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Research Article

# SCREENING OF PLANT ESSENTIAL OILS FOR ANTIFUNGAL ACTIVITY AGAINST *MALASSEZIA FURFUR*

**ARORA POOJA[1]*, DR. NANDA ARUN[2], DR. KARAN MANINDER[3]**

**Swami Devi Dyal Institute of Pharmacy, Golpura, Barwala, Distt. Panchkula, [2]Department of Pharmaceutical Sciences, Maharshi Dayanand University, Rohtak, [3]University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.**
**Email: poojaarora1975@yahoo.com**

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**ABSTRACT**

Dandruff is a common scalp disorder affecting almost half of the population at the prepubertal age and of any gender and ethnicity. The fungus *Malassezia furfur,* a lipophilic yeast is widely accepted as the causative agent of dandruff, which due to its lipase activity releases proinflammatory free fatty acids causing dermal inflammation and tissue damage. Currently available treatment options of chemical origin have various limitations, either due to poor clinical efficiency or compliance issues. Also, these drugs are unable to prevent recurrence, which is the most common problem. Due to this, attention is shifting towards herbal remedies with aromatic plants and their essential oils being widely popular for their empirically antifungal properties. The present study was aimed to evaluate the potential inhibitory effects of essential oils of indigenous plants on *M. furfur*. The antifungal activity of four aromatic oils of kapur tulsi, cinnamon, eucalyptus, cajeput along with one fixed oil of karanj was screened alone or in combinations using tea tree oil and ketoconazole as standards. Out of five selected oils, three oils showed activity in the order cinnamon oil >kapur tulsi oil >cajeput while karanj oil and eucalyptus oil were inactive against the fungus. The minimum inhibitory concentration (MICs) of the active oils was evaluated using broth dilution method. The cinnamon oil, kapur tulsi oil and cajeput oil on evaluating in different combinations showed synergistic effect with a mixture of cinnamon oil and kapur tulsi oil exhibiting the best activity. The study reports effectiveness of kapur tulsi oil against *M. furfur* for the first time and further the synergistic combinations of oils is also being reported for the first time. The findings provide promising information on the potential use of essential oils for the treatment of dandruff.

**Keywords:** Dandruff, *Malassezia furfur*, Essential and fixed oils, Antifungal activity

## INTRODUCTION

Dandruff, a clinical condition caused by *Malassezia s*pecies is of great cosmetic concern all over the world [1]. It is characterized by white to whitish yellow, dry, loose scaling and mild pruritis of the scalp. Dandruff may gradually progress through redness, irritation and increasing scaling of the scalp to seborrhoeic dermatitis and both conditions are considered to be the same disorder of different severity[2].

*Malassezia* species are yeasts that comprise part of the micro flora of human skin. Especially, they are abundant in regions supplied with sebaceous glands because of their lipid requirement for growth. Several skin problems like pityriasis versicolor, folliculitis, atopic dermatitis including dandruff are caused by *Malassezia* species under suitable environmental conditions. Taxonomic revision has divided the genus *Malassezia i*nto seven different species- *M. globosa*, *M. resticta*, *M. obtuse, M. sloofia, M. sympodialis, M. furfur* and *M. pachydermatits[3].* Among these, *M. furfur* is one of the main causative agents of dandruff. Currently available treatment options for the management of dandruff include therapeutic use of zinc pyrithione, salicyclic acid, imidazole derivatives, glycolic acid, steroids, sulphur and tar derivatives. Of all the synthetic agents, ketoconazole (an imidazole derivative) has become the leading contender among various treatment options of dandruff[4,5]. It is a broad-spectrum antimycotic agent active against *Malassezia*and it is also effective in many dermatomycoses, including pityriasis versicolor[6]. However, these agents have certain limitations, either due to poor clinical efficacy or due to the compliance issues. Furthermore, these drugs are unable to prevent recurrence, which is the most common problem[7]. Such complications have prompted the search for novel and effective antifungal agents of natural origin.

