



Antifungal activity of Amla extracts against dandruff causing pathogens (*Malassezia* sp.)

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Abstract

Amla (*Emblica officinalis*) is a vitamin C rich fruit has a wide range of medicinal properties known from the ancient period. The wide utility of amla fruits for antimicrobial activity has been extensively studied by many authors. In our present study, the antifungal activities of different extracts of amla were tested against dandruff causing isolates viz., *Malassezia furfur* and *Malassezia globosa*. The investigation was carried out from 25 female students with a complaint of hair fall and dandruff in the Department of Microbiology, Annamalai University. A total of 25 samples tested, only 2 samples showed positive for the presence of *M. furfur* and *M. globosa*. Among the three extracts used, fresh fruit juice exhibited higher antidandruff activity against selected pathogens in all the concentrations used but the best results shown in higher concentration. Thus, the amla fruit extract would be potential source of inhibitory substances and their inhibitory effect was directly proportional to the concentration of the extract tested.

Keywords: *Emblica officinalis*, *Malassezia furfur*, *Malassezia globosa*, antimicrobial activity.

Introduction

Medicinal plants are rich sources of bioactive compounds such as alkaloids, flavonoids and phenolic compounds (Kannan *et al.*, 2009). Most of the traditionally used medicinal plants has got the therapeutic properties and has been used for improving immune system as well as to cure various ailments. Plants are reservoir of effective chemotherapeutic and can provide valuable sources of natural antimicrobial compounds (Balandrin *et al.*, 1985). *Emblica officinalis* Gaertn. *Phyllanthus emblica* Linn. (Euphorbiaceae) is widely distributed in tropical and subtropical countries. The fruit of *Emblica officinalis* commonly known as amla is highly used in traditional Indian medicine (Scartezzini *et al.*, 2006). The fruit contains mainly tannin, gallic acid, ellagic acid, phyllembic acid and emblicol, vitamin C, alkaloids of phyllantidine and phyllantine, pictine and mineral

(Shrivastava, 2006; Trivedi, 2006). Apart from vitamin C, amla also contains cytokine like substances such as Zeatin (Zhao *et al.*, 1987; Pillay, 1958).

Amla is useful in vitiated conditions of tridosha, diabetes, inflammation, peptic ulcer, dyspepsia, cough, asthma, hepatopathy, leprosy, jaundice, diarrhea, dysentery, menorrhagia, grayness of hair, cardiac disorders (Gupta priya *et al.*, 2012; Madhuri *et al.*, 2011; Anil kumar *et al.*, 2012). *Emblica officinalis* is also used as antipyretic, analgesic, cytoprotective, antitussive, and gastro protective.

Dandruff is a common scalp disorder affecting almost half of the population in the pre-pubertal age and of any gender and ethnicity (Ranganathan and Mukhopadhyay, 2010). Hair fall is very common in

dandruff sufferers (Paul, 1999; Ravichandran *et al.*, 2004). For people with dandruff, skin cells may mature and be shed in 2–7 days, as opposed to around a month in people without dandruff. The result is that dead skin cells are shed in large, oily clumps, which appear as white or grayish patches on the scalp, skin and clothes (DeAngelis *et al.*, 2005). The potential non-microbial causes for dandruff are excessive exposure to sunlight, minimal irritation of the scalp due to over shampooing, frequent combing, use of certain cosmetic products and exposure to dust and dirt, although experimental evidence is lacking (Pierard-Franchimont *et al.*, 2006; Jo *et al.*, 2005; Pierard *et al.*, 1995).

Malassezia furfur (Pityrosporum ovale), a lipophilic fungus affects the hair and causes diseases called dandruff (Ranganathan *et al.*, 2001) and also called Pityriasis versicolor, Tinea circinata, Seborrheic dermatitis (Rippon, 2000). *Malassezia* sp. play a role in pathogenesis of this condition along with stress, fatigue, weather extremes, oily nature of skin, use of shampoos, immune suppressed status (AIDS) and neurological disorders. *M. restricta* and *M. globosa* are generally considered to be the causative agents of dandruff (Zisova, 2009). The species implicated vary depending on geographical location of the host. *M. furfur*, *M. sympodialis*, *M. obtusa* and *M. slooffiae* are the other species associated with this condition (Gupta *et al.*, 2004). In majority of the herbal antidandruff shampoo preparations, amla is the inevitable component. Thus, the aim of the present study was to evaluate the antifungal efficiency of *E. officinalis* against dandruff causing pathogen from affected individuals using different solvents.

