



RESEARCH ARTICLE

MICROBIOLOGY

EVALUATION OF SOME MEDICINAL PLANTS FOR THEIR DANDRUFF CONTROL PROPERTIES**K. P. BALAKRISHNAN^{1*}, NITHYA NARAYANASWAMY¹, SOOSAMMA MATHEWS², KARUNA GURUNG²**¹ ITC Research and Development Centre, Peenya Industrial Area Phase 1, Bangalore 560 058, India.² Department of Botany, Mount Carmel College (Autonomous), Palace Road, Bangalore 560 052, India.**K. P. BALAKRISHNAN**ITC Research and Development Centre, Peenya Industrial Area Phase 1, Bangalore
560 058, India.**ABSTRACT**

Dandruff caused by *Malassezia* spp is controlled by antifungal agents. In the present study four medicinal plants- *Terminalia bellerica*, *Terminalia chebula*, *Emblica officinalis* and *Lantana camara*- were selected to check their antifungal activity. Their efficacy was tested using different concentrations of extracts in various solvents. The antifungal activity of the plant extracts was determined by well diffusion method. Among the four plants screened, *Terminalia bellerica* and *Terminalia chebula* exhibited good antifungal action against the test organism. *Emblica officinalis* and *Lantana camara* did not show significant antidandruff activity. Aqueous and ethanolic extract of a combination of *Terminalia bellerica* with *Lantana camara*, and *Terminalia chebula* with *Lantana camara* were tested for their synergistic antidandruff activity. Results of the synergistic studies indicated that synergy exists between ethanolic extracts of *Terminalia chebula* and *Lantana camara*. From the study, it was found that *Terminalia bellerica* and *Terminalia chebula* act as the good sources of antidandruff agents.



KEY WORDS

Dandruff, antifungal, *Malassezia* spp, *Terminalia bellerica*, *Terminalia chebula*, well diffusion

INTRODUCTION

Dandruff is a common scalp disorder affecting almost half of the pubertal population of any ethnicity in both genders but most prevalent in male population between the age group of 20 and 60 years (Ravichandran *et al.*, 2004). It is a major cosmetic problem that causes very great public health concern both in developed and developing countries (Faergemann, 1997; Sunenshine *et al.*, 1998). 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 (Paul, 1999).

Malassezia (formerly called *Pityrosporum*), a yeast like lipophilic basidiomycetous fungus, is considered to be the chief cause of dandruff problem which is present as scalp commensal (Ranjith *et al.*, 2002). Lipid dependant *Malassezia* yeasts are commonly found on human skin, in particular, on the upper body, where the sebum secretion is highest (Leeming and Notman, 1987; Marcon and Powell, 1992). Although dandruff is associated with scalp, flakes may also appear on face, nose and eyebrows, as well as on the skin behind the ears and neck. Due to the impact of the male hormone testosterone, the sebaceous glands are stimulated to secrete more sebum, which enhances the microbial growth and the associated formation of dandruff on the scalp.

More than seven species of *Malassezia* (i.e. *M. globosa*, *M. restricta*, *M. obtusa*, *M. sloofiae*, *M. sympodialis*, *M. furfur* and *M. pachydermatis*) exists and their growth is enhanced by the hypersecretion of sebum and hyperproliferation of the stratum corneum. To date, the species *M. globosa*, *M.*

restricta and *M. furfur* have been most closely associated with dandruff in humans (Gupta *et al.*, 2004).

Malassezia furfur is an important causal factor for dandruff. The lipase activity of *Malassezia* hydrolyses the sebum triglycerides which results in excess release of oleic acid (chemotactic substances) that attracts the neutrophils towards it. As a result, neutrophils release the reactive oxygen species and cytokines that aggravate the scalp by causing dermal inflammation and tissue damage (DeAngelis *et al.*, 2007; Troller, 1971). Moreover, the released oleic acid accelerates the hyperproliferation of keratinocytes resulting in the deregulation of keratinisation. As a result, the corneocytes present in the epidermis clump together to form large flakes on the skin (Nazzaro-Porro and Passi, 1976). Therefore, treatment is the need of the hour for people suffering from dandruff.

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, ciproprox olamine and others. They act by removing the scales, reduce *Malassezia* species adherence to corneocytes and inhibit its growth.

