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Studies on the antidandruff activity of the essential oil of *Coleus amboinicus* and *Eucalyptus globulus*

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ABSTRACT

Objective: To determine the effect of the essential oil of *Coleus amboinicus* and *Eucalyptus globules* as potential antidandruff agent. **Methods:** Fresh leaves of *Coleus amboinicus* and *Eucalyptus globules* were collected from the Horticultural Research Station, Yercaud, Salem, Tamil Nadu, India. The collected leaves were washed thoroughly and shade dried for 7–10 days. The essential oils from both the dried plant leaves were obtained by hydro distillation procedure. The chemical compositions of hydro distilled essential oils were analyzed by Gas Chromatography–Mass spectrometry (GC–MS). The pure essential oils and the essential oils diluted with coconut oil of the two plants were used to check antidandruff activity against the dandruff causing fungus *Malassezia furfur* by agar well diffusion assay. **Results:** The average yield of essential oil from *Coleus amboinicus* (0.89%) and *Eucalyptus globules* (0.77%). The results of GC–MS revealed that the presence of eight compounds from *Coleus amboinicus* essential oil and five constituents from *Eucalyptus globules* representing 90.45% and 96.32% of the total oil respectively. The major component was identified as Thymol (phenolic rich compocnd) (41.3%) in *Coleus amboinicus* and in *Eucalyptus globules* the major constituents was 1–8, Cineol (91.61%). In the antidandruff activity the broad range of inhibitory zone was observed in *Eucalyptus globules* (37mm) oil compare to *Coleus amboinicus* (31mm). Ketaconazole based shampoo used as a standard. **Conclusion:** This study would direct to the establishment of a natural compound from *Coleus amboinicus* and *Eucalyptus globules* as an antidandruff agent which can be used for the production of potential antifungal drug and novel pharmaceutical and cosmeceutical leads.

1. Introduction

Plants based medicines are important therapeutic weapon to cure human diseases. Plants are of relevance to pharmacology. Pharmacological properties of medicinal plants may be used as leads in developing novel therapeutic agents[1]. Today herbal products and extracts are widely used to control various human diseases. Medicinal plants are providing an efficient local aid to the health care and disease free life and they contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments[2]. The uses of plant derived products as diseases control agents have been studied, since they

tend to have low mammalian toxicity, less environmental effects and wide public acceptance. Essential oils are concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from plants. They may provide potential alternatives to the control agents currently used because the compositions of essential oils are rich of bioactive chemicals[3]. In Tunisia, many plant extracts and essential oils have been shown to exert biological activity in vitro and in vivo, which justified research on traditional medicine focused on the characterization of antimicrobial activities[4–6]. *Coleus amboinicus* is commonly known as Indian / country borage. It is a large succulent herb with aromatic leaves. It's wide spread genus containing 300 species and is found in different parts of tropical Africa, Asia and Australia. It is widely used in making native medicines. In India this herb is traditionally applied externally for burns and insect bites, while internally it is used as a carminative and to control asthma[7]. The essential oil extracted from *Coleus amboinicus* show antibacterial and antifungal activity[8–9]. The genus *Eucalyptus*, (family:

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Myrtaceae) is native to Australian region. The genus *Eucalyptus* comprises wellknown plants of over 600 species of trees. *Eucalyptus globulus* is increasingly used in traditional medicine for various medical implications such as antibacterial, anti-inflammatory, and antipyretic effects. The plant is popular for this, it is cultivated in subtropical and Mediterranean regions more than other species. The essential oil of leaves of *Eucalyptus* species has been the object of several studies antibacterial, antioxidant, antihyperglycemic and antifungal activity^[10]. Dandruff is often defined as increased scaling of the scalp, representing the more active end of physiological desquamation^[11]. Dandruff (pityriasis capitis, seborrheic dermatitis confined to the scalp) is a disease that has been around for centuries despite several treatment options. Almost every day new players are entering the market with various antidandruff products, perhaps due to an increase in the incidence of the dandruff all over the world^[12]. *Malassezia furfur* is an important causal factor for dandruff^[13]. Several studies on the prevalence of dandruff across the world have shown a prevalence of dandruff of up to 50% in the general populations^[14]. Therefore, the treatment is the need of the hour for people suffering from dandruff. In the present investigation, two essential oils were extracted from *Coleus amboinicus* and *Eucalyptus globules* were used to compare the antifungal activities against the dandruff causing fungus *Malassezia furfur* both in its pure form and diluted with coconut oil.

