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COMPARATIVE EFFICACY OF TEA TREE OIL NANOEMULGEL AND TEA TREE OIL GEL AGAINST CANDIDA ALBICANS.

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ABSTRACTS:

Fungal skin infections are caused by different types of fungi, including dermatophytes and yeasts. Increased use of antibiotics and immunosuppressive drugs such as corticosteroids are major factors contributing to higher frequency of fungal infections. Fungi can infect almost any part of the body including skin, nails, respiratory tract, urogenital tract, alimentary tract, or can be systemic. Anyone can acquire a fungal infection, but the elderly, critically ill, and individuals with weakened immunity, due to diseases such as HIV/AIDS or use of immunosuppressive medications, have a higher risk. Nanoemulsion based gel is a promising approach. The present study was aimed to compare an in vitro efficacy of nanoemulgel, tea tree oil gel and placebo carbopol 934 P gel by cup-plate method. Tea tree oil loaded nanoemulgel was formulated using 1% w/w carbopol 934P in optimized nanoemulsion formulation. The antifungal study was carried out using Candida albicans strain (MTCC NO: 227). The zone of inhibition for tea tree oil nanoemulgel (37±1.3 mm) was found to be significantly higher ($p \leq 0.05$) as compared to tea tree oil gel (19±1.5 mm) and placebo carbopol 934 P gel (00±1.1 mm). Based on the observations, it was concluded that tea tree oil in nanoemulgel formulations due to its nanosize is able to inhibit the growth of candida albicans more efficiently as compared to tea tree oil normal gel.

Keywords: Tea tree oil gel, nanoemulgel, carbopol 934 P, cup plate method, C. albicans

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Introduction

Fungi are identified to be a cause of serious infection with increased frequency during the past two decades.[1]Over 40 million people have suffered from fungal infections.[2] Progression of infections can be rapid and serious due to compromise with immune function.[3]

Fungal infections can range in severity from superficial to life-threatening. For example, fungal infections affecting only the top layers of the skin are readily treatable and have a relatively limited impact on quality of life. However, if a fungal infection enters systemic circulation, consequences can be deadly.[4,5]

Although several species of fungi are potentially pathogenic in humans, *candida* (esp. *Candida albicans*) is the organism responsible for most fungal infections. *Candida*, which is normally present within the human body, is usually harmless. *Candida* is a type of fungus that can cause an infection in skin also. In normal conditions, skin may host

small amounts of this fungus, but problems arise when it begins to multiply and creates an overgrowth. Candida skin infections can occur on almost any area of the body, but are more commonly found in intertriginous regions—where two skin areas may touch or rub together—such as armpits, the groin, skin folds, and the area between the fingers and toes. The fungus thrives in warm, moist, and sweaty conditions. Normally, the skin acts as an effective barrier against infection, but any cuts or breakdown in the superficial layers of the skin may allow the fungus to cause infection. The prognosis for *candidal* infections is often very good. Generally, the condition isn't serious and can be easily

Table 1: Zone of inhibition for different formulations against Candida *albicans* strain

Strain No.	Formulation	Zone of Inhibition (mm) Mean ± S.D (n=3)			Inference
		24 hrs	48hrs	72 hrs	
MTCC No. 227	Tea tree oil Nanoemulgel	37± 1.3	36.5± 1.3	36±1.4	Fungicidal action
MTCC No. 227	Tea tree oil gel	19± 1.5	18.5±1.3	17.8±1.6	Fungicidal action
MTCC No. 227	Placebo carbopol 934 P gel	00	00	00	No action

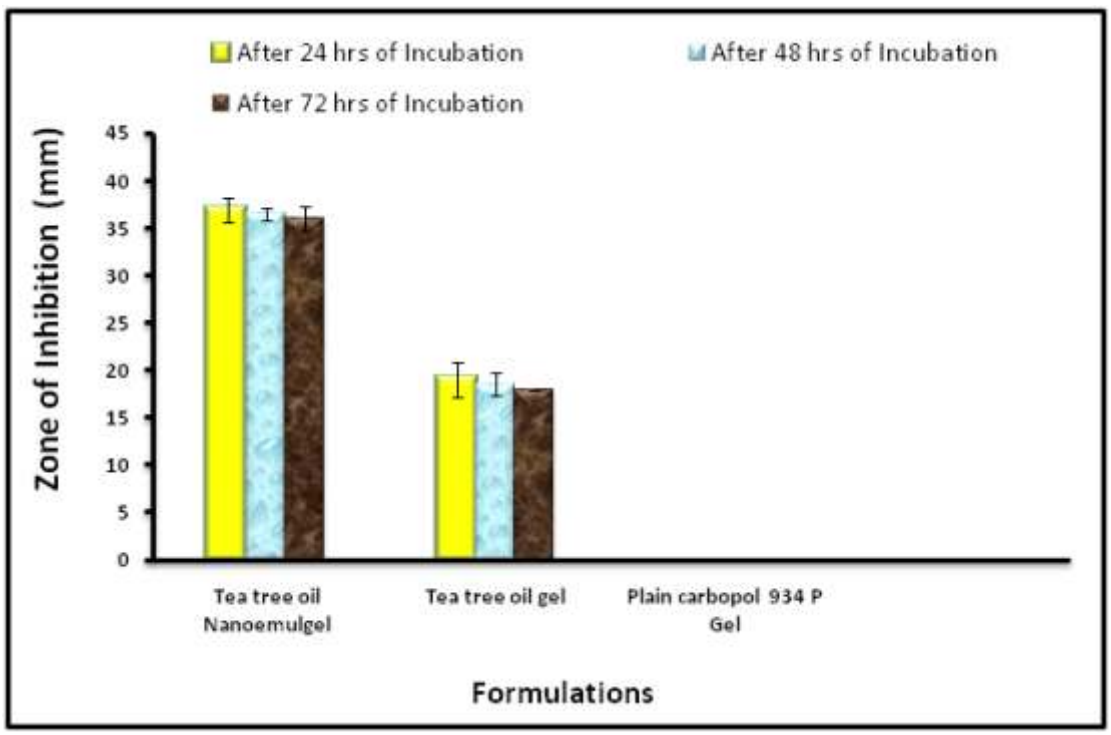
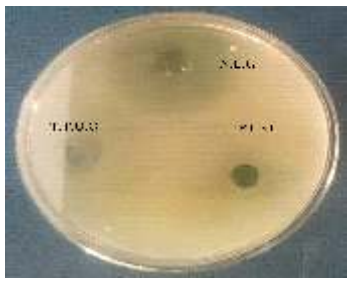
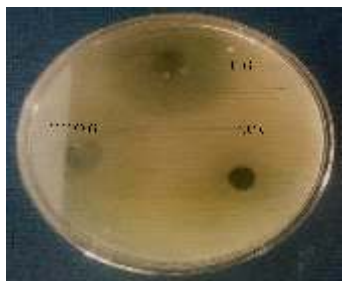


Figure 1: Comparison of zones of inhibition for different formulations during *in vitro* anti-fungal activity against *Candidaalbicans* (MTCC No: 227)

Table 2: Observations of Zones of Inhibition for different formulations evaluated against strain of *Candida albicans* at different time intervals

Incubation Time (hrs)	Observations of Zone of Inhibition for different formulations
	***Candida albicans* (MTCC No. 227)**
24	
48	
72	

Where, N.E.G = Nanoemulsion Gel, P.C.G = Placebo carbopol 934 P Gel, T.T.O.G= Tea tree oil Gel

treated.[6]

Fungal Infections can also invade deeper tissues as well as blood causing life threatening systemic infections. Therefore, it is very necessary to treat not only the superficial infections, but also the deeper ones.[7]

Tea tree oil has been used medicinally in Australia for more than 80 years, with uses relating primarily to its antimicrobial [8,9] and anti-inflammatory properties.[10] The oil is obtained by steam distillation from the Australian native plant *Melaleuca alternifolia*, and contains 100 components, which are mostly monoterpenes, sesquiterpenes and related alcohols. Compositional ranges for 14 of the major components are stipulated in the International Standard (ISO 4730) and as such, oils compliant with the standard vary little in chemical composition. Tea tree oil shows promise as a topical antifungal agent, with recent clinical data indicating efficacy in the treatment of dandruff and oral candidiasis.[11] Data from an animal model also indicate that it may be effective in the treatment of vaginal candidiasis.[8] These clinical uses are supported by a wealth of *in vitro* susceptibility data.[8,12] Further *in vitro* work has shown that tea tree oil and components cause the leakage of intracellular compounds and inhibit respiration in bacteria.[13] In the present study, the efficacy of tea tree oil loaded nanoemulgel is compared with tea tree oil loaded gel and placebo carbopol 934 P gel using cup and plate microbiological assay method.

Materials and Methods

Nanoemulsion and Nanoemulgel components

Tea tree oil was procured from Sigma Aldrich Pvt Ltd (Bangalore, India). Carbopol 934P was purchased from Sigma Aldrich Pvt Ltd (Bangalore, India). Transcutol P was obtained as a gift sample from Gattefosse (Saint Priest, Cedex, France). Tween 20 was purchased from Central Drug House, New Delhi, India. All other chemicals and reagents were of analytical grade and procured from Merck (Mumbai, India) and S.D. Fine Chem. (Mumbai, India).

Strain, growth media and culture conditions

Candida *albicans* MTCC No. 227 was procured from IMTECH (Institute of microbial technology, Chandigarh). Candida *albicans* was grown in suspension of YME (Yeast Malt Extract) and incubated at 28°C in B.O.D incubator shaker. Where necessary, the concentrations of viable cells in suspensions were confirmed by viable counts.

Preparation of nanoemulgel, tea tree oil gel and placebo carbopol 934 P gel

The tea tree oil nanoemulgel was prepared by using aqueous titration method by incorporating carbopol 934 P as a gelling agent. Tea tree oil gel and placebo carbopol 934 P gel were also prepared at Khalsa College of Pharmacy, Amritsar.

In vitro antifungal activity using cup and plate method

This study was done as per the procedure given by Maebashi *et al* 1995 and Vijaya*et al* 2014. From the candida albicans suspension ($1x10^7$cfu/ml), 50 µl suspension was taken and spread on Sabouraud dextrose agar (SDA) plates aseptically with the help of sterile cotton swab. The plates were rotated through an angle of 60° after each application. Finally the swab was pressed round the edge of the agar surface. It was allowed to dry at room temperature with the lid closed. Then, three wells of about 3mm diameter were punched using sterile core borer into the agar medium and filled with tea tree oil nanoemulgel (1g), tea tree oil normal gel (1g) and placebo carbopol 934 P gel (1g) respectively. The plates were kept in refrigerator for 2 hours to facilitate uniform diffusion of the drug. Then the plates were incubated at 28ºC for 18-24hrs. Observation was made for zone of inhibition around the well. The zones of inhibition obtained for tea tree oil nanoemulgel, Tea tree oil gel and placebo carbopol 934 P gel were compared.[14,15]

Statistics

Results were expressed as mean± standard deviation (S.D). The data obtained from various groups were statistically analysed using Graph Pad Instat 3, using two tailed paired t-test. Values at $p \leq 0.05$ were considered significant.

Result and Discussion

In vitro anti-fungal activity by using cup and plate method

In vitro anti-fungal activity was evaluated by using cup and plate method.

Determination of zones of inhibition by using cup and plate method

The zones of inhibition that appeared around the formulations evaluated on the S.D.A plate were measured. The zone of inhibition for tea tree oil nanoemulgel (37±1.3 mm) was found to be significantly higher ($p \leq 0.05$) as compared to tea tree oil gel (19±1.5 mm) and placebo carbopol 934 P gel (00mm). The larger zone of inhibition for tea tree oil loaded nanoemulgel could be attributed to the presence of tea tree oil in nanosize in the gel, which resulted in a greater diffusion of tea tree oil through S.D.A which inturn resulted in a higher penetration of tea tree oil through fungal cell walls, which ultimately resulted in higher fungicidal effect due to greater inhibition of synthesis of ergosterol, a sterol, which is required for maintaining the integrity of cell wall of fungi. The results are given in table 1, table 2 and figure 1.

Inference: The zone of inhibition for tea tree oil nanoemulgel (37±1.3 mm) was found to be significantly higher ($p \leq 0.05$) as compared to tea tree oil gel (19±1.5 mm) and placebo carbopol 934 P gel (00 mm). It can thus be concluded that nanoemulgel of tea tree oil significantly increases the antifungal activity of tea tree oil against Candida Albicans as compared to tea tree oil gel and placebo carbopol gel.

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