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Extraction and Evaluation of Bio - active plant products against HAMRSA

Research Article

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Abstract

Microbial drug resistance is increasing worldwide and is currently considered as major threat to human healthcare. To overcome this problem, several new drugs are being tried to manage and to control the pathogenesis of these microorganisms. Currently, natural sources are being investigated for novel antibiotics. Medicinal plants are good sources of bio-active compounds and chemical structures that have potential beneficial effects. *Azadiracta indica* (Neem) is a multipurpose tree with more health benefits; the plant shows antimicrobial effects. Azadiractin (AZA) is one of the main bio-active compounds present in neem. AZA shows anti malarial activity, and anticancer activity. *Ocimum sanctum* L (Tulasi) has anti-stress and anti-oxidant activity. Eugenol (EUG) bio-active compound of Tulasi, has therapeutic potentials in cardio vascular system, central nervous system, blood bio chemistry, and immune system. The main objective of this study is to check the antibacterial activity of bio-active compