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Antimicrobial Properties of Essential Oil Extracted from *Azadirachta indica* Leaves on Common Skin Pathogens

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Abstract

Antibiotic resistance is rising to dangerously high levels and spreading globally at an alarming rate mainly due to misuse and over prescription of antibiotics. Hence, scientists are studying medicinal plants to find potential alternatives in reducing the antibiotic resistance. In this study, *Azadirachta indica* essential oil was extracted from dried leaves due to several findings reported that the phytochemicals present in *A. indica* leaves had the ability to treat common skin pathogens including *Staphylococcus aureus, Staphylococcus epidermidis, Propionibacterium acnes*, methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE). The results showed that the extracted *A. indica* essential oil had better antimicrobial effects on MRSA and MRSE compared to cefoxitin as a reference point. The extracted oil also had significant antimicrobial activity when tested against *P. acnes*. Stability of the extracted essential oil was studied to confirm the stability of phytochemicals in the extracted *A. indica* essential oil at 4ºC for a month and the results were similar to the freshly extracted *A. indica* essential oil.

Keywords

Antimicrobial property, Natural Herbs, Skin diseases

Introduction

Bacterial infections are among the leading causes of morbidity worldwide. This is compounded by the overuse and misuse that has led to the emergence of and subsequent increase in antibiotic resistance (Rivera, Loya, & Ceballos, 2013). Researchers have found that plants contain various bioactive chemicals and have the antimicrobial activity against bacterial strains. According to Zeenat, Ravish, Ahmad, & Ahmad (2018), *Azadirachta indica* is one of the common medicinal plants used by the Indian Ayurvedic and Unani systems of medicine due to the wide therapeutic benefits including anti-bacterial, anti-fungal and anti-viral activities. *A. indica* has been shown to contain a rich source of phytochemicals including azadirachtin, nimbidin, salannin, quercetin and steroids (Asif, 2012). In this study, the main aim was to extract essential oil from *A. indica* leaves using ethanol and to determine the phytochemical content in this extract. This extracts were used

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to screen for antimicrobial activity against common bacterial pathogens from the skin which included *Staphylococcus aureus, Staphylococcus epidermidis*, MRSA, MRSE and *Propionibacterium acnes*. The zones of inhibition that were formed in the agar well diffusion assay showed the effectiveness of the *A.* indica essential oil against skin pathogens.

Methodology

Preparation of crude essential oil of Azadirachta indica

Fresh *A. indica* leaves were obtained from a residence in Nilai. The leaves were washed three times with deionised water and dipped in 85% ethanol to remove dirt before being left to dry under the sun for 3 days. The leaves were then mechanically ground until fine powder was produced and stored in a sterile Schott bottle at 4ºC until further use.

Essential oil extraction using a Soxhlet apparatus

The powdered *A. indica*, 30 grams, was filled into a thimble and placed into the Soxhlet extractor and extracted with 75% ethanol for 4 hours at a temperature of approximately 69ºC (Tesfaye & Tefera, 2017). The extract was then concentrated using a rotary evaporator. The final, concentrated liquid product was dispensed into a sterile universal bottle and stored at 4ºC until further use (Redfern, Kinninmonth, Burdass, & Verran, 2014). Commercial neem essential oil (Chiltan Pure) was used as a control.

Phytochemical screening

Ethanol extracted *A. indica* essential oils were subjected to phytochemical screening including tests for alkaloids, phytosterols, fixed fats and oils, flavonoids, saponins and terpenoids as per the methodology cited in Thilagavathy et al., 2015.

Agar well diffusion assay

A suspension of each bacteria tested diluted obtain 5 x10^5 CFU/mL and spread onto Mueller-Hinton agar plates (Citron et al., 2005). Plant essential oil was dispensed into wells made in the agar plates. The diameter (mm) of the inhibition zone was measured after incubation at 37℃, overnight. The agar well diffusion assay was done in triplicates.