The potential of essential oils as antimicrobial agents is well established. Essential oils are mixtures of volatile secondary metabolites isolated from different parts of aromatic plants[8]. Tea tree oil is widely used natural antidandruff agents in many marketed formulations like anti dandruff shampoos, ointments, creams and lotions. Nenoff *et.al,* 1996 demonstrated that the essential oil of tea tree inhibits the growth of *Mfurfur*[9]. An antidandruff shampoo containing 5% tea tree oil is reported to be effective in the treatment of mild to moderate dandruff[10].

The aim of this study was to evaluate the essential oil of cinnamon, cajeput, kapur tulsi, eucalyptus and a fixed oil obtained from karanj seeds against a pathogenic fungi *Malassezia furfur* and develop a new formulation based on validated antimicrobial activity of selected oils alone or in combination.

Cinnamon oil is obtained from the bark of the plant *Cinnamomum zeylanicum* Blume (Lauraceae). The bark yields 0.35% oil containing cinnamaldehyde and eugenol as major constituents. Presence of cinnamylacetate, linalool, 1,8-cineol, p-cymene, cuminaldehyde is also reported. The oil is carminative, stimulant, aromatic, powerful germicide and an active fungicide[11].

Cajeput oil is obtained from the leaves of *Melaleuca leucadendron* Linn (Myrtaceae). The oil is rich in 1,8-cineole (40-65%). The other constituents of the oil include p-cymene, linalool and terpinene-4-ol. The oil is used to cure migraine, cold, influenza, stomachache, psoriasis, eczema, acne and rheumatism. The antifungal activity of the oil extends its utility as sterlizer or preservative[12].

Eucalyptus oil is obtained from the leaves of *Eucalyptus globulus* Labill (Myrtaceae). The oil contains over 80% cineol, with other constituents as p-cymene, alpha-pinene, limonene, geraniol and camphene. The oil is widely used in curing headache, body pains, fever, chronic bowel complaints and dysentery. It is greatly valued for its antiseptic and disinfectant property and is used especially in the treatment of infection of upper respiratory tract and in certain skin diseases [13,14].

Kapur tulsi oil is obtained from the leaves of *Ocimum kilimandsharicum* (Lamiaceae). The major components present in the oil are camphor (56.07%), limonene, camphene, 4- terpineol, beta-ocimene, linalool, alpha-terpineol and L-phellandrene. The oil is used as indigenous medicine for variety of ailments like cough, bronchitis, viral infection, anorexia and healing of wounds. It also possesses insect repellent and antimicrobial property [15,16].

Karanj oil is obtained from the seeds of the plant *Pongamia glabra* Vent (Papilionaceae). The main constituent of the seed oil is karanjin. Other constituents reported in the oil are pongapin, pongaglabrone, kanjone, ovalitenone, glabrin etc. The oil from seeds is used in leucoderma, cutaneous infections including herpes and scabies. Internally, the oil is used as stomachic and in dyspepsia. The

oil possesses antibacterial activity and it is an ingredient of hair oil prescribed by Sushruta for baldness[17].

In the present study the antifungal activity of above mentioned oils was evaluated and compared with both tea tree oil and ketoconazole against the fungus *M. furfur* in varying combinations to find out such composition which gives enhanced activity preferably synergistically over individual oils.

## MATERIALS AND METHODS

### Collection of plant material

The aromatic plants were selected on the basis of their ethno-medicinal importance and literature survey. The authenticated plant material for extraction of kapur tulsi oil, cajeput oil and eucalyptus oil was collected from Medicinal Plant Garden of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh. Their specimens were also submitted to the Museum-cum-Herbarium of University Institute of Pharmaceutical Sciences, Punjab University. The cinnamon bark and karanj seeds were procured from local market of Ambala Cantt. The drug samples were authenticated by Dr. H.B Singh, Chief scientist and Head, RHMD, NISCAIR, New Delhi vide ref. no. NISCAIR/RHMD/Consult/2011-12/1807/107. The essential oils under study were isolated by hydro distillation process using Clevenger's apparatus. The fixed oil from karanj seeds was isolated by expression method. The gift sample of tea tree oil and ketoconazole were provided by Care Cosmetics, Kala Amb, Himachal Pradesh. Details of the oils are mentioned in Table 1.

