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Comparative Analysis of Anti-Fungal Activity of Tea Tree Oil (Melaleuca alternifolia) and Tea Seed Oil (Camellia sinensis) against Trichophyton tonsurans

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Abstract: ***Trichophyton tonsurans is a ubiquitous, anthropophilic dermatophyte, responsible for infections of the scalp and sometimes of the glabrous skin or nails. It is a highly prevalent organism causing dermatophytosis around the world with no initial symptoms. Tea Seed Oil is known to treat several skin related problems. Similarly, Tea Tree Oil is also known to treat various skin related problems. So, the present study is based to compare the effect of both these essential oils on the growth of Trichophyton tonsurans. Disc diffusion assay was employed to compare the effect of both the oils. No inhibitory effect was observed in case of Tea Seed Oil while there was considerable inhibitory effect, observed on the growth of Trichophyton tonsurans, in case of Tea Tree Oil.***

Keywords: Trichophyton tonsurans, Dermatophytosis, Disc Diffusion Assay, Zone of Inhibition

I. INTRODUCTION

Trichophyton tonsurans is a ubiquitous, anthropophilic dermatophyte, responsible for infections of the scalp and sometimes of the glabrous skin or nails. Tinea corporis due to T. tonsurans appears as small, erythematous, scaly plaques, often measuring only cm in diameter, have no central clearing, and are similar in appearance to eczema. Tinea capitis due to T. tonsurans can be subdivided into three types: the seborrheic subtype, which is primarily characterized by the presence of dandruff and crusts; the kerion celsi subtype; and the black dot subtype. Unlike dermatophytosis caused by other dermatophytes, the clinical features of infection due to T. tonsurans are not very apparent initially mostly infects children; however, it can also cause disease in adults who are close contact with those children [1]. Thus, it is difficult to diagnose and it requires a high index of suspicion from the examination physician. Epidemiological data regarding T. tonsurans infections have emerged from different studies and countries; T. tonsurans is the commonest cause of tinea capitis in the Americas, Europe, Asia, and Australia and is responsible for more than 60% of cases. It has replaced Microsporum canis and Microsporum audouinii as the main pathogen causing tinea capitis.

Tea seed oil, known as oriental olive oil, is unique to Asia and is valued for its high nutritional and health benefits. It belongs to the family Theaceae. Its fatty acid composition is similar to that of olive oil, with large amounts of monounsaturated fats. Tea seed oil has received prominent attention due to its abundant functional ingredients, such as sterol, squalene, phenols, vitamin E and so on, which could play a role in scavenging free radicals and reducing the risk of cardiovascular disease [2]. This plant has also been traditionally useful in treating inflammations, asthma, and and fighting cancer. It is also useful in wound ulcers, coughs, bronchitis, burning sensation, diarrhoea, dysentery, leprosy, fever, hair fall, greyness of hair and various skin diseases [3].

The essential oil of Melaleuca alternifolia, also called tea tree oil or melaleuca oil, is known in Australia, and increasingly overseas, as a natural topical antiseptic. Tea tree oil contains c. 100 components, which are largely monoterpenes, sesquiterpenes and related alcohols. [4] Anecdotally, tea tree oil is known as an excellent treatment for fungal infections, in particular vaginal candidiasis and dermatophytoses.[5] This has prompted several in vitro investigations into the anti-candidal properties of the oil.[6–8] In contrast, there have been few comprehensive in vitro studies of the effects of tea tree oil on filamentous fungi, including dematophytes.

The aim of the present study is to compare the effect of Tea Seed Oil and Tea Tree Oil on the growth of Trichophyton tonsurans.

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II. MATERIALS & METHODS

A. Chemicals

1) *Growth Media:* To test the biological activity of the products, Sabouraud Dextrose Agar (SDA) was purchased from HiMedia Laboratories (Mumbai, Maharashtra, India). They were prepared and used according to the manufacturers' instruction. The media were solubilized in distilled water and sterilized by autoclaving at 121◦C, 1.0 atm. for 15 min.
2) *Reagents:* Ketoconazole (200mg) was obtained commercially from Adley Formulations (Chandigarh, India). 100% natural and therapeutic grade Tea Tree Oil and Tea Seed Oil was procured from Kalp (Surat, Gujrat).
3) *Micro-Organism:* A culture of *Trichophyton tonsurans* (MTCC 8475) was procured fom the Microbial Type Culture Collection and Gene Bank (MTCC) (Chandigarh, India) for its susceptibility testing studies. All cultures were maintained on SDA slants at. Subcultures were maintained in Sabouraud medium to ensure purity and viability until the testing was performed. 15 days old cultures on SDA slants at 25◦C were used to prepare the fungal inoculum to be used in the antimicrobial assays.
4) *Inoculum Preparation:* Inocula were prepared according to M27-A3 and M38-A2 documents determined by the National Committee for Clinical Laboratory Standards [9-10] Briefly, the stock inocula suspension was prepared from *T. tonsurans* culture plated on SDA and incubated at temperature of 25ºC for 15 days. After incubation, sterile normal saline solution (0.9%, 3mL) was added to the agar plate and the culture was gently swabbed to dislodge the conidia from the hyphal mat.The suspensions of conidia with hyphal fragments was then transferred to a sterile tube, and the volume of suspension was adjusted to 10 mL with sterile normal saline solution.The resulting suspension was strongly agitated using a Vortex apparatus for 15 s. The inoculum was standardized in sterile normal saline 0.9% spectrophotometrically to optical densities that ranged from 0.085 to 0.100 (approximately 80 to 82% transmittance).
5) *Anti-fungal Susceptibility Test:* The filter paper disc method of Wannisorn, et al.[11] was used with some modification for the study of antidermatophytic properties of essential oils. In present investigation standard size whatman no. 1 filter paper discs 6.0 mm in diameter, sterilised by pre dry heat at 140°C in an oven for one hour were used. 20 ml sterilised Sabouraud's dextrose agar medium was taken in each autoclaved petridish and allowed to solidify. To determine the susceptibility of the T. tonsurans and the antifungal activity of essential oils. 100µL of the fungal suspension was taken from the test tube and uniformly spread on sterile SDA Petri dishes. After inoculums got completely absorbed by agar, a single sterile filter disc was placed on the culture medium. The discs were impregnated with 5µL of each essential oil viz. Tea Seed Oil and Tea Tree Oil respectively. After 30 minutes, the plates were inverted and incubated at temp between 25°C for 15 days. Ketoconazole impregnated discs were used as a reference standard which served as a positive control. Discs without any oil or drug impregnated served as a negative control. At the end of the incubation period, the inhibition halo diameters were measured using calipers and expressed in millimeters. When the inhibition halo observed was equal or higher than 10 mm, it was considered as positive antifungal activity. Three replicates were kept in each case and the average values of the inhibition zones were were determined. The activity of oil was measured by the following formula:

$$\text{Activity Index} = \frac{\text{Inhibition Zone Of The Sample}}{\text{Inhibition Zone Of The Standard}}$$

6) *Statistical Analysis:* The experiment has three replicates and therefore, three determinations were conducted and mean of the variable and standard deviation were recorded.

III. RESULT & DISCUSSION

The table shows the results for antifungal susceptibility test for Tea Tree Oil and Tea Seed oil on Trichphyton tonsurans. The results clearly indicate indicates that after the completion of the incubation period inhibition zones were observed in case of Tea Tree Oil having the mean diameter sizes of 20.33 ± 1.35mm, which were comparable with the inhibition zone obtained in petri-plates containing Ketoconazole that has been taken as positive control which had the mean diameter size of 25.67 ± 1.68 mm. On the other hand, there was no inhibition zone observed in petri-plates containing discs impregnated with Tea Seed Oil. No inhibition was observed in negative control experiment. These results clearly indicate that Tea Seed Oil do not have any effect on the growth of Trichophyton tonsurans, in contrast, Tea Tree Oil have good antifungal activity against the test organism.

Table 1: Comparative Analysis Antifungal Susceptibility of Tea Tree Oil with Tea Seed Oil

OIL	ZONE OF INHIBITION (in mm)	ACTIVITY INDEX
TEA TREE OIL	20.33 ± 1.35	0.79
TEA SEED OIL	0.00 ± 0.00	0.00
Ketoconazole	25.67 ± 1.68	1
NEGATIVE	0.00 ± 0.00	0.00

IV. CONCLUSION

So, from the present study it is clear that the essential oil from Camellia sinensis i.e., Tea Seed Oil do not have any effect on the growth of Trichophyton tonsurans; on the other hand, essential oil from Melaleuca alternifolia i.e., Tea Tree Oil have inhibitory effect on the growth of the test organism. Thus, this study further suggest to evaluate the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Tea Tree Oil along with the testing of its toxicity, both in vitro and in vivo, against Trichophyton tonsurans; so that Tea Tree Oil may be used as a therapy against the infections caused by it.

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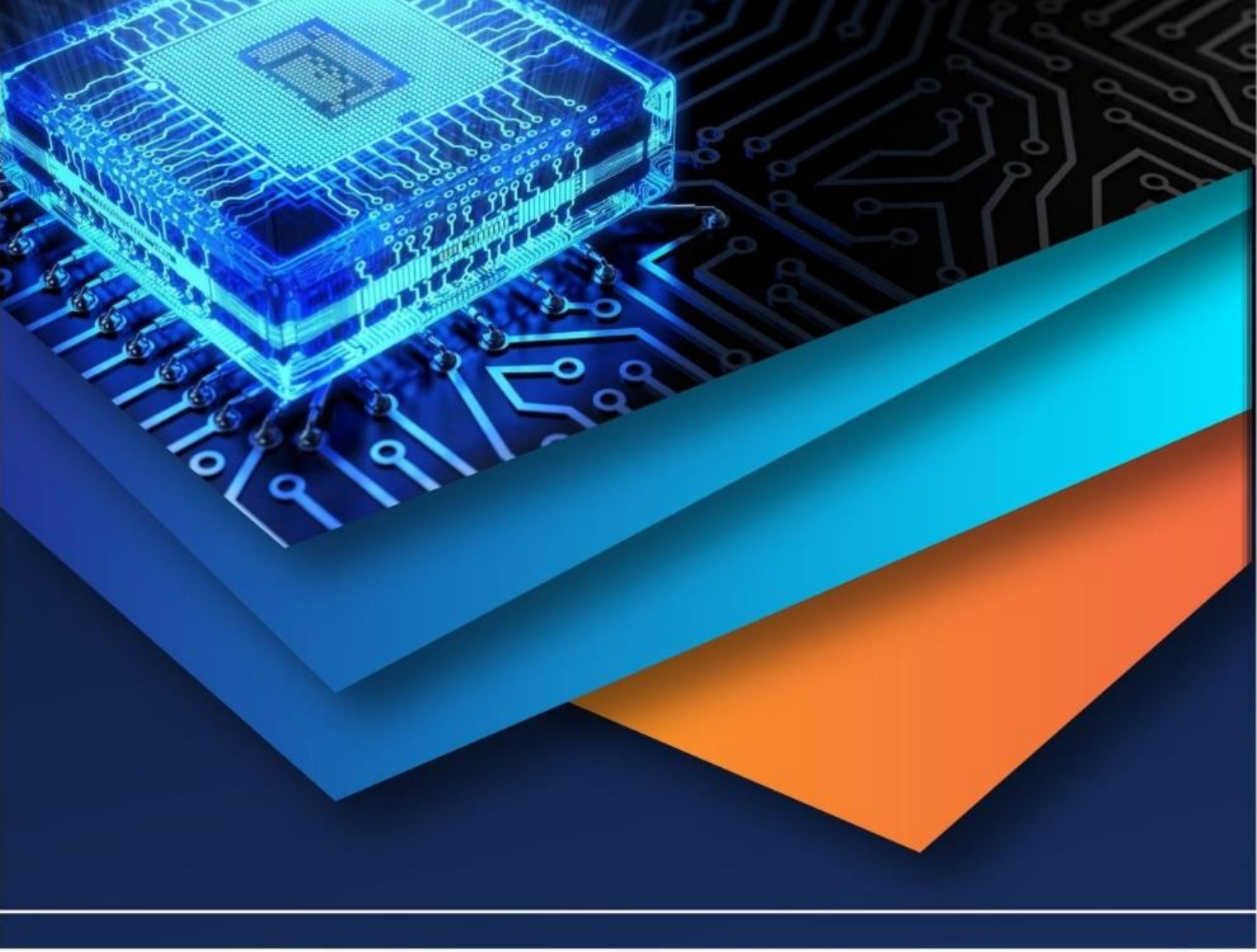

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