ANTI-FUNGAL ACTIVITY OF SELECTED PLANT EXTRACTS AGAINST *MALASSEZIA GLOBOSA*

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ABSTRACT
Dandruff is a common disorder affecting the scalp condition. Dandruff cannot be completely eliminated but can only be managed and effectively controlled. Currently available products in the market have poor efficacies or recurrence of symptoms. Plant extracts possess various active compounds that may help to overcome emergence of resistance in causative organism. Hence, our present study is an attempt to make a polyherbal mixture (rich in alkaloids, flavanoids, phenolic compounds, terpenoids, tannins etc.) incorporated in hair oil for better antidandruff efficacy. In the present study we found methanolic extract of pomegranate fruit rind, *Azadirachta indica* and *Datura metel* shows much better inhibition against *Malassezia globosa* at 1 mg/ml. Polyherbal hair oil showed MIC at 50 mg/ml.
KEYWORDS: Dandruff, *Malassezia globosa*, Antidandruff, Hair oil, Pomegranate fruit rind,

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INTRODUCTION

Dandruff is a common scalp disorder caused by *Malassezia globosa* affecting almost half of the population at the pre-pubertal age and of any gender and ethnicity but most prevalent in male population between the age group of 20 and 30 years. It is a major cosmetic problem that causes very great public health concern both in developed and developing countries. It is characterized by slight to moderate scaling of the scalp with varying degrees of irritation or erythema associated with the sensations of dryness. The characteristic flaking and scaling of the scalp suggest impairment in the desquamation process. In most of the dandruff sufferers, hair fall is a very common problem (Ravichandran *et al*., 2004).

Lipase catalyses the hydrolysis of the ester bonds of triacylglycerols, thereby releasing free fatty acids. Besides their important role in biotechnology, these reactions have been discussed as potential virulence factors in pathogenic bacteria and fungi. (Sascha Brunke *et al*., 2006).

In recent years there has been an increasing search for new antifungal compounds due to lack of efficacy, side effects and or resistance associated with some of the existing drugs. Much attention has been paid to plant-derived antifungal compounds based on the knowledge that plants have their own defense systems against fungal pathogens (Fontenelle, 2007).

Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids found to have antimicrobial properties. Many of the spices and herbs used today have been valued for their antimicrobial activity in addition to their flavor and fragrance properties (Ravikumar, *et al.*, 2010). Treating fungal infections is becoming more and more challenging. The use of plants for curing diseases was inevitable as is already proven by seeing the problems associated with synthetic antibiotics. Rind of some plants such as *Punica granatum* which are generally treated as wastes are true antibiotics as they are available for no cost, have no side effects and the most important benefit is that antibiotic resistant pathogens will be killed by these new and natural antimicrobials because they will take at least a few decades to get mutated and become resistant to them. (Jahir, *et al.*, 2011).

There is a continuous urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Contrary to the synthetic drugs, antimicrobial of plant origin are not associated with many side effects and have an enormous therapeutic potential to treat many infectious diseases (Mehrotra *et al.*, 2010). In the present study we have focused to bring out a new polyherbal mixture for antidandruff treatment.

MATERIALS AND METHODS

Test organism

Malassezia globosa of clinical origin was used for the study. The cultures were sub-cultured and maintained on Sabouraud dextrose agar (SDA) slants and stored in refrigerator at 4^{o}c.

Inoculum preparation

Inoculum of *M. globosa* was prepared by inoculating in 5 ml of Sabouraud dextrose broth and incubated at 30^{o}C for 2 days.

Preparation of the extracts

Fresh leaves of *Azadirachta indica* (Meliaceae) (AI), *Datura metel* (Solanaceae) (DM) and fruit rind of *Punica granatum* (Lythraceae) (PG) were collected and authenticated. All the samples were washed, dried in shade and powdered. The powdered sample of AI, DM and PG were separately extracted with ethanol, chloroform, ethyl acetate, methanol using soxhlet apparatus. Then the extract was filtered and concentrated on water-bath at low temperature. The final extract residue was re-dissolved in the respective solvent and made as required quantity.

Preparation of polyherbal mixture

Among the tested solvent extract methanol shows better activity. Hence we have selected 0.5% of methanolic extract of all the three plants and made a polyherbal formulation in coconut oil base.

Minimum Inhibitory Concentration (MIC) Test

MIC was determined by incorporating various concentrations of the extract (0.5-50

mg/ml) and polyherbal mixture (10-100 mg/ml) in 10 ml of SDA. The medium with extract was emulsified with Tween 80 thoroughly and was allowed to solidify at room temperature. Olive oil was overlaid and 100 µl of the inoculum (10^8 cfu/ml) was inoculated on each plate. The plates were incubated at 30°c for 5 days. Negative control with solvents was maintained (Sureshkumar *et al.*, 2010).