A wide variety of antifungal agents are available for the treatment of dandruff but a complete cure is far from reach. Further, most of the available drugs are either fungistatic in action or are expensive in nature. Besides, fungal resistance to synthetic antibiotics in clinical use is rising, and it often develops rapidly (Rocha *et al.*, 2004). With this in mind, the potential of natural antifungal agents was



investigated. The antifungal activity of pepper extract, basil extract, neem extract, basil oil, clove oil and tea tree oil have been well documented by Lee *et al.*, 2010. Our study focuses on the antidandruff activity of certain herbs and its synergistic effect against *Malassezia furfur*.

MATERIALS AND METHODS

Collection of Plant samples:

The four medicinal plants used in this study were collected from the Ethnobotanical garden of Mount Carmel College, Bangalore. They were authenticated by the Botanist of Mount Carmel College, Bangalore, India.

Sr.No	Botanical name	Family	Part used
1	<i>Terminalia bellerica</i>	Combretaceae	Fruit coat
2	<i>Terminalia chebula</i>	Combretaceae	Fruit coat
3	<i>Emblica officinalis</i>	Euphorbiaceae	Fruit coat
4	<i>Lantana camara</i>	Verbenaceae	Leaves

Preparation of plant extracts:

The plant samples (leaves, and fruits) were dried in the shade and crushed thoroughly in a blender to get the coarse powder. 1.5g/ 2g / 2.5 g of herbal powder was taken and dissolved in 10ml of water/ethanol/petroleum ether. The mixture was heated in a boiling water bath at 80°C for 15 minutes. The mixture was cooled at room temperature and centrifuged at 4000 rpm for 10 minutes. The supernatant was filtered and the filtrate was collected and used for the analysis.

Microorganism used

The test organism used in this study was *Malassezia furfur* (MTCC 1374). The culture was obtained from Microbial Type Culture Collection, Chandigarh, India.

Media Preparation

The media and other microbiology accessories were obtained from Himedia. Leeming Notman agar and broth were used for the culture of *Malassezia furfur* (Mirinda *et al.*, 2007).

Preparation of Standard (ZPTO)

Zinc pyrithione (ZPTO) was dissolved in Dimethyl sulphoxide. The working standard concentration of ZPTO was 50 µg/ml.

Preparation of McFarland Standard

McFarland Standard was prepared as described in NCCLS, 1997.

Antifungal susceptibility testing-well diffusion method

Malassezia furfur strain (MTCC 1374) was grown on Leeming Notman broth supplemented with 2 % of pure olive oil for 2-7 days at 30°C in an incubator. *Malassezia furfur* was maintained on the same medium described previously, at 4°C, with subcultures being carried out on a monthly basis. Inoculum suspensions were prepared by the method as described by Rukayadi *et al.*, 2006. Two to three loopful colonies of organism was transferred into 5 ml of Leeming Notman broth. The inoculated tubes were incubated at 30°C for 48 hrs in an incubator. After incubation, the cultured organism was centrifuged at 5000 rpm at 4°C for 5 minutes in a sterile centrifuge tube, followed by washing these pellets thrice with 1 ml of Phosphate Buffered Saline (PBS). The turbidity of suspension was adjusted with 0.5 McFarland standards.

The antifungal activity of the plant extracts was carried-out by the method of (Kumar *et al.*, 2007). One hundred microlitres of suspension containing 5 X10⁶ CFU/ml of *Malassezia furfur* was spread uniformly using a sterile glass spreader on Leeming Notman

agar and the inoculum was allowed to dry for 5 minutes. Different concentrations of plant extract (15%, 20% and 25 %) and the standard zinc pyrithione (50µg/ml) was prepared. One hundred µl of the plant extract and 20 µl of the standard were loaded into the wells. The loaded plates were kept one hour for diffusion and then incubated for **48 hrs** at 30°C in an incubator in an inverted position. At the end of incubation, inhibition zone formed around the disc was measured using HIMEDIA antibiotic zone scale. All the studies were performed in **triplicates**.

Statistical analysis

Samples were analysed in triplicates and the results are given as Mean±SD.

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 (Tim cushnie and Lamb, 2005). In addition, medicinal plant extracts are the promising sources of antifungal drugs, even though they have relatively mild effect against human

pathogenic fungi when compared with commercial synthetic antifungal drugs (Hammer et al., 1999; Faleiro et al., 2003). This study emphasizes the importance of medicinal plants as an alternative anti-*Malassezia* agent against pathogenic fungi causing dandruff.

