EFFECT OF CRUDE EXTRACT MIXTURES OF FIVE SELECTED MEDICINAL PLANT SPECIES ON *Malassezia sp.*

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ABSTRACT

Plants contain a variety of phytochemicals which can be used for curing various kinds of diseases. In this study, crude extract mixtures of five medicinal plant species Citrus aurantifolia, Curcuma domestica, Trigonella foenumgraceum, Cassia alata, Azadirachta indica were investigated for antidandruff activity against dandruff causing agent Malassezia sp. The methanol extracts of five plants were combined in designated ways, in which every combination had an equal amount of each plant extract. Malassezia sp fungi was isolated and incubated in a modified PDA medium supplemented with olive oil and coconut oil. Disc diffusion method was used to test the antifungal activity of the plant extracts and the mean inhibition zone was measured in mm. Data were analyzed using one way ANOVA and Tukey's pairwise mean comparison was applied to compare the mean at P=0.05. The combined crude extracts A (Citrus aurantifolia + Azadirachta indica), B (Azadirachta indica + Curcuma domestica) and F (Azadirachta indica + Curcuma domestica + Citrus aurantifolia) having the highest zone (28.00 mm, 23.25mm and 24.92 mm respectively) of inhibition followed by combinations E (Azadirachta indica + Curcuma domestica + Cassia alata) (18.67mm), C (Citrus aurantifolia + Cassia alata) (15.00mm) and D (Citrus aurantifolia + Trigonella foenumgraceum) (15.00mm). All combinations of crude extracts had good antifungal activity but there is a considerable disparity in the potency of their antifungal activity. This may be due to the variation in concentration of active compound present in the crude extracts against fungi. Among the commercial antidandruff shampoos tested, the best antidandruff shampoo was Head&Shoulder (22.1mm). This was followed by 'Clear' (20.4mm) and 'Lifebuoy' (18.5mm). All these shampoos contain Zinc pyrithione (ZPT) which is reported to be anti-malassezial agent. The experiment showed that the antifungal activity of five plant extracts in the form of various combinations and commercial antidandruff shampoos was significantly different. Herbal-based shampoos are more effective in terms of safety and ease of manufacturing and in an economic point of view to control dandruff which is a major cosmetic problem.

Keywords: Medicinal Plants, crude extracts, Antifungal activity, *Malassezia sp.*

1. INTRODUCTION

Complementary and alternative medicine is gaining more and more attention worldwide as a mean of finding solutions for miseries of ill health where contemporary medicine has failed. Meantime concerns are also raised by many parties on the safety and efficacy of such treatments and practices creating a gap between the beliefs and available evidence in relation to complementary and alternative medicine (Jeyachandran et al. 2010).

Sri Lanka has a unique history in relation to complementary and alternative medicine practices. Indigenous medicine, Ayurveda, Siddha, Unani, and other systems are widely practiced in Sri Lanka. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance (Arora et al. 2013). Various natural plant extracts are known for their antidandruff properties. Evaluation of antifungal properties of such plant extracts can be

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done and they can be used effectively as an alternative to chemical agents in various antidandruff formulations. Along with anti-fungal properties, plant extracts are also known for their conditioning properties which will be fruitful in maintaining the overall health of scalp and hair (Balakrishnan et al. 2011).

Dandruff is a chronic scalp condition, which involves excessive shedding of dead skin cells from the scalp. It is caused by a fungus called *Malassezia restricta* and *Malassezia globosa*. *Malassezia* formerly called *Pityrosporum* which is a yeast causing infection of skin and scalp. Dandruff is the shedding of dead skin cells from scalp (Shuster, 1984). Dandruff is a common scalp disorder affecting almost half of the population at the post-pubertal age and of any gender and ethnicity. Dandruff is a condition characterized by flaking of skin (most commonly scalp skin) resulting from rapid turnover and release of skin cells. Dandruff is reliant on three factors that favor its survival and reproduction of the yeasts. They are: Sebum production, Microbial metabolism and Susceptibility of individual.

The increasing antimicrobial resistance exhibited by microorganisms causing dandruff infections has led to extensive research on the therapeutic potential of anti-dandruff herbal plants. Due to the side effects of the commercial shampoo, currently people like to use complementary and alternative medical treatment to control the dandruff cases. These medicinal systems mostly associate with plant base ingredient/ secondary metabolite for controlling dandruff. In natural, plants or herbs do not contain such kinds of serious side effects on the skin and can often act more slowly, because it gives the body its natural time of restoration and recovery (Laura Garces, 2014). Often several different herbs are used together. Practitioners say that the principles of synergy or combinations of plants claim that combining herbs improves efficacy and reduces adverse effect. This contrasts with conventional practice, where polypharmacy is generally avoided whenever possible (Vickers and Zollman, 1999).

The experiment was mainly aimed to study the effect of crude extract mixtures of five medicinal plant species (*Azadirachta indica*, *Curcuma domestica*, *Trigonella foenumgraceum*, *Citrus aurantifolia* and *Cassia alata*) on *Malassezia* sp causing dandruff. In addition, a relative study was carried out to compare the effect with that of synthetic antidandruff shampoo.

2. MATERIALS AND METHOD

2.1 Plant materials and extraction procedure

Table 1 shows the description medicinal plants used for extraction. The different parts of the plants as mentioned in the Table 1 were collected and shade dried.

Table 1: Medicinal plants used for crude extractions

Plants	Family	Plant parts used	Figure
Azadirachta indica	Meliaceae	Leaf	
Curcuma domestica	Zingiberaceae	Rhizome	
Trigonella foenumgraceum	Fabaceae	seeds	
Cassia alata	Fabaceae	Leaf	
Citrus aurantifolia	Rutaceae	Exocarp	

The dried plants were ground into fine powder. Five gram of fine powder of each medicinal plant separately added into 50ml of pure ethanol and kept at orbit shaker for 72 hrs. The filtered extracts were concentrated using rotary evaporator and crude extracts were preserved at 4°C (Reddy et al. 2010).

b. Isolation of dandruff causing agent - *Malassezia* sp

Samples were collected by scraping the scalp cells of the subjects suffering from dandruff. The isolate was inoculated on a modified medium by PDA agar supplemented with olive oil and coconut oil (2ml/ liter) by spread plate technique (Chua et al. 2005). The plates were incubated at 37˚C for 7 days

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c. Testing antifungal activity against *Malassezia* sp

Equal amount of different extracts was combined as shown in Table 2. Antimicrobial effect on *Malassezia* sp. was carried out through disc diffusion method (Sibi et al. 2012). Sterile paper disc were prepared using Whatman filter paper (Grade 3) and they were soaked in 100µl of each combination separately and discs were air dried before placing on the inoculated plates. The plates were incubated at 37˚C for 7 days. The radius of the zone of inhibition was measured in millimeters and recorded against the corresponding combination. Experiments were carried out with three replicates per combination of extract.

Table 2: Combination of crude extract mixtures

Crude extract Combination (A – F)
A. *Citrus aurantifolia + Azadirachta indica*
B. *Azadirachta indica + Curcuma domestica*
C. *Citrus aurantifolia + Cassia alata*
D. *Citrus aurantifolia + Trigonella foenumgraceum*
E*. Azadirachta indica + Curcuma domestica + Cassia alata*
F*. Azadirachta indica + Curcuma domestica + Citrus aurantifolia*

d. Testing the antifungal activity of commercial antidandruff shampoo

Three popular commercial antidandruff shampoos ('Head&Sholuder', 'Clear' and 'Lifebuoy') were selected and their controlling activity against *Malassezia* sp was tested as described in the part c of this section. The concentration of shampoo was adjusted to 0.25ml/ 5ml (50% v/v) distilled water and a homogenized solution was prepared.

2.9 Statistical analyses

One way analysis of variance (ANOVA) was done following the completely randomized design (CRD). Data were analysed in Minitab 16.1 (2007) at $P \leq 0.05$ of significant level. The mean differences of the treatments were adjusted by Turey's pairwise comparisons.