Materials and Methods

Collection of plant material

The fresh fruits of *Embllica officinalis* were collected from local market of Chidambaram, Cuddalore district. Matured fruits with Greenish yellow colour, spherical shaped, hard on appearance with six vertical stripes or furrows were selected for the present study.

Preparation of plant extracts

The collected fruit samples were washed with distilled water and ethanol to remove dust particles and surface micro flora. The fruit pieces were dried and powdered. Cold extraction method was followed using different solvents (distilled water and ethanol) and finally the extracts were filtered through Whatman No. 1 filter

paper. The filtrate obtained was evaporated and was stored at 4°C in refrigerator until use (Taylor *et al.*, 1996). Fresh fruit juice was also used in the present study.

Collection of clinical samples

The present study was conducted in the Department of Microbiology, Annamalai University. About 25 female subjects were included in this study. The infected region was first washed with 70% ethanol followed by scraping with the sterile forceps then transferred into a dark sampling paper. The samples were then taken to the laboratory for further analysis and identification studies like Direct microscopy, catalase, Esculin hydrolysis, urease and tween utilization test (Shuaibu Isa *et al.*, 2013).

Antidandruff assay

The 48 hr old cultures of the fungal isolates on SDS plates were suspended in 5ml of sterile saline were used as inoculums. The turbidity of the suspension was adjusted to the Mcfarland 0.5 turbidity standard. The broth culture of *Malassezia furfur* and *Malassezia globosa* was swabbed over the Sabouraud's dextrose agar by using sterile cotton swabs. Sterile 5mm diameter Whatman No. 1 filter paper discs were dipped in plant extracts and Clotrimazole (reference antibiotic) were placed equidistantly on the plates. The plates were incubated at $30 \pm 2^\circ\text{C}$ and the zone of inhibition was observed after 7 days. Control was maintained with filter paper discs dipped in distilled water.

Results and Discussion

Among the 25 samples collected from suspected cases, only 2 samples showed positivity in KOH mount preparation and were subjected to isolation and identification procedures. Based on morphological, cultural and physiological characteristics they have been confirmed as *Malassezia furfur* and *Malassezia globosa* respectively. Karhoot *et al.* (2012) isolated and identified different species of *Malassezia* in Iraqi patients with pityriasis versicolor by following standard identification procedures. Shuaibu Isa *et al.* (2013) have isolated *Malassezia globosa* from dandruff among female students of Gombe State University. Identification of the isolates was done by comparing morphological and physiological characteristics. In this regard, 75% of the isolates obtained were *Malassezia globosa*. Shokohi *et al.* (2009) studied the distribution of *Malassezia* species in patients with pityriasis versicolor in Northern Iran. Identification had

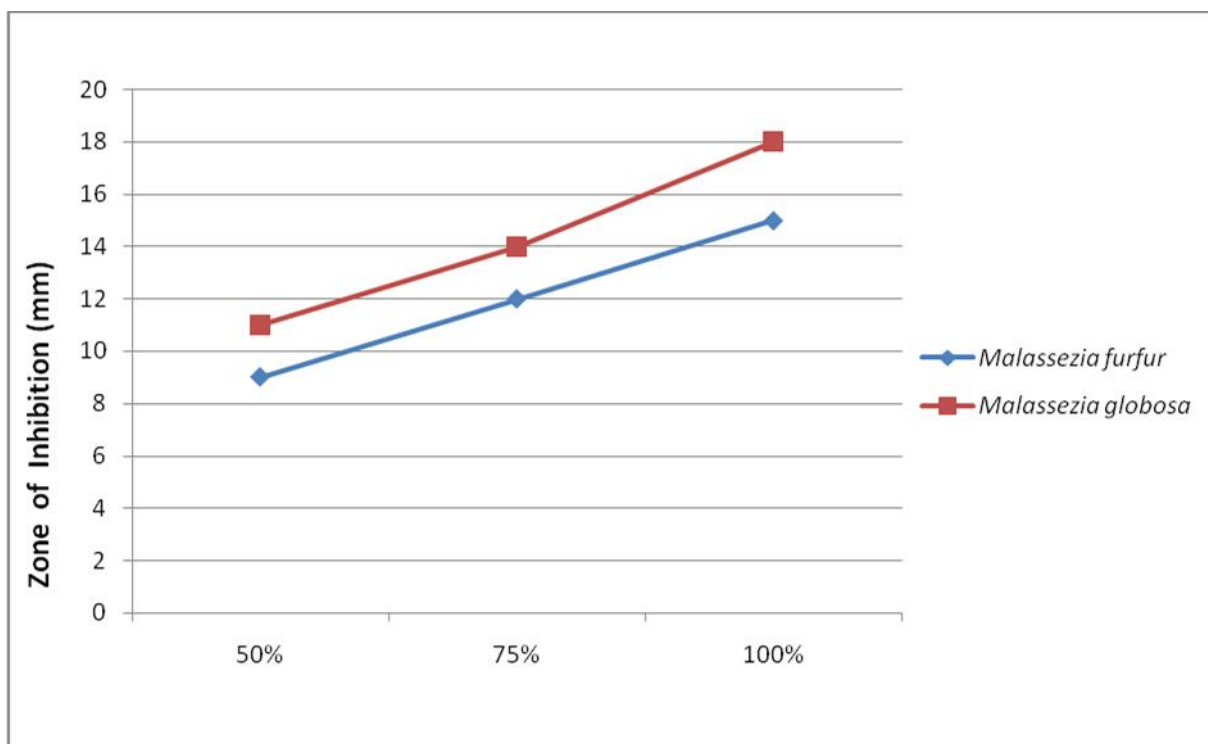
done by using morphological, biochemical, physiological as well as Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) methods.