**Table 1: Oils and their source**

| S. No. | Name of the oil | Source | | Plant part used |
|---|---|---|---|---|
| 1. | Cinnaman oil | *Cinnamomum zeylanicum* Blume | Lauraceae | Bark |
| 2. | Kapur Tulsi oil | *Ocimum kilimandscharicum* Baker | Lamiaceae | Leaves |
| 3. | Eucalyptus oil | *Eucalyptus globulus* Labill | Myrtaceae | Leaves |
| 4. | Cajeput oil | *Melaleuca leucadendron* Linn | Myrtaceae | Leaves |
| 5. | Tea tree oil | *Melaleuca alternifolia* | Myrtaceae | Leaves |
| 6 | Karanj oil | *Pongamia glabra* Vent | Papilionaceae | Seeds |

### Chemicals and reagents

### *Malassezia furfur* culture

Pure culture of *Malassezia furfur* (MTCC: 1374) was obtained from Institute of Microbial Technology (CSIR), Chandigarh. The culture was maintained in Emmons modified medium.

### Evaluation of antifungal activity

Antifungal effect of all the selected oils against *M.furfur* was evaluated by Disc diffusion method[18]. Standard size Whatman No.1 filter paper discs, 6.0 mm in diameter, sterilized in dry heat at 140ºC in an oven for 1 hour were used to determine antifungal activity. Sabourauds dextrose agar medium was prepared with 1% corn oil. After sterilization, it was poured into sterilized petri plates and allowed to solidify. Using a sterile cotton swab, culture was swabbed on the surface of medium plates. Sterilized filter paper discs were soaked in neat, undiluted (100 %) concentration of different oils. The discs saturated with each oil were aseptically placed over plates containing medium with the help of sterilized forceps. Along with the five test oils, discs saturated with tea tree oil and ketoconazole, both taken as standards were also aseptically placed over plates containing medium for comparison of antifungal activity of test oils. Three replicates were maintained for each sample. The plates were incubated at 32°C and the zone of inhibition was observed after 4 days. Control was maintained with filter paper discs dipped in distilled water.

### Determination of minimum inhibitory concentration (MIC)

The MIC of the oils showing antifungal activity was determined by the micro dilution broth method (National Committee of Clinical Laboratory Standard, 1993)[19]. A stock solution of 25.6 mg/mL of the active oils was prepared in Mueller Hinton broth (Difco). Further, serial double dilutions were made in a range between 25.6 mg/mL to 0.05 mg/mL. A volume of 100 µL aliquot of each dilution and Mueller Hinton broth without oil (positive and negative controls) were put in the wells of a microtiter plate. The inoculated microtiter plates were then incubated at 37°C for 72 h. After examining the turbidity visually, 40 µL of 0.02 mg/mL of 2,3,5 triphenyl tetrazolium chloride (TTC) was added to each microplate well and incubated again at 37 °C and re-examined after 30 mins. The MIC was calculated as the lowest concentration of the oil that prevented growth of the culture. All samples were examined in duplicate in three separate experiments. The MIC of the active oils was compared with the MIC of tea tree oil and ketoconazole and the results are shown in Table 2. Further, combinations of oils were also evaluated to check their antagonist or synergistic effects. The combinations which were evaluated are (a) cinnamon oil + kapur tulsi oil (b) cinnamon oil + cajeput oil (c) cinnamon oil + kapur tulsi oil + cajeput oil. The results are shown in Table 3. The combinations were also subjected to the determination of their MIC.

### Statistical analysis

The difference in the zone of inhibition of the oils against the fungus was determined by Student-T test. A 'p' value of less than 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

The antifungal activity of certain bioactive compounds from medicinal plants has attracted a lot of attention within the scientific community largely as a result of the growing problem of multidrug resistance among pathogenic fungi. In addition, medicinal plant oils are the promising sources of antifungal drugs. Based on these facts, the present study emphasizes the importance of plants essential oils as an alternative antimalassezia agent against pathogenic fungi causing dandruff.