3. RESULTS AND DISCUSSION

Dandruff is a common disease caused by *Malassezia* species especially *Malassezia furfur*. The fungi hydrolysis the sebum of the hair and convert it into free fatty acids which cause hair loss and scalp. The combined crude extracts A, B and F having the highest zone (28.00 mm, 23.25mm and 24.92 mm respectively; figure 1) of inhibition followed by combinations E (18.67mm), C (15.00mm) and D (15.00mm) (Table 3).

Combination A Combination B

Combination F

Figure1. Antifungal effect of crude extracts on *Malassezia* sp.

All combinations of crude extracts had good antifungal activity but there is considerable variation in the potency of their antifungal activity may be due to the concentration of active compound against fungi. This work agrees to the work of previous author (Parthasarathy et al. 2014). Among the commercial antidandruff shampoos tested, the best antidandruff shampoo was Head&Shoulder (22.1mm). This was followed by 'Clear' (20.4mm) and Lifebuoy (18.5mm) as they all contain antifungal compounds like Zinc Pyrithione (Table 4).

Therefore, comparatively certain combination of plant extracts showed a high zone of inhibition than the shampoos. However, this findings were slight controversial to the study carried out by Obasi Chinelo et al. (2018), in which it was revealed that the combined extracts of *Citrus aurantifolia* and *Azadirachta indica* inhibited the growth of *Malassezia* sp and the diameter of mean inhibition zone was 13mm and commercial antidandruff shampoo, Head&shoulder had less inhibition (10mm) than other shampoo used in the experiment. The experiment showed that the antifungal activity of five plant extracts in the form of various combinations and commercial antidandruff shampoos was significantly different.

Table 3. Mean inhibition of crude extract mixture on Malassezia species

Plant Combination	**Mean inhibition radius/ mm**
Comb-A *Citrus aurantifolia + Azadirachta indica*	28.00 a
Comb-B *Azadirachta indica + Curcuma domestica*	23.25bc
Comb-C *Citrus aurantifolia + Cassia alata*	15.00e
Comb-D *Citrus aurantifolia + Trigonella foenumgraceum*	15.00e
Comb-E *Azadirachta indica + Curcuma domestica + Cassia alata*	18.67d
Comb-F *Azadirachta indica + Curcuma domestica + Citrus aurantifolia*	24.92b

(Tukey's pairwise comparison of mean inhibition at P=0.05)

Table 4. The effect of antidandruff commercial shampoo on *Malassezia* sp.

Antidandruff Shampoo	**Mean inhibition radius /mm**
Head&Shoulder	22.1a
Clear	20.4a
Life Buoy	18.5b

(Tukey's pairwise comparison of mean inhibition at P=0.05)

Malassezia sp. growth inhibition was higher than by plant extracts than synthetic shampoo. In addition, the inhibition zones of antidandruff shampoos at low concentrations almost matched with those of plant extracts. It is further stated that the plant extracts showed a considerable activity against dandruff causing organism *Malassezia* sp. and can be used to treat dandruff and this would can make a way forward to use the plant extracts widely for the antidandruff activity against *Malassezia* sp. since it is cheap and less side effect due to its herbal properties. Although, Mistry et al. (2016) has stated that herbal anti-dandruff shampoos can be used with the safe side of health but, they demonstrate slow or less effectiveness in controlling dandruff compared to synthetic ones.

Shreya Kothari et al. (2018) has reviewed the use of the poly-herbal anti-dandruff shampoo with reference to their basic concept, benefits, and challenges. They have presented that although the herbal based products are safe and free from side effect, it is seen that many product natural claims are still based on extensively on synthetic functional ingredient. Nevertheless, herbal-based shampoos are more effective in terms of safety and ease of manufacturing and in economic point of view to eliminate dandruff which is a major cosmetic problem.

4. CONCLUSION

This study has evidenced that medicinal plant extracts could be used to combat dandruffs and their efficiencies was not doubtful .The findings and knowledge obtained from the study could be advanced in the production of poly-herbal mix that could be added in hair oil or shampoos for better anti-dandruff activity. Also, it could be even encourage the use of whole plant extracts in the form of effective combination after further confirmation for treatment of dandruff rather than synthetic products which can cause problem to hair health.

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