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Control of *Propionibacterium acnes* using Essential oil

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Abstract : In the present investigation the antimicrobial effect of 19 plant essential oils have been studied on *Propionibacterium acnes*, the bacterium causes human skin disease, the Acne vulgaris. Out of 19 essential oil, 5 oils showed inhibitory effect against this bacterium. These plant oils used are from *Thymus vulgaris* (thyme), *Eucalyptus globulus* (eucalyptus), *Syzygium aromaticum* (clove bud), *Cinnamomum verum* (cinnamon) and *Melaleuca alternifolia* (tea tree), they have shown effective inhibition zone in mm as 29.66±0.5, 25.00±0.0, 55.33±0.5, 18.00±0.0, 13.66±0.5 respectively. The anti acne cream was prepared containing 2% of each of the effective essential oil, stearic acid (24.2%), cetyl alcohol(1.20%), triethanolamine (1.32%), propylene glycol(0.95%), isopropyl myristate (1.20%), glycerine (2.00%), borax (1.90%). Anti acne cream formulated showed the anti activity against *P. acnes*.

Key words : *Propionibacterium acnes*, Acne vulgaris, Minimum inhibitory concentration, Anti acne cream.

Introduction

Acne vulgaris is common human skin disease, affecting particularly adolescents but also affect adults as well. Although overall health is not impaired it is not a trivial disease; it produces cutaneous and emotional scars. The symptoms of acne also include whiteheads, blackheads and pimples causing disfiguration ensuing psychological, social and economic problems (Ravichandran *et al.*, 2004).Webster (2002) reviewed the literature on acne vulgaris and reported that it is caused due to follicular inhabitant of *Propionibacterium acnes*, which acts as a harmless commensal incapable of tissue invasion or serious infection. He further suggested that *Propionibacterium acnes* activates complements and became inflammatory when comes in contact with immune system. The organisms produce metabolites as subaceous trigycerides, consuming glycerol fraction by discarding fatty acids. Tropical therapy has been recommended for the management of acne vulgaris with specific limitations (Ravichandran *et al.*, 2004; Jerajani *et al.*, 2004). Gislene *et al.*(2000) reported antibacterial activity of some plant extracts and phytochemicls on antibiotic-resistant and antimicrobial treatment of *P. acnes*. Gubelin *et al*. (2006) isolated from inflammatory acne and suggested antimicrobialability against *Propionibacterium acnes*. Cox *et al*. (2000) studied the role of essential oil of *Melleuca alternifolia* (tea tree oil) against *Propionibacterium acnes* as antimicrobial action. The antibacterial activity of clove essential oil against *Propionibacterium acnes* was noticed by Fu *et al*. (2009). Athkomkulchai *et al*. (2008) and Abbasi *et al*. (2010) gave formulations of anti acne creams from plant oils and reported their efficacy avoiding use of systemic antibiotics.

The present study is an attempt to use essential oils as antiacne formulations are very good therapeutic use for treating acne.

Materials and Methods

Collection and maintenance of culture -

Pure culture of *Propionibacterium acnes* MTCC 1951 was obtained from Institute of Microbial Technology, Chandigarh, India. The culture was maintained in Peptone yeast extract glucose broth. (Moore and Cato, 1963)

Plant essential oil -

Plant essential oils were obtained from commercial outlet of Dr. Urjita Jain Herbal ltd, Mumbai.

Determination of effect of plant essential oil on growth of *P. acnes.*

Kirby Bauer disc diffusion method was used to determine the effect of plant essential oil on the growth of *P.acnes* (Casida,1993). The broth culture of *P. acnes* was seeded in peptone yeast extract glucose agar containing 1% tween 80. Plates were allowed to solidify. Sterile discs were dipped in plant essential oil and placed onto the agar plates. Erythromycin was used as a control. The plates were incubated at 37°C under anaerobic conditions. The zone of inhibition was observed after 48 hours.

Determination of Minimum inhibitory concentration of effective essential oil –

Broth dilution method was used to determine the Minimum inhibitory concentration. The effective plant essential oil were selected and dilutions were prepared

in the range of 0.1% - 1% using PYG broth containing tween 80. 0.1 ml of the culture was inoculated in each tube. Sterile mineral oil was added in each tube to create anaerobic conditions. The tubes were incubated at 37°C under anaerobic conditions. The results were recorded after 48 hours (Zu *et al.*, 2010).

Formulation of anti acne cream -

Stearic acid was melted and then required quantity of paraffin oil was added to it at a temperature of 70-80°C preventing it from boiling. After mixing, cetyl alcohol was added and the contents were homogenized. With continuous stirring triethanolamine, propylene glycol, isopropyl myristate, glycerine was added in this mixture. Whole material was kept warm during the entire procedure.