In the present investigation the antifungal activity of four essential oils and one fixed oil were examined and compared with the standard antifungal agents tea tree oil and ketoconazole against the fungus *Malassezia furfur.* The recorded results are presented in Table 2. Among the five selected oils only three essential oils exhibited antifungal activity against the fungus with MIC values ranging from 32 to 128 µg/ml. The bark oil of cinnamon showed the highest activity against the fungus and other oils showing good inhibition were the leaf oil of cajeput followed by leaf oil of kapur tulsi. Although cinnamon oil was found to possess best antifungal activity among the test oils but it was lesser than the activity shown by the standard tea tree oil and ketoconazole.

Some studies have concluded that combinations of essential oils have greater antimicrobial activity than their individual components and studies also suggested that the components of essential oils are responsible for providing synergestic or potentiating effects probably[20-22]. Hence cinnamon oil in combination with cajeput oil and kapur tulsi oil in ratio 1:1 was evaluated against *M. furfur*. In an investigation of different combinations, it was noted that both cajeput oil as well as kapur tulsi oil were showing good synergism against the *M. furfur* when combined with the cinnamon oil. But most of the antimicrobial activity was appeared to be associated with the combination of cinnamon oil and kapur tulsi oil as shown in Table 3. These two oils in combination were more effective against the fungus *M. furfur* than when they were used separately and the combination was showing better activity than the activity shown by

standard tea tree oil and ketoconazole alone.The MIC of the combinations got significantly reduce to 4µg/ml from 32-128 µg/ml of individual oils.

The antimicrobial activity of cinnamon oil and kapur tulsi oil could be associated with cinnamaldehyde and camphor as these are the main components of respective oils. These components have been shown to have antimicrobial activities against common laboratory target strains in other studies[23-27]. Their mechanism of action has most often been attributed to the disturbance of microbial membranes, disrupting the proton motive force, electron flow, and active transport and resulting in the coagulation of intracellular contents[28-29].

**Table 2: Antifungal activity of oils against *Malassezia furfur* by the disc diffusion assay**.

| S. No. | Essential oil | Zone of Inhibition (mm) | MIC (µg) |
|---|---|---|---|
| 1. | Cinnamon oil | 14 ±0.51 *** | 32 |
| 2. | Cajeput oil | 12 ± 0*** | 64 |
| 3. | Kapur tulsi oil | 8± 0.057 *** | 128 |
| 4. | Eucalyptus oil | 00 | *ND |
| 5. | Karanj oil (Fixed | 00 | *ND |
| 6. | oil) | 17.5 ±0.404*** | 32 |
| 7. | Tea tree oil | 22 ±0.057 | 16 |
| | Ketoconazole | | |

*ND = not detected, ***p value- 0.0001,

**Table 3: Antifungal activity of combination of selected oils against *Malassezia furfur* by Disc diffusion assay**

| S. No. | Combinations of oils | Zone of Inhibition (mm) | MIC (µg) |
|---|---|---|---|
| 1. | Cinnamon oil +Kapur tulsi | 23.3 ±0.20 *** | 4 |
| 2. | Cinnamon oil + Cajeput oil | 21± 0*** | 16 |
| 3. | Cinnamon oil + Kapur tulsi + Cajeput oil | 20± 0.08 *** | 16 |

***p value- 0.0001

## CONCLUSION

Dandruff induced by *M.furfur* represents a fastidious cutaneous problem because of aesthetic problems. The prolonged chemical based treatments and the high rate of recurrence suggest the opportunity of alternative treatment. So, in this study selected essential oils were screened for antifungal activity against *M.furfur*. In conclusion, the present investigation demonstrated that binary mixture of cinnamon oil and kapur tulsi oil showed excellent antifungal activity against the fungus. The combined effects of essential oils can provide an effective alternative to synthetic prophylactic or therapeutic treatments. This is in agreement with findings of previous research on antimicrobial effects of combinations of different essential oils on fungal species. Accordingly the combination of cinnamon oil and kapur tulsi oil is a promising source of antifungal agents that may be useful for formulation of cosmetics and in the development of new treatments for dandruff. Further the authors have formulated an antidandruff shampoo by incorporating cinnamon oil and kapur tulsi oil as main antidandruff ingredients. The evaluation study of the formulation is under progress**.**

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