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ANTIBACTERIAL ACTIVITY OF CERTAIN MEDICINAL PLANTS AGAINST ACNE-INDUCING BACTERIA

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ABSTRACT

The present investigation was conducted to evaluate the antibacterial efficacy of five medicinal plants against *Propionibacterium acnes*. The various solvent extracts of herbs namely *Azadirachta indica, Vitex negundo, Annona squamosa, Cymbopogon citratus* and *Terminalia chebula* were prepared by decoction method. The antiacne potential of various solvent extracts of herbs were tested along with the standard Clindamycin in a dose dependant manner by disc diffusion method. The results of the study found that aqueous and ethanolic extracts of *Terminalia chebula* exhibited the outstanding antiacne activity when compared to all other herbal extracts. Moderate zone of inhibition was observed in the medicinal plants namely *Azadirachta indica, Vitex negundo* and *Annona squamosa*. Inhibition zone was not detectable in any of the solvent extracts of *Cymbopogon citratus*. The findings of this study have identified that *Terminalia chebula* acts as the promising source of antiacne agent which could be useful in the treatment of acne vulgaris.

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KEY WORDS

Propionibacterium acnes, Terminalia chebula, clindamycin, disc diffusion method, acne vulgaris

INTRODUCTION

Acne vulgaris is the most common disorder of human skin that affects upto 80% of individuals in their lives (Dreno and Poli, 2003). The areas most vulnerable to acne are the largest oil secreting glands present in the face, back and trunk (Van De Kerkhof, 2006). It is a chronic inflammatory disease of multifactorial etiology affecting more than 85% of teenagers and frequently continues into adulthood. The influencing factors of acne include excess sebum secretion, hyperkeratinization of the hair follicle, oxidative stress and the release of inflammatory mediators (Katzman and Logan, 2007; Nourin and Ballard, 2006).

Acne is related to abnormalities in sebaceous gland function particularly in teenage (White, 1998). The hypersecretion of hormone androgen stimulates higher sebum secretion in sebaceous gland (Henderson et al., 2000). The secreted sebum normally contains a mixture of lipids, squalene, wax and cholesterol both in free and in ester forms and triglycerides that naturally provide a skin barrier function (Pilgram et al., 2001). However, the resulting abnormalities in sebaceous glands due to hormonal effects alter sebum composition and decrease linoleic acid content (Downing et al., 1986). Thus, the normal skin barrier function is impaired. In addition, the deficiency of linoleic acid in the follicle promotes the growth of normal flora like *P.acnes*. Proliferation of *P.acnes* leads to inflammatory lesions and severe acne (Holland et al., 1998). In addition, *Staphylococcus epidermidis* and *Pitryosporum ovale* are present in acne lesions (Shehadeh, and Kligman, 1963).

Although the other microorganisms are present in acne lesions, the prevalent bacterium implicated in the clinical course of acne is *Propionibacterium acnes*. It is a gram positive, anaerobic bacterium that mostly resides in the pilosebaceous follicles of the skin. Although *P. acnes* is a member of the normal skin commensal, it plays a critical role in the development of acne (both inflammatory and non-inflammatory) when it becomes overgrown and colonizes the pilosebaceous unit.

The main components of pilosebaceous unit on the skin are the keratinocytes and sebocytes which can be activated by *P. acnes* leading to the production of pro-inflammatory products. These include lipases, proteases, hyaluronidases and chemotactic factors (Heymann, 2006). Among these products, lipase is an important factor in causing inflammation. They act by metabolising the sebaceous triglycerides into fatty acids, which chemotactically attract neutrophils.

As a consequence, neutrophils which are attracted to the acne lesion constantly release inflammatory mediators such as reactive oxygen species (Leyden, 1997). Although reactive oxygen species perform a useful function in the skin barrier against acne microbes (Boh, 1996; Cals-Grierson and Ormerod, 2004), excess formation affects skin condition by activating neutrophil infiltration. The reactive oxygen species include singlet oxygen, superoxide radical anion, hydrogen peroxide, hydroxyl radical, lipid peroxide and nitric oxide.They play a critical role in irritation and disruption of the intergrity of the follicular epithelium leading to the development of

inflammatory acne as well as tissue injury (Jame et al., 1999). This toxic reactive oxygen species can also act as second messengers in the induction of several biological responses like the generation of cytokines which also plays a crucial role in the development of inflammatory lesions and severe acne. Therefore, treatment of acne is an important requisite for acne patients.

Acne treatment involves the correcting of the altered pattern of follicular keratinization, decreasing sebaceous gland activity, decreasing *P. acnes* population and producing anti-inflammatory effect.