2. Materials and methods

2.1. Collection of plant materials

Fresh plant leaves of *Coleus amboinicus* and *Eucalyptus globules* were collected from the Horticultural Research Station, Yercaud, Salem, Tamil Nadu, India. The plant leaves were taken to the Laboratory.

2.2. Preparation of powder form leaves

The plant leaves were air-dried under the shade (25–29°C) until the leaves become crispy dry and powdered.

2.3. Extraction of essential oils

The components of the half of the dried leaves were extracted for the essential oil as follows. In first batch of oil extraction 125 grams of the leaves powder was put in a rounded bottom flask, 500 ml of distilled water was added for each batch of extraction and then subjected to hydro distillation in a manual set up. After about one hour the 1st extraction was obtained in the collecting column, the quantity of the oil is read off directly from the graduated column and recorded. The used leaves are then poured out

and 150 grams fresh set of leaves is used and once again the process was repeated for another batch of leaves and so on. The oil recovered was dried over anhydrous sodium sulphate and kept in the refrigerator at 4 °C before use.

2.4. Percentage yield of Oil

The amount of extracted oils was determined and percentage yield of the extracted oils from each sample on the basis of various leaves samples by using following formula:

$$\% \text{ yield of oil} = \frac{\text{Weight of oil}}{\text{Weight of dried powder}} \times 100$$

2.5. Gas chromatography–mass spectrometry (GC–MS) analysis conditions

The analysis of the essential oils were performed using a Hewlett Packard 5890 II GC, equipped with a HP–5 MS capillary column (30 m, 0.25 mm, film thickness 0.25 μm) and a HP 5972 mass selective detector. For GC–MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290°C, respectively. Column temperature was initially kept at 50°C for 3 min, then gradually increased to 150°C at a 3°C/min rate, held for 10 min and finally raised to 250°C at 10°C/min. Diluted samples (1/100 in acetone, v/v) of 1 μl were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, NBS75K library data of the GC–MS system and literature data. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature^[15].

2.6. 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.

2.7. Media Preparation

The media and other microbiology accessories were obtained from Himedia. Sabarouds dextrose broth and sabarouds dextrose agar were used for the culture of *Malassezia furfur*.

2.8. Dilution of extracted oils

The extracted both oils were diluted with coconut oil at different concentrations such as 75%, 50% and 25% respectively.

2.9. Fungal inoculum preparation

The *Malassezia furfur* culture was maintained in sabarouds dextrose agar plates, slants and was further subculture before use. The mother inoculum was maintained at 30°C for about 7 days.

2.10. Anti fungal activity

The essential oils extracted from *Coleus amboinicus* and *Eucalyptus globules* were tested against the dandruff causing fungus *Malassezia furfur* using agar diffusion method. About 20–25 ml of potato dextrose agar medium for each Petriplate cooled to 45°C and was added to presterilized plates (150 mm in size). After this 0.1 ml of 12–16 hrs old cultures of fungal strain was spreaded over the agar plates. Petri plates were allowed to dry. About 3 wells in each plates of 6mm diameter were punched in agar surface with the help of sterilized cork borer for sphere for placing the extracted pure oil and mixed

with coconut oils samples of different plants. About 20 μ l of oil samples were added in separate wells. In the negative control well 20 μ l of distilled water was added and in the positive control ketoconazole antidandruff shampoo was placed for the comparison of result. The plates were kept for incubation at 30°C for about 48 to 72 hours. After 72 hours of incubation the zone of inhibition was clearly visible and the diameter of the zone was measured and tabulated.

3. Results

Two essential oils were obtained from *Coleus amboinicus* and *Eucalyptus globules* by hydrodistillation method by two extractions. In the first extraction of *Coleus amboinicus* the percentage yield was 0.88% ml/g for 125g and in the second extraction the yield was 0.9% ml/g for 150g and the average yield was 0.89% ml/g. For the *Eucalyptus globules* the percentage yield was 0.72%ml/g for 125g and 0.83% ml/g for 150g in the first and second extraction respectively. The

Table 1.
The yield of the essential oils from *Coleus amboinicus* and *Eucalyptus globules*

Extraction	Coleus amboinicus		Eucalyptus globules	
	First Extraction	Second Extraction	First Extraction	Second Extraction
Weight of leaves (g)	125	150	125	150
Volume of oil after one hour of distillation(ml)	1.1	1.35	0.9	1.25
Percentage yield(% ml/g)	0.88	0.9	0.72	0.83
Average yield(% ml/g)	0.89	0.77		

Table 2.
The main components identified in the Essential oils of *Coleus amboinicus* used in this study.

Compound	(KI) HP-5	% Essential oil	Mode of Identification
β -Caryophyllene	1423	4.20	RI, GC/MS
Carvacrol	1304	13.25	RI, GC/MS
Thymol	1295	41.3	RI, GC/MS
Methyl eugenol	1205	2.10	RI, GC/MS
Terpinolene	1166	3.75	RI, GC/MS
1,8-Cineole	1040	21.45	RI, GC/MS
β -Pinene	979	1.20	RI, GC/MS
α -Pinene	935	3.20	RI, GC/MS

Table 3.
The main components identified in the Essential oils of *Eucalyptus globules* used in this study.

Compound	(KI) HP-5	% Essential oil	Mode of Identification
α -Terpineol	1192	0.28	RI, GC/MS
1-8,Cineole	1040	91.61	RI, GC/MS
Myrcene	991	0.53	RI, GC/MS
β -Pinene	979	1.40	RI, GC/MS
α -Pinene	935	2.50	RI, GC/MS

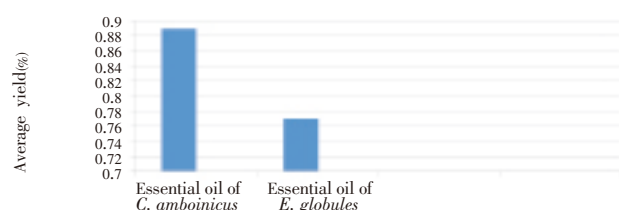


Figure 1. Average yield of essential oils

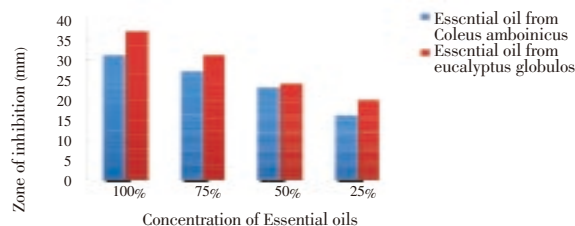


Figure 2. Antidandruff activity of essential oils.

average yield was 0.77%ml/g represented in table 1. Eight compounds from *Coleus amboinicus* essential oil and five constituents from *Eucalyptus globules* oil were identified by gas chromatography–mass spectrometry (GC–MS) which representing 90.45% and 96.32% of the total oil, respectively. The main constituents identified in the essential oil of *Coleus amboinicus* are Thymol, 1, 8–Cineole, Carvacrol, β –Caryophyllene, Terpinolene, Methyl eugenol, β –Pinene. In *Eucalyptus globules* the major components were identified as 1–8, Cineole, α –Pinene, β –Pinene, Myrcene, α –Terpineol table 2 (A and B). In the antidandruff activity results indicate that the pure essential oil of *Coleus amboinicus* showed an inhibitory zone of 31mm and *Eucalyptus globules* exhibited a zone of 37mm. The diluted forms with coconut oil at different concentrations showed an activity of 27mm, 23mm,16mm, of *Coleus amboinicus* and 31mm, 24mm, 20mm of *Eucalyptus globules* at 75%, 50%, 25% respectively Figure 1 and 2. Ketaconazole based shampoo was used as standard. The pure essential oils were more efficient and had the best antidandruff effect compared to the standard.

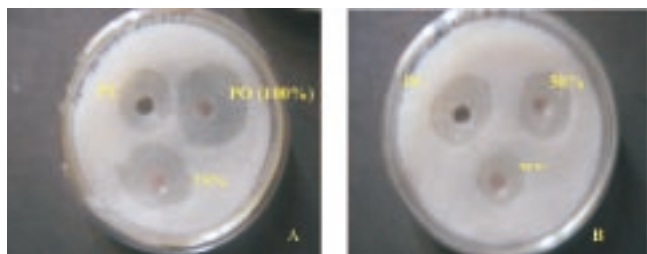


Figure 3. Anti dandruff activity of pure and diluted essential oil of *Coleus amboinicus*
 PO – Inhibitory zone of pure essential oil; PC – Inhibitory zone of Positive control; 75% – 75 μ l of essential oil diluted with 25 μ l of coconut oil; 50% – 50 μ l of essential oil diluted with 50 μ l of coconut oil; 25% – 25 μ l of essential oil diluted with 75 μ l of coconut oil.

