¹⁰. It has long been used in Indian medicinal practice as astringent, carminative, stomachic, vermicide, etc. It is believed to be a good remedy for visceral obstructions and extract of the fruit is used in leprosy, diarrhoea, circulatory and respiratory disorders and menorrhagia^{11,12}. Tender fruits are used as astringent, refrigerant, stomachic, in dry cough, diseases of kidney and spleen, loss of voice, styptic, astringent to bowel, tonic, useful in the treatment of leucorrhoea, burning sensation, epistaxis, blood disorder, urinary discharges, fatigue, leprosy, intestinal worms and carminative. They are also useful in miscarriage, spermatorrhoea, epididymitis, cancer, myalgia, scabies, haemoptysis, intrinsic haemorrhage, excessive thirst¹³⁻¹⁶. The plant possesses potent inhibitory activity against six species of fungi, viz. *Trichophyton mentagrophytas*, *Trichophyton rubrum*, *Trichophyton soundanense*, *Candida albicans*, *Candida krusei* and *Torulopsis glabrata*^{17,18}.

The present study was undertaken to investigate and determine the antidandruff activity of ethanolic extract of *S.mukorossi* seed coat and *F. racemosa* fruit peel against *Trichophyton rubrum*. Protein interaction studies using in silico methods has also be conducted in the present experimentation.

Materials and Methods

Collection of Plant Material

Fresh plant parts were collected from Visakhapatnam region. The Plant material was thoroughly washed in water and dried plant material was grinded to powder and 150 gms of the dried plant material was used for the extraction.

Extraction Process

Freshly collected plant was dried under shade and powdered. The powdered material [150g] was extracted with ethanol (hot extraction using Soxhlet Apparatus) and the extract obtained was concentrated to a small volume under vacuum (50°C) and then dried in vacuum desiccators. The dried extract (76g) was fractionated and the fractions were concentrated and dried in desiccators.

Soxhlation Process

Soxhlation is an extraction process by which the plant material is going to be mixed with the particular solvent and the plant extract is obtained by maintaining particular temperature. Weigh the plant materials. Then take 150gms of weighing powder packed in a filter paper and place in a soxhlet apparatus. Take 200ml of solvent in round bottomed flask and place it to the downside of the soxhlet, and top side of the soxhlet immerse the condenser contains the outlet and inlet pipe line for continuous water supply to avoid evaporation of solvents. The total set up have to placed in heating panel and allow it to boil for six hours at desired temperature.

Microorganism

The fungal species used in the present study is *Trichophyton rubrum* (MTCC 296) is obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India

Determination of antibacterial activity by well diffusion assay

Antibacterial tests were carried out by well diffusion method¹⁹. The density of the fungal suspension was standardized by using Mac Farland standard method. The 6 mm wells on inoculated Sabouraud dextrose agar plates were filled with 40 µl of the ethanolic extract at the concentration of 4mg/well. Penicillin was used (4mg/well) as positive reference standard. The agar plates were incubated at 37°C for 48 hours. In the present study, the antidandruff activity of ethanolic extract of *S. mukorossi* (seedcoat) and *F. racemosa* (fruit coat) separately against dandruff tested and their inhibitory activity was quantitatively assessed by the presence or absence of inhibition zones and zone diameters.

Protein-Protein interaction

In the complex systems like plants, there will be many protein components that connect and interconnect with other protein components forming networks. These networks of protein interactions can be analyzed using String, a protein network server. In the present work, MEK1 and MEK2 are submitted as input for the server so that the interaction studies of networks can be known for the mechanism of action in dermatophyte *Candida albicans* and two yeast related species *Pichia stipitis* (or *Scheffersomyces stipitis*) and *Saccharomyces cerevisia* that also has relatedness with *Trichophyton rubrum*.

Results and Discussions

Antifungal activity of certain bioactive compounds from medicinal plants has shown attention within the scientific community due to growing problem of multidrug resistance among pathogenic fungi²⁰.

Anti-dandruff activity has been conducted for ethanolic extract of *S. mukorossi* (Seed Coat) and *F. racemosa* (Fruit Peel) against *T.rubrum*. Both the plant extracts has shown good activity against *T.rubrum* (**Table 1**)

Several proteases of dermatophytes have elastinolytic, keratinolytic and/or collagenolytic activities with gene family (MEP1, MEP2 and MEP3) coding for three endometalloproteases belong to fungalysins¹⁹. The modelled keratinase protein to *Trichophyton rubrum* has been shown in **Figure 1**. Protein interaction studies in *Candida albicans*, *Pichia stipitis* and *Saccharomyces cerevisiae* has been shown in **Figure 2-4**. The protein interaction studies in MEK1 and MEK2 has network of interactions with other proteins like NADP-specific glutamate dehydrogenase (GDH3) potential transcriptional activator with GATA-type Zn finger (GLN3), uroporphyrinogen decarboxylase (HEM12), pyruvate kinase (PYK1), glutamine synthetase (GLN1), S-adenosylmethionine synthetase (SAM2), 3-phosphoglycerate kinase (PGK1), components for the initiation of protein synthesis (IFM1), Transcriptional activator (MSS1), etc.

Protein interaction studies have shown good results with aging and disease¹⁹. Various components present in the plant species shows cure²¹ for the occurrence of aging and diseases and provides good health to living beings. Hence ethanolic extract of *Sapindus mukorossi* seed coat and *Ficus racemosa* fruit peel shows protein mechanisms in the control of dermatophytes like *Trichophyton rubrum*.

CONCLUSION

The ethanolic of *S. mukorossi* (Seed Coat) and *F. racemosa* (Fruit Peel) has shown anti-dandruff activity against *T.rubrum*. The Mechanism of action with MEP1 and MEP2 in dermatophyte *Candida albicans* and two yeast related species *Pichia stipitis* and *Saccharomyces cerevisia* shows different. Further studies has to be done for the activity of compounds and mechanism of action against *T.rubrum*

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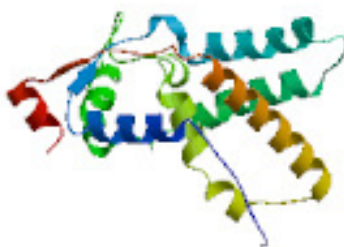
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Table 1: Antidandruff activity

Plant extract/ Antibiotic	Trichophyton rubrum	
	Conc (in μ l)	ZI(in mm)
Sapindus mukorossi	10	No zone
	20	10
	30	11
	50	16
Ficus racemosa	10	No zone
	20	No zone
	30	10
	50	12
Penicillin	50	No zone