In the present investigation, the antifungal activity of four plants against *Malassezia furfur* at various concentrations (15%, 20% and 25%) were examined along with the standard antifungal agent zinc pyrithione (50µg/ml). Zinc pyrithione has been used widely for treating skin disease caused by *Malassezia*. Figure 1 and Plate 2 shows the antifungal activity of *Terminalia bellerica* at different concentrations in various solvents. The aqueous and ethanolic extract of *Terminalia bellerica* showed dose-dependent antidandruff activity. The aqueous extract of *Terminalia bellerica* exhibited a strong inhibition of 28 mm at 25 % concentration whereas the ethanolic extract showed 25mm inhibition zone in the same concentration. No inhibition zone was observed for the petroleum ether extract in any of the concentrations tested. The zone of inhibition for zinc pyrithione was found to be 23mm (Plate 1).

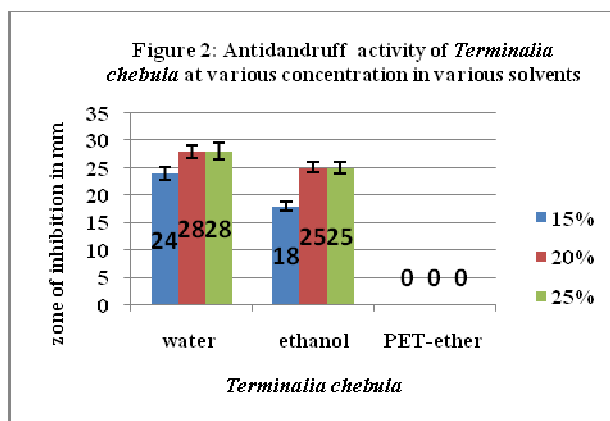
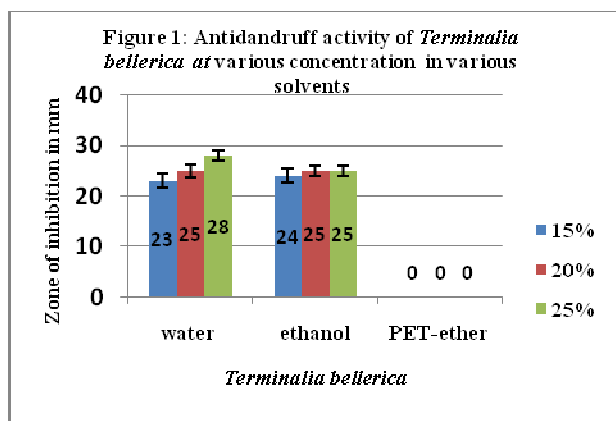


Figure 2 and plate 3 illustrates the antidandruff activity of *Terminalia chebula* in various solvents at different concentrations. The elevation in the inhibitory effect was

observed with increasing concentrations of sample. The aqueous extract possessed the similar inhibitory zones in both 20 % and 25 % concentrations. The same trend was

also noticed in the ethanolic extract. No zone was found for petroleum ether extract in any of the concentrations studied.

Emblica officinalis was tested for determining its anti-*malassezia* activity at different concentrations in various solvents (Figure 3 and Plate 4). The ethanolic extract of *Emblica*

officinalis had strong inhibitory effect at 25% concentration (22 mm). Similar zones (21 mm) were found for aqueous extract at 20% and 25% concentrations. Petroleum ether extracts showed no zone in any of the concentrations tested.