The antidandruff effect of different extracts (ethanol, aqueous and fresh juice) of *E. officinalis* was examined against the test microbes viz., *M. furfur* and *M. globosa* by disc diffusion method and the results were shown in the Figures 1, 2 and 3 respectively. The results shown in Figure 3, clearly indicated that fresh juice of *E. officinalis* was found most effective against the *M. furfur* and *M. globosa* with the diameter of the highest zone of inhibition being 22 mm and 20 mm

respectively. Further, the results of aqueous and ethanol extract showed appreciable antifungal activity.

Data recorded in figures (Figures 1, 2 and 3) also showed that results of 100% concentration of different extracts exhibited maximum activity when compared to other concentrations viz., 50% and 75%. Our results are comparable with Dhanalakshmi *et al.* (2013). They studied the antidandruff activity of *Vernonia cinerea* leaf extracts and suggested that increasing concentrations of all the extracts exhibited increased trend of antifungal activity. The results for antidandruff activity of ethanol extract of *Emblca officinalis* was 18 mm zone against *Malassezia globosa* at 100% and the minimum zone of inhibition of 15 mm was recorded against *Malassezia furfur*.

Figure-1 Inhibition effects of ethanol extract of *Emblca officinalis* fruits against dandruff causing pathogens at different concentration



The inhibition zones of methanol extract of flowers and leaves of *Jasminum sambac* and essential oil flowers showed potential for antifungal activity with zones of 11.10 ± 1.92 , 12.90 ± 1.68 and 13.06 ± 0.26 mm respectively and minimum inhibitory concentration (MIC) values of 80 mg/mL and 50%, respectively (Santhanam *et al.*, 2014).

Hemamalini *et al.* (2014) determined the antidandruff activity of lyophilized aqueous and ethanolic extracts of *Phyllanthus emblica* Linn on two fungi (*Malassezia furfur* and *Malassezia obtusa*) isolated from dandruff and pityriasis vesicolor. The aqueous extract showed better activity as 6.25 µg/ml for *Malassezia furfur* and the ethanolic extract showed better activity for the organism *Malassezia obtusa* as 25 µg/ml.

Figure-2 Inhibition effects of aqueous extract of *Emblica officinalis* fruits against dandruff causing pathogens