Results and Discussion

Phytochemical screening

In this study, *A. indica* essential oils was extracted with 80% (v/v) ethanol. Phytochemical testing was carried out to identify the different phytochemicals present in this extract specifically those that contribute to antimicrobial activity. These included alkaloid, phytosterols, fixed oils and fats,

flavonoids, saponins and terpenoids. The results from this showed that ethanol extracted essential oil from *A. indica* contained alkaloids, fixed fats and oils and terpenoids.

Anitimicrobial activity of A. indica against Gram positive bacteria.

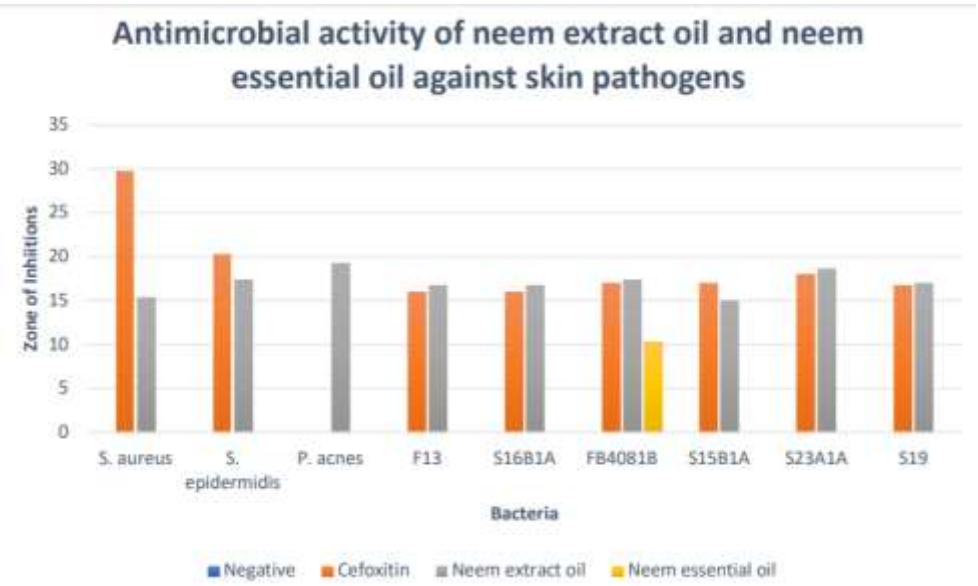

Figure 1. Antimicrobial activity of neem extract oil and neem essential oil against skin pathogens.

The dried leaves of *A. indica* were extracted using 80% ethanol to produce an extracted essential oil. From the results, the essential oils extracted with ethanol were found to be more effective against the bacteria tested compared to commercially prepared *A. indica* essential oil (Figure 1). In general, the extracted neem essential oil was found to be effective against most of the bacteria tested. Only *S. aureus, S. epidermidis* and S15B1(A) showed little or no activity towards the neem extracted oil. According to Ghosh et al. (2016), the commercially available essential oil from *A. indica* was effective against *S. aureus* which is not concurrent with this study where *S. aureus* did not show any zones of inhibition. Studies have reported that *A. indica* contains high concentrations of secondary metabolites including glycosides, alkaloids, compounds play an important role in antimicrobial activity by disrupting the cell membrane or protein synthesis of an organism which could contribute to the effectiveness of *A. indica* against these Gram positive bacteria. This plant has already been used as gel formulation to treat psoriasis which is a skin related disease which caused by the clinically important pathogens (Fatima et al, 2014). These leaves have shown great antimicrobial activity against oral microorganism because of the high content of antimicrobial or bioactive compound such as nimbidin which was effective against Staphylococci and Salmonella species (Kumari et al, 2014).

Conclusions

The antimicrobial effectiveness of *A. indica* were tested against clinically important skin pathogens. From our results, it can be concluded that the antimicrobial activity of *A. indica* was effective against the Gram positive bacteria tested. The results suggest the potential use of *A. indica* ethanol extracted essential oil can be used for development of skincare products in future.

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