Antifungal activity by agar well diffusion method

SDA plates were overlaid with olive oil and inoculated with *M.globosa* by spreading on the surface of the media. A well was made in the center of the medium and the extract (10 µg/µl) was loaded in the well. Ketoconazole (10 µg) was maintained as positive control. The plates were incubated at 32°c for 3-4 days. The antifungal activity was assessed by measuring the diameter of the zone of inhibition (in mm) (Sivasakthi *et al.*, 2011).

Phytochemical analysis:

Phytochemical analysis was carried out to find the presence of various groups of compounds *viz.* alkaloids, glycosides, terpenoids, steroids, flavonoids, phenolic compounds and tannins (Siddiq and Ali, 1997).

RESULTS AND DISCUSSION

The results of present investigation are presented in graph-1 & 2 and table-1.

Dandruff is a common embarrassing scalp disorder affecting the people globally. The aim of dandruff treatment is to reduce the number of the *M.globosa* on the scalp, and the goals of therapy are to reduce morbidity and prevent complications (Ravichandran, *et al.*, 2004). Dandruff is caused by the commensal lipophilic yeast and hence cannot be completely eliminated but can only be managed. Recent years for the treatment of dandruff, a variety of the synthetic chemicals with anti-malassezial activity such as Ketoconazole, Zinc pyrithione, Octopirox, Climbazole (Faergemann, 1996) and natural ingredients like Hyamarka (veppalai), Tea tree oil, Neem, Rosemary oil and Chamomile oil are commercially used in shampoos, lotions, creams and hair oils. However, these shampoos have to be used for a long period and leave scalp skin dry (which leads to frequent scalp irritation), and are also known to discolor light colored hair. Thus, with the available therapies, the problem of complete symptomatic control and clinical cure is usually not achieved and dandruff usually recurs on stopping these treatments (Phadke *et al*. 2000).

Plants contain innumerable active compounds which exhibit many diverse effects on biological systems. The neem tree has been used for centuries in India systems medicine and is sometimes referred to as the "Village Pharmacy" **(**Wendy *et al.*, 2011). *Datura metel* (Thorn apple, Devil trumpet, Solanaceae) widely used to cure diseases such as asthma, cough, convulsion and insanity. The plant also finds application in the treatment of catarrh, diarrhea and skin diseases and in the treatment of burns (Sivasakthi *et al.*, 2011). Pomegranate is rich in polyphenols that includes flavonoids, tannins (both condensed and hydrolysable). Hydrolysable tannins are found rich in the fruit-rind and are showed predominant antioxidant activity (Saad *et al.*, 2010).

The literature review reveals that there is lack of anti-malassezial activity of above plants individually. Hence, the present study was attempted to screen the antidandruff activity of above plants individually and also in polyherbal mixture.

The results of present study showed that the both methanolic and chloroform extracts of *Azadirachta indica* exhibit MIC at 1 mg/ml in and that of *Datura metel* at 5 mg/ml. Methanolic extract of *Punica granatum* showed MIC at 1 mg/ml. The ethyl acetate extract of all the three plants exhibit low activity when compared to other solvents.

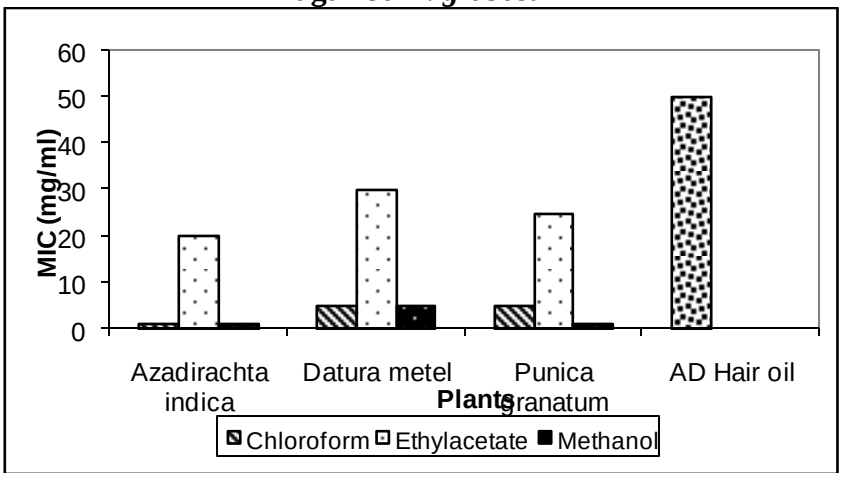
Ali *et al*., (2011) had earlier reported the MIC at 10 mg /ml of methanolic extract of pomegranate peel against *Candida albicans.* Another study had showed a maximum zone of inhibition (28 mm) for 9% ethanolic extract *Punica granatum* (Vasudha Pai *et al*., 2011). However, there is no earlier report for antidandruff activity of pomegranate fruit rind extract.