**Figure 1: Modeled structure of Keratinase protein of Trichophyton rubrum**

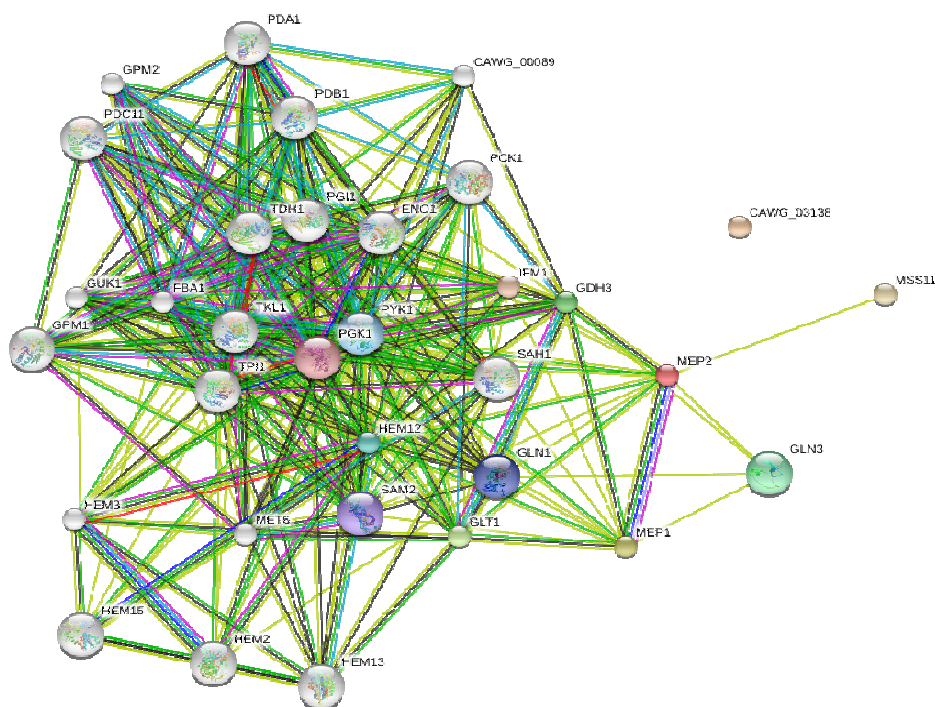


Figure 2: Protein interaction studies in *Candida albicans* (may show similar mechanism with *Trichophyton rubrum*)

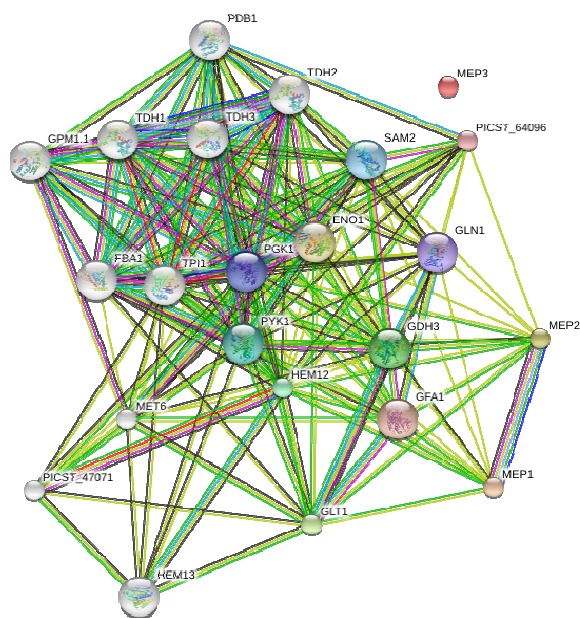


Figure 3: Protein interaction studies in *Pichia stipitis*

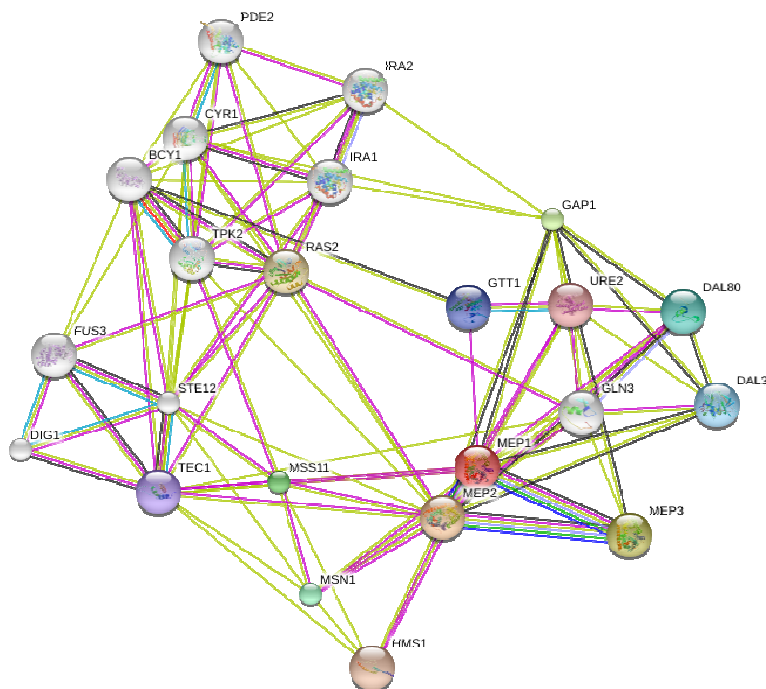


Figure 4: Protein interaction studies in *Saccharomyces cerevisiae*