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Control of *M. furfur* using Plant Essential Oil

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Plants have traditionally provided a source of hope for novel drug compounds. The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Seborrheic dermatitis is a common papulosquamous disorder of the skin. Dandruff, a less severe form of Seborrheic dermatitis, affects a greater proportion of the population. The exact pathogenesis of Seborrheic dermatitis is unknown, however colonization of the lipophilic yeast, *Malassezia furfur* and an inflammatory reaction to this yeast each seem to play a role in disease etiology. Essential oils have been shown to possess antimicrobial properties. The effect of plant essential oils on the growth of *M. furfur* was evaluated and reported. Among the 19 different plant essential oil tested, *Salvia sclarea*, *Cinnamomum verum*, *Cymbopogon flexuosus*, *Eucalyptus globulus*, *Thymus vulgaris*, *Melaleuca alternifolia*, *Syzygium aromaticum*, *Rosmarinus officinalis*, *Matricaria recutita*, *Cymbopogon martinii*, *Pogostemon cablin*, *Cedrus atlantica* and *Vitus vinifera* oil were found to be effective. Antidandruff shampoo was formulated using the lead extracts.

Key word: *M. furfur,* Essential oil, Minimum inhibitory concentration, Anti dandruff shampoo.

Skin reflects ones general health. It can get affected by environmental factors, stress, dehydration, sun exposure etc. In youth skin is smooth, with fine pores, and soft, but with age it becomes dry, wrinkled, and marked. Between these two normal conditions there are short periods of change and irritations of the normal skin caused by hormonal changes, illness, and lifestyles. Dandruff is one of the most common skin problems. *Malassezia furfur*, yeast like lipophilic basidomyceteous fungus is considered to be the chief cause of dandruff. This organism is the commensal flora of the scalp and skin region. It is believed that *M. furfur* converts the sebum lipid into fatty acids and triglycerides. These fatty acids may presumably accelerate hyperproliferation of keratinocytes. Management of dandruff must essentially contain the control of the causative agent *M. furfur*. Dandruff is characterized by scaling of the scalp and is frequently associated with seborrhea and seborrhea is the precursor of seborrheic dermatitis. *M. furfur* feed on the dermal lipid and proteins and facilitates lipase activity which releases proinflammatory free fatty acids [1]. The spread of multi drug resistant strains of fungus and the reduced number of drugs available makes it necessary to discover new classes of antifungal compounds[2]. Antidandruff shampoo was formulated using lead plant extracts. The study depicted that the antidandruff formulations are very good therapeutic compositions for treating dandruff. Anti dandruff shampoo showed the anti *M. furfur* activity.

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MATERIALS AND METHODS

Collection and maintenance of culture

Pure culture of *Malassezia furfur* MTCC 1374 was obtained from Institute of Microbial Technology, Chandigarh, India. The culture was maintained on Sabouraud's Dextrose medium containing Corn oil.

Determination of antimicrobial activity

Plant essential oil were obtained from commercial outlet of Dr. Urjita Jain Herbal Ltd, Mumbai. Kirby Bauer disc diffusion method was used to determine the effect of plant essential oils on the growth of *M. furfur*[3]. The broth culture of *M. furfur* (MTCC 1374) was swabbed on Sabouraud's agar containing 1% Tween 80. Sterile discs were dipped in plant extracts and placed onto the agar plates. Ketoconazole was used as a control. The zone of inhibition was observed after 48 hours[4].

Determination of MIC of effective essential oil

Agar cup method was used to determine the MIC. The effective plant essential oils were selected and dilutions were prepared in the range of 0.1% - 1% using sterile 1% Tween 80. The culture was swabbed on the sterile Sabouraud's Dextrose agar containing 1% Tween 80 plates and wells were bored using sterile borer. Various dilutions were added in the wells and plates were incubated at 30±2°C to record the MIC value after 48 hours.

Formulation of anti dandruff shampoo

Required quantities (Table 1) of dioctyl sodium sulfosuccinate and glycerine are heated together in a water bath at 95 °C until a clear solution is formed. Sodium lauryl sulphate is dissolved in water and left overnight in a closed vessels. Both the above solutions are mixed and plant extract is added and mixed slowly with gentle stirring to get uniform mixture[5,6]

Evaluation of anti dandruff shampoo's activity against *M. furfur*

Kirby Bauer disc diffusion method was used to determine the effect of formulation on the growth of *M. furfur*[3]. The broth culture of *M. furfur* was swabbed on sterile Sabouraud's agar containing 1% Tween 80. Sterile discs was dipped in shampoo and placed onto the agar plates. The zone of inhibition was observed after 48 hours[4,7].