In the present study, polyherbal mixture in coconut oil base showed MIC at 50 mg/ml. Krishnamoorthy *et al.,*(2006) reported antidandruff activity of Dano – a polyherbal hair oil containing *Azadirachta indica, Cassia alata* and *Wrightia tinctoria* showing MIC at 50 mg/ml. It has been earlier reported that the localized immune elicitation activity of bitter fraction of neem offers better protection against relapse of dandruff for prolonged period of time (Ranganathan *et al.*,1996).

In another study, using a polyherbal hair oil with 6 herbs (*Albizia amara, Achyranthes aspera, Cassia fistula, Cassia auriculata, Datura stramonium* and *Azadirachta indica*) showed antifungal activity with MIC of 1 & 5 mg / ml against *P.ovale* & *C. albicans* respectively (Sureshkumar et al., 2010).

Sivasakthi *et al*., (2011) reported a zone of inhibition (11 mm) in methanolic extract of *Datura metel* against *Candida spp.* In ZOI studies, we observed the maximum inhibition in methanolic extracts of all three plants. A zone diameter of 6, 10 & 25 mm at 100 mg /ml for *Datura metel* , *Azadirachta indica*, & *Punica granatum* extracts respectively were recorded.

Graph 1: Minimum inhibitory concentration of Plants extracted in different solvents against *M. globosa*



Graph 2: Zone of inhibition of different solvent extracts of plants against *M. globosa*

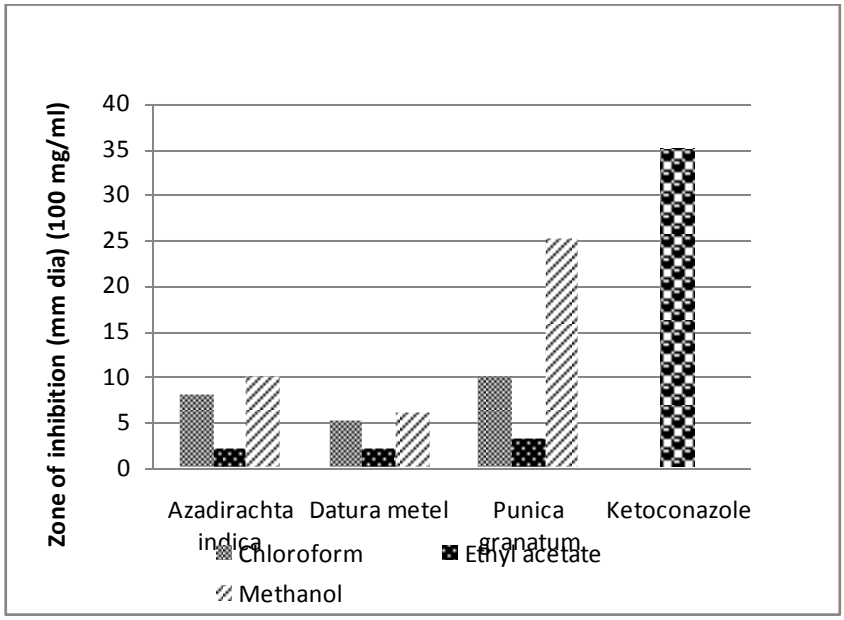


Table – 1. Phytochemical analysis of *Azadirachta indica, Datura metel* and *Punica granatum*

Chemical constituents	*Azadirachta indica*	*Datura metel*	*Punica granatum*
Alkaloids	-	+	-
Glycosides	-	-	-
Steroids	-	-	-
Flavonoids	+	+	+
Tannins	+	-	+
Saponins	-	-	-
Terpenoids	+	-	+
Phenolics	+	-	+

+ Present - Absent

CONCLUSION

Recently much attention has been paid to plant-derived antifungal compounds based on the knowledge that plants have their own defense systems against fungal pathogens. Our current study reports the use of herbal actives against dandruff. In conclusion, the above plants extract and polyherbal oil shows promising activity against *M.globosa*. Detailed studies on combination of formulations and *in vivo* studies are however required to confirm the functional anti-dandruff activity of the product.

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