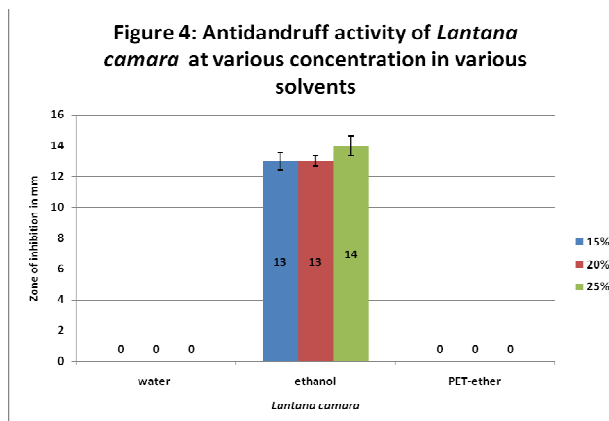
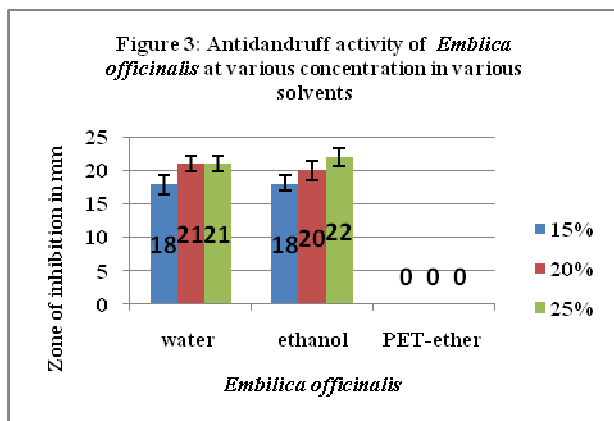
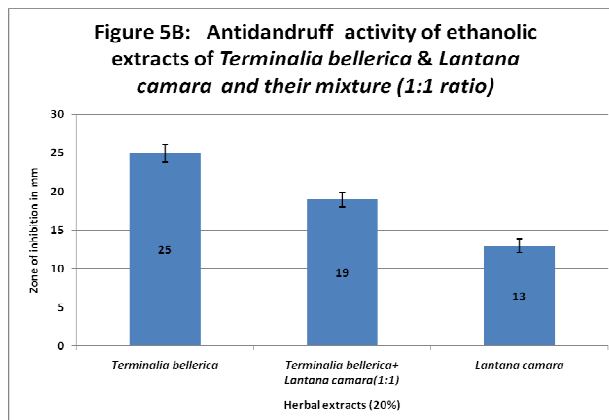
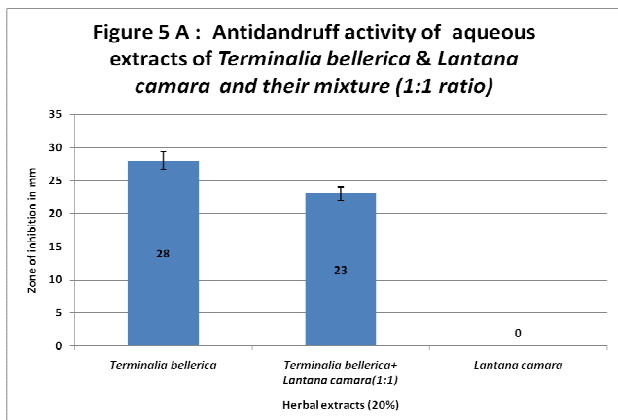


Figure 4 shows the antidandruff activity of *Lantana camara* at various solvents. The antidandruff activity was seen in ethanolic extract of *Lantana camara* and it was in a

dose dependant manner. Aqueous and petroleum ether extracts had no detectable zone in any of the concentrations tested.



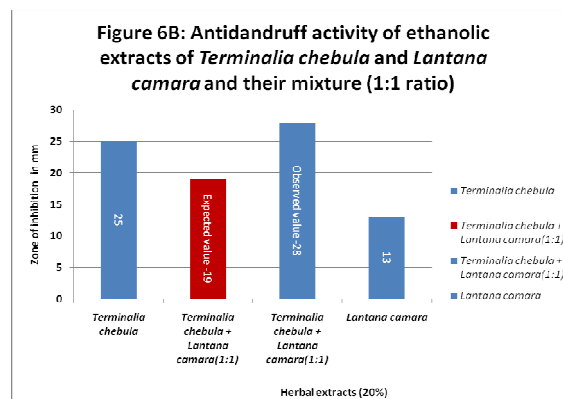
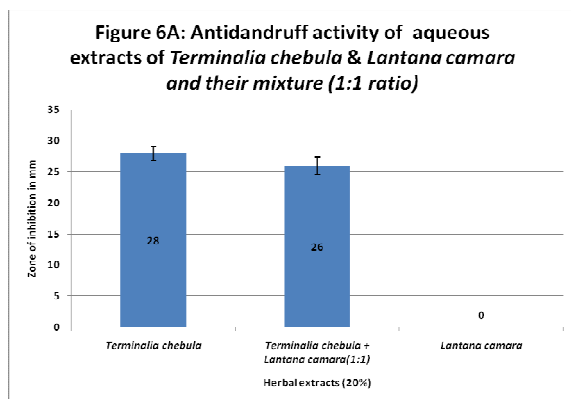
An attempt was made to find out the synergistic antidandruff actives from the screened plants. For this antidandruff study, *Terminalia bellerica* and *Terminalia chebula* were chosen since it showed promising antidandruff activity. *Lantana camara* was selected for this study since it has shown minimal antidandruff activity among the screened herbs. The synergistic antidandruff studies were performed by making the

aqueous mixtures of *Terminalia bellerica* & *Lantana camara* and ethanolic mixtures of *Terminalia bellerica* & *Lantana camara* in equal proportions (Figure 5A and Figure 5B). The results showed no synergy between *Terminalia bellerica* and *Lantana camara* in any of the solvents tested.