Antibiotics are generally employed to inhibit inflammation or to kill bacteria (Odou et al., 2007; Nakatuji et al., 2009). Some of the antibiotics used in acne treatment include tetracycline, erythromycin, roxithromycin, clindamycin, benzoyl peroxide and azelaic acid. Long term use of these antibiotics cause significant antibiotic resistance and multiple drug resistance in acne patients (Eady et al., 2003). Moreover, *P. acnes* form biofilms (bacteria in a distinct metabolic state attached to the cell surface) which increases its resistance to antimicrobial agents (Coenye et al., 2007). These problems of resistance may cause the failure of antibiotic treatment for acne. In order to overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as an alternative treatment for acne. In addition, the medicinal plants/ naturally derived compounds are believed to be safer than synthetic compounds (Cordell, 2002). In this study, five medicinal plants have been investigated for their antiacne potential against *P. acnes*.

MATERIALS AND METHODS

Plant Material:

The five medicinal plants used in this study were collected from the herbal garden of Mount Carmel College in Bangalore. They were authenticated by the botanist, Mount Carmel College, Bangalore, India. The following list of medicinal plants was used for the study.

S.No	Botanical name	Family	Part used
1	*Azadirachta indica*	Meliaceae	Leaves
2	*Vitex negundo*	Verbenaceae	Leaves
3	*Annona squamosa*	Annonaceae	Seeds
4	*Cymbopogon citratus*	Poaceae	Leaves
5	*Terminalia chebula*	Combretaceae	Fruits without seed

Preparation of Plant Extracts:

The various parts of plant materials were made into fine powder.1 gram of herbal powder were dissolved in 10ml of water, ethanol and Petroleum ether. The mixture was heated in a boiling water bath at 80 degree centigrade for 15 minutes. The mixture was cooled to room temperature and centrifuged at 6000 rpm for 10 minutes. The supernatant was filtered and the filtrate was collected and used for the analysis.

Microorganism and Media

The test organism used in this study was *Propionibacterium acnes* (MTCC 1951). The bacterium was obtained from IMTECH (Institute of Microbial Technology), Chandigarh, India. The media and other microbiology accessories were obtained from Himedia.

Antibacterial Susceptibility testing-Disc Diffusion Method

The method of Bauer et al., 1966 was adopted for testing the efficacy of herbal extracts against the test organism. *P. acnes* was incubated in brain heart infusion broth (BHI broth) with 1% glucose for 72 hours under anaerobic conditions and adjusted to yield approximately 1.0 X 10^8 CFU/mL. Aliquots of molten BHI with glucose agar were used as an agar base. The molten agar in the plates was allowed to solidify for 5 mins. The inoculum suspension (100 µl) was swabbed uniformly and allowed to dry for 5 mins. The different concentrations of plant extracts (5mg/disc, 7 mg/disc and 10 mg/disc) and standard clindamycin (0.2 µg/disc, 0.4 µg/disc and 0.6µg/disc) were loaded on 6mm sterile disc. The petriplates containing the loaded disc were kept in refrigerator at 4ºC for 1hour diffusion. After diffusion, the petriplates were incubated for 72 hours at 37^0C under anaerobic conditions. At the end of the incubation, the zone of inhibition, that appears as a clear zone around the loaded disc were observed and measured in mm using HIMEDIA antibiotic zone scale.

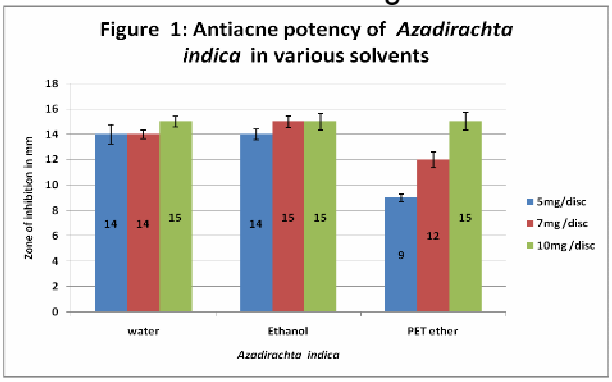
Statistical analysis

Samples were analysed in triplicates and the results were given as Mean ± SD.

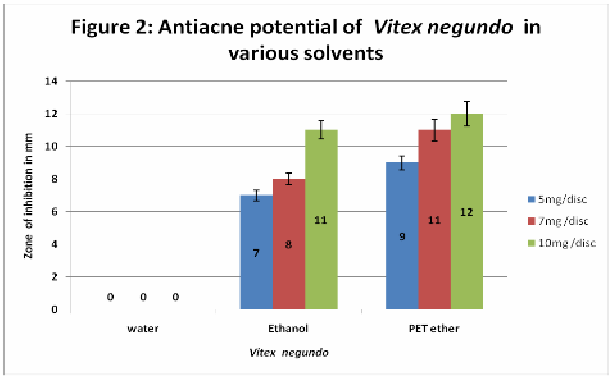
RESULTS AND DISCUSSION

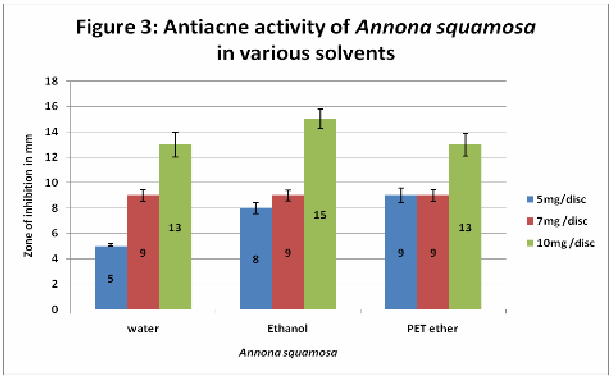
The antiacne potential of five herbs at various concentrations (5 mg/disc, 7 mg/disc and 10 mg/disc) was investigated for their antibacterial activity against *P. acnes* along with the standard Clindamycin (Figure 6). The results showed that 4 herbs could inhibit the growth of acne inducing bacteria in a dose dependant manner. Among the medicinal plants studied, aqueous and ethanolic extract of *Terminalia chebula* showed strong inhibitory effects (Figure 4). Interestingly, the ethanolic extract of *Terminalia chebula* exerted the maximum inhibition zone of 32 mm at 10 mg concentration. Petroleum ether extract of *Terminalia chebula* had no detectable activity against *P. acnes*.

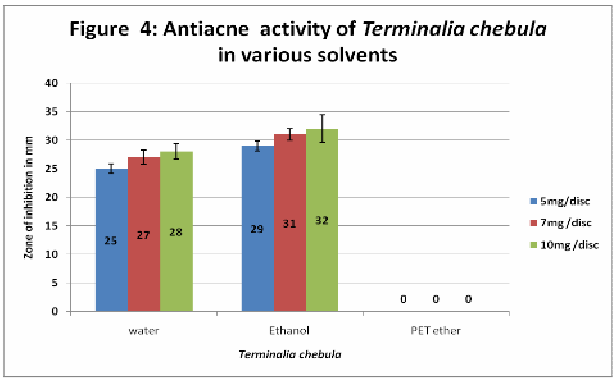
Figure 1: Antiacne potency of *Azadirachta indica* in various solvents

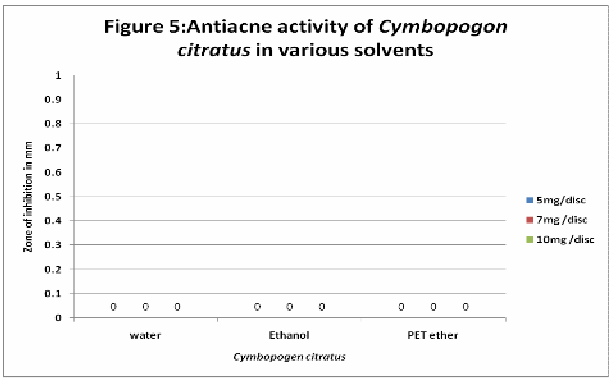


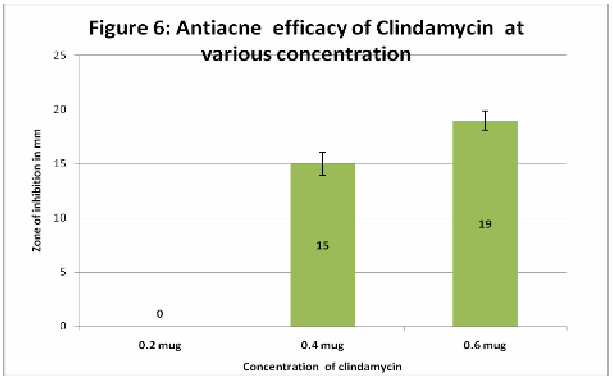
Azadirachta indica and *Annona squamosa* exhibited moderate inhibitory activity against the test organism in all the three solvents (Figure 1 and Figure 3). The ethanolic and petroleum ether extracts of *Vitex negundo* also had a moderate inhibitory effect whereas the aqueous extract showed no zone of inhibition (Figure 2). The antiacne activity of *Cymbopogon citratus* was not detectable in the three solvents tested (Figure 5).











In conclusion, the study indicates that the ethanolic extract of *Terminalia chebula* possess good anti-acne activity which can be used as a promising alternative source instead of antibiotics in acne treatment.

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