RESULTS AND DISCUSSION

Among the 19 different plant essential oil tested, *Salvia sclarea*, *Cinnamomum verum*, *Cymbopogon flexuosus*, *Eucalyptus globulus*, *Thymus vulgaris*, *Melaleuca alternifolia*, *Syzygium aromaticum*, *Rosmarinus officinalis*, *Matricaria recutita*, *Cymbopogon martinii*, *Pogostemon cablin*, *Cedrus atlantica* and *Vitus vinifera* oil were found to be effective. *Azadirachta indica*, *Piper nigrum*, *Zingiber officinale*,

Table 1. The composition of anti dandruff shampoo

Ingredient	% Weight
Dioctyl sodium sulfosuccinate	14.00
Glycerine	53.00
Sodium lauryl sulphate	4.00
Plant extract	2.00

Table 2. Antimicrobial activity of plant oils against *M. furfur*

Essential oil	Average zone size	Essential oil	Average zone size
Piper nigrum	No Zone	*Simmondsia chinensis*	No Zone
Cedrus atlantica	47.00±0.0 mm	*Cymbopogon flexuosus*	80.00±0.0 mm
Matricaria recutita	17.66 ±0.5 mm	*Vitus vinifera*	15.33±0.5 mm
Cinnamomum verum	80.00±0.0 mm	*Azadirachta indica*	No Zone
Salvia sclarea	84.66±0.5 mm	*Cymbopogon martinii*	80.00±1.0 mm
Syzygium aromaticum	36.66±0.5 mm	*Pogostemon cablin*	30.00±0.0 mm
Curcuma longa	No Zone	*Rosmarinus officinalis*	20.33±0.5 mm
Eucalyptus globulus	55.33±0.5 mm	*Melaleuca alternifolia*	40.66±1.1 mm
Zingiber officinale	No Zone	*Thymus vulgaris*	44.66±1.1 mm

Ketoconazole 10 mcg/disc (Standard) showed the zone of inhibition of 25.33±0.5 mm.

Curcuma longa, and *Simmondsia chinensis* oil were found to be ineffective these results have been compiled in Table 2.

In terms of MIC value *Matricaria recutita* oil was most effective. The MIC value for *Matricaria recutita* oil was 0.2%, and that for *Salvia sclarea* oil was 0.4%, *Thymus vulgaris* and *Cymbopogon flexuosus* had an MIC value of 0.5%, *Melaleuca alternifolia*, *Eucalyptus globulus* and *Cinnamomum verum* showed 0.6%, *Syzygium aromaticum* oil MIC was found to be 1% . *Pogostemon cablin*, *Cymbopogon martinii*, *Vitus vinifera* oil and *Cedrus atlantica* oil did not show any zone of inhibition even at 2% concentration. This can be due to the less effectivity of these oils or less solubility of these oils in Tween 80.

Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances[8]. The permeability of bacterial membranes and the intracellular distribution of the oil constituents are key elements that influence the diffusion and the action of the essential oil, hydrophobic component into the cell[9].

The study depicted that the antidandruff formulations are very good therapeutic compositions for treating dandruff. Anti dandruff shampoo showed the anti *M. furfur* activity. The zone sizes observed after 48 hrs. incubation are tabulated in Table 3.

The antibacterial properties of essential oil have been attributed to the presence of phenolic components. Different modes of action are involved in the antimicrobial activity of essential oils. The activity may be in part be due to their hydrophobicity responsible for their partition into lipid bilayer of the cell membrane, leading to an alteration of permeability and a consequent leakage of cell contents. As typical lipophiles essential oils pass through the cell wall and cytoplasmic membrane disrupt the structure of the different layers of polysaccharides, fatty acids and phospholipids and permeabilize them. Cytotoxicity appears to be characterized by such membrane damage[10]. Essential oil inhibits respiration and increase permeability of bacterial cytoplasmic and yeast plasma membranes. They also cause potassium ion leakage. The ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely source of its lethal action at the minimum inhibitory levels[11, 12]. The main component of *Thymus vulgaris* essential oil is thymol and that of *Cinnamomum verum* is eugenol, which possesses notable anti-bacterial and anti-oxidant effects. These two constituents may also be responsible for the antibacterial and cytotoxic activities of *Thymus vulgaris* or *Cinnamomum verum* essential oil.

Table 3. Evaluation of anti dandruff shampoo's activity against *M. furfur*

Plant extract added in shampoo	Average zone size
Matricaria recutita	10.66±0.5 mm
Cinnamomum verum	55.00±0.0 mm
Salvia sclarea	11.00±0.0 mm
Syzygium aromaticum	15.00±0.0 mm
Eucalyptus globulus	18.00±0.0 mm
Cymbopogon flexuosus	11.66±0.5 mm
Rosmarinus officinalis	11.66±0.5 mm
Melaleuca alternifolia	14.33±0.5 mm
Thymus vulgaris	14.66±0.5 mm

Botanical therapies are often considered as therapeutic alternatives as agents for safer choice than conventional therapy. Botanical based cosmetics are said to possess the ability to detoxify, hydrate, strengthen, stimulate, relax and balance the skin and hair[13]. Shampooing is the most common form of hair treatment. Shampoos are primarily been products aimed at cleansing the hair and scalp. A more radical approach in popularizing herbal shampoo would be to change consumer expectations from a shampoo, with emphasis on safety and efficacy[14].

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