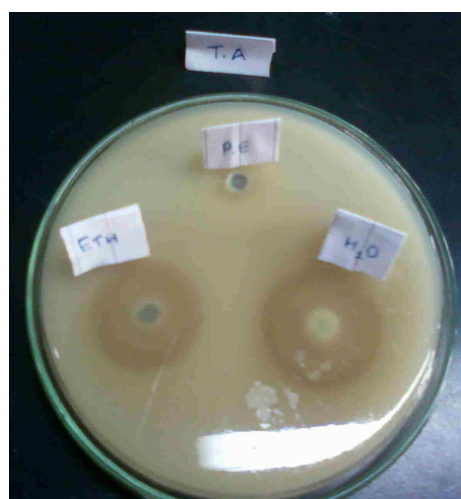
Figure 6A, 6B and Plate 5 shows the antidandruff activity of the aqueous extracts of *Terminalia chebula* & *Lantana camara* and

ethanolic extracts of *Terminalia chebula* & *Lantana camara* respectively. The results indicated that there was no synergy between aqueous extracts of *Terminalia chebula* and

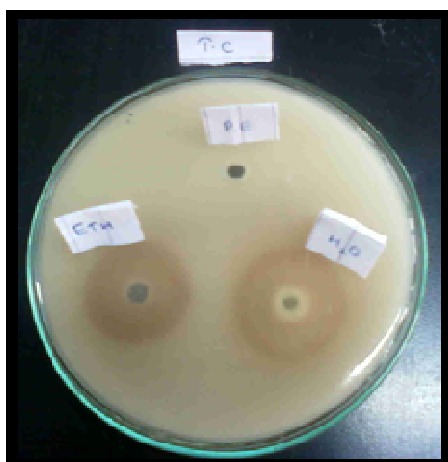
Lantana camara whereas the ethanolic extracts of *Terminalia chebula* and *Lantana camara* exhibited synergistic antidandruff effect.



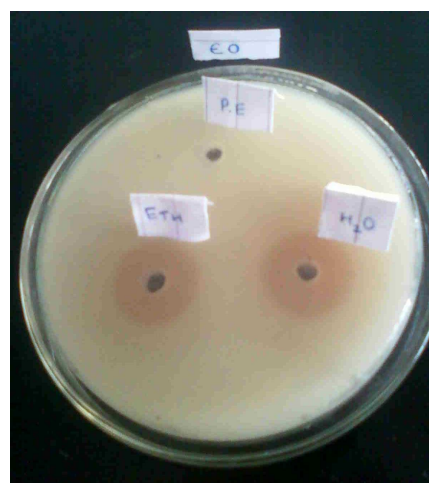
STANDARD ZPTO (Plate. 1)



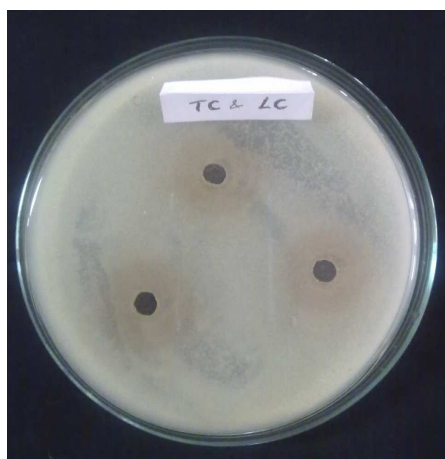
Terminalia bellerica (Plate .2)



Terminalia chebula (Plate 3)



Emblica officinalis (Plate.4)



Terminalia chebula + *Lantana camara* (Plate.5)

The study implies that aqueous and ethanolic extract of *Terminalia bellerica* and *Terminalia chebula* possessed outstanding antidandruff activity among the herbs screened. They were found to be similar in its inhibitory potential. Petroleum ether extract had no detectable

inhibitory effect in any of the plant extracts studied. The results of synergistic studies have found that synergy was observed between ethanolic extracts of *Terminalia chebula* and *Lantana camara*.

CONCLUSION

It can be concluded that the very significant antidandruff activity of *Terminalia bellerica* and *Terminalia chebula* present them as valuable natural antidandruff agents for controlling the growth of *Malassezia furfur*. The synergistic

effect of *Terminalia chebula* and *Lantana camara* is also an interesting observation which can well be applied as a development strategy in hair care products, targeting the control of dandruff.

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