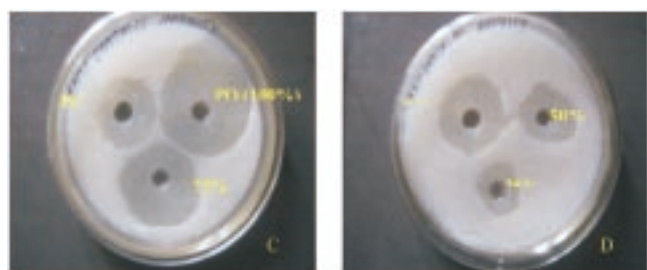


Figure 4. Anti dandruff activity of pure and diluted essential oil of *Eucalyptus globules*
 PO – Inhibitory zone of pure essential oil; PC – Inhibitory zone of Positive control; 75% – 75 μ l of essential oil diluted with 25 μ l of coconut oil; 50% – 50 μ l of essential oil diluted with 50 μ l of coconut oil; 25% – 25 μ l of essential oil diluted with 75 μ l of coconut oil.

4. Discussion

The traditional use of plants as medicine provide the basis for indicating which essential oils and plant oils may be useful for specific medical conditions are safe and without

side effects. The use of essential oils for the prevention and treatment of infection has been gaining popularity within the research field over the past decade[16]. In the present study two essential oils were extracted from *Coleus amboinicus* and *Eucalyptus globules*. The *Coleus amboinicus* had the highest yield with an average of 0.89 % ml/g followed by *Eucalyptus globules* with an average yield of 0.77% ml/g (graph 1). It is clear from the results that different plants containing essential oils have different quantities or yield. Armando (2009) reported the extraction of essential oils from the various plants[17] and the result of present study showed very similar to their report. The chemical composition and retention time of essential oils of *Coleus amboinicus* and *Eucalyptus globules* are presented in table 2. The GC–MS analysis revealed the presence of constituents of essential oils of *Coleus amboinicus* and *Eucalyptus globules* are listed in order of their elution order on the HP–5 MS capillary column. In total of eight compounds were identified from the oil of leaves of *Coleus amboinicus* representing (90.45%) of the total oil. The major constituent was Thymol (41.3%) followed by 1, 8–Cineole (21.45%) and β –Pinene (1.20%) as minor constituents were detected. A comparison of the constituents of the essential oil of *Coleus amboinicus* of this region from other different regions[18] showed that the Thymol was found as a major compound. In the essential oil of *Eucalyptus globules* five components were identified which comprise 96.32% of the total oil. The major constituent was 1–8,cineole (91.61%). Other minor constituents were β –Pinene (1.40%) Myrcene (0.53%) and α –Terpineol (0.28%). 1,8–cineole (91.61%) and alpha–pinene (2.50%) were the main components of *E. globules* essential oil tested in the present work. In fact, multiple studies have been reported on the chemical composition of the essential oils of *Eucalyptus* species belonging to different regions in the world. The chemical compositions of the leaf oils of *Eucalyptus* from various parts of the world have been reported and the 1.8–Cineole was identified as the major component in from samples growing in Taiwan, Uruguay, Algeria, Burundi, Congo, Mozambique, Greece, Australia, Tunisia, Italy, Nigeria, Turkey and Morocco[19]. The presence of three constituents viz., 1, 8–Cineole, β –Pinene and α –Pinene are the similar of *C. amboinicus* and *E. globules*. To develop environment–friendly alternatives to synthetic fungicides for the control of dandruff causing fungus, the interest on essential oils has been increased. A survey of literature reveals that there are many essential oils which possess antifungal activity[20–21]. With this interest we investigated the antidandruff activities of 2 essential oils as volatile compounds against *Malassezia furfur* by exposure to pure essential oils and essential oils diluted with coconut oil by agar well diffusion method. The zone of inhibition was clearly visible and the diameter of the zone was measured and tabulated. The pure essential oil of *Eucalyptus globules* showed greater inhibitory zone (37mm) than the inhibitory zone (31mm) of essential oil of *Coleus amboinicus* represented in graph 2. The both oils showed

maximum activity as compared to standard. The presence of rich phenolic compounds like Thymol, 1,8-Cineole, β -Pinene and α -Pinene present in the *C. amboinicus* and *E. globules* contribute to the bioactivity. The encouraging results indicate the *C. amboinicus* and *E. globules* might be of potential therapeutic values.

Conflict of interest statement

We declare that we have no conflict of interest.

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