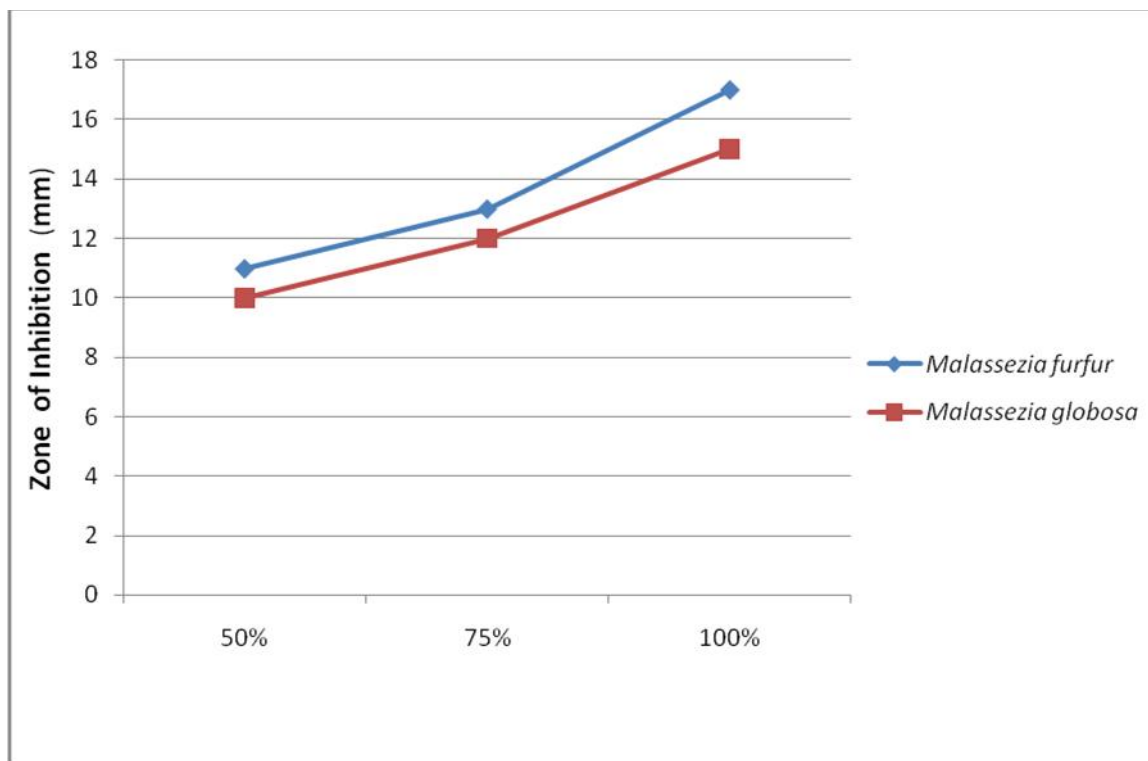
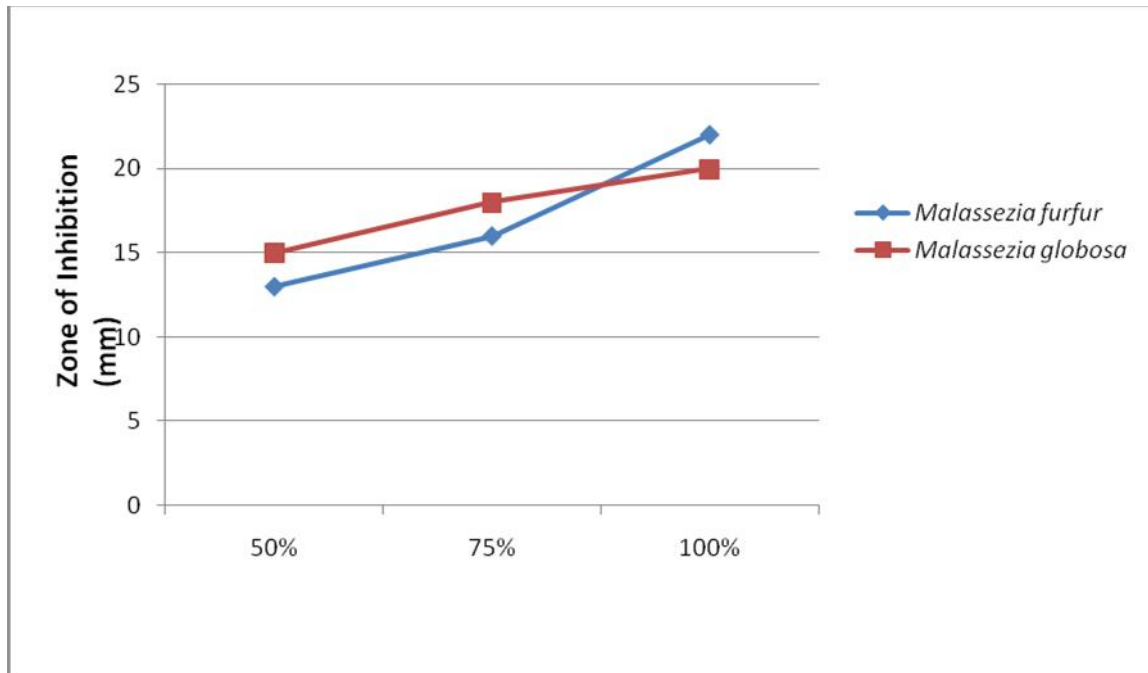


Figure-3 Inhibition effects of Fresh fruit juice of *Emblica officinalis* against dandruff *Malassezia* sp.



Vijayakumar *et al.* (2006) proposed modified medium for the culturing of *M. furfur*. Growth of the fungus in the influence of different temperature, pH and salinity was also studied determined. Plant extracts of 19 species were screened against the growth of the fungus by using disc diffusion method. In conclusion, the

amla fruit extract would be potential source of inhibitory substance for selected dandruff infection causing pathogens. Their antidandruff potency is mainly influenced by the extracting solvents as well as their concentrations which are used.

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