In another vessel, essential oil and borax were mixed in distilled water and this suspension was heated at 80-90°C. This suspension was added into the above oily phase mixture and the contents were thoroughly homogenized. Anti acne creams containing 2% of each of the effective essential oil were prepared (Abbasi *et al.*, 2010; Athikomkulchai *et al.*, 2008). The composition of the antiacne cream is given in Table 1.

Results and discussion:

Among the 19 different plant essential oils tested, *Thymus vulgaris* (thyme), *Eucalyptus globulus* (Eucalyptus), *Melaleuca alternifolia* (Tea tree), *Cinnamomum verum* (Cinnamon), and *Syzygium aromaticum* (clove bud) are effective these results have been compiled in Table 2.

MIC value for Thyme was 0.5%, Cinnamon was 0.08%, Eucalyptus was 0.6%, Tea tree was 0.8%. MIC of Clove bud oil could not be determined correctly as the dilutions made were turbid making it difficult to observe the bacterial growth and to determine the MIC value. Similar observation is reported by Carson and Riley (1994) while determining MIC of Tea tree oil. These plant oils are *Thymus vulgaris* (thyme), *Eucalyptus globulus* (eucalyptus), *Syzygium aromaticum* (clove bud), *Cinnamomum verum* (cinnamon) and *Melaleuca alternifolia (*tea tree) have shown effective inhibition zone as 29.66±0.5mm, 25.00±0.0mm, 55.33±0.5mm, 18.00±0.0mm, 13.66±0.5mm respectively. The anti acne cream with each essential oil (2.00%) containing stearic acid (24.2%), cetyl alcohol (1.20%), triethanolamine (1.32%), propylene glycol (0.95%), isopropyl myristate (1.20%), Glycerin (2.00%), Borax (1.90%) was suggested. Anti acne cream formulated showed the anti *P. acnes* activity. In terms of MIC cinnamon oil was found to be most effective.

Anti acne cream also showed the activity against *P. acnes*. The study depicted that the anti acne formulations are very good therapeutic compositions for acne. Our observations confirm the findings of Athkomkulchai *et al*. (2008) and Abbasi *et al*. (2010). Anti acne cream containing Eucalyptus oil formulated by Athkomkulchai *et al*. (2008) showed the zone of inhibition of 8.0±0.0 mm whereas in the present study zone size of 14.0±0.0 mm was observed.

Essential oil inhibits respiration and increase permeability of bacterial cytoplasm and yeast plasma membranes. They also cause potassium ion leakage. The ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely source of its lethal action at the minimum inhibitory levels (Cox *et. al.*, 2000).

Clove essential oil exhibited significant activity

Table 1: The composition of anti acne cream

Ingredient	% Weight
Stearic acid	24.20
Cetyl alcohol	1.20
Triethanolamine	1.32
Propylene glycol	0.95
Isopropyl myristate	1.20
Glycerin	2.00
Borax	1.90
Essential oil	2.00

Table 2: Antimicrobial activity of plant oils against *P. acnes*

Plant essential oil	Average Zone in mm
Eucalyptus globulus	25.00±0.0
Matricaria recutita	-
Cinnamomum verum	55.33±0.5
Syzygium aromaticum	18.00±0.0
Pogostemon cablin	-
Vitus vinifera	-
Cedrus atlantica	-
Cymbopogon flexuosus	-
Melaleuca alternifolia	13.66±0.5
Thymus vulgaris	29.66±0.5
Rosmarinus officinalis	-
Curcuma longa	-
Zingiber officinale	-
Piper nigrum	-
Salvia sclarea	-
Azadirachta indica	-
Simmondsia chinensis	-
Cymbopogon martinii	-
Citrus limon	-

against *P. acnes*, promises potential in vivo activity. The bacteriostatic mechanism involves damage to the cell walls and membranes of bacteria. At longer incubation times, cytoplasm proteins may diffuse from the cytoplasm. Alternatively, essential oil might inhibit protein synthesis (Fu *et al*, 2009). The results of the present study support their theory.

The main component of thyme essential oil is thymol and that of cinnamon is eugenol, which possesses notable anti-bacterial and anti-oxidant effects. These two constituents may also be responsible for the antibacterial and cytotoxic activities of thyme or cinnamon essential oil.

In addition to their use for food and cosmetics, the potential of essential oil for the treatment of acne merits further exploration in the future (Zu *et al*. 2010). Traditional herbal treatment in combination with cosmetic care is a well established basis of acne therapy. Some herbal products may have the potential to replace standard chemical therapy in a mild to moderate cases because of their good efficacy and higher tolerability (Reuter *et al.*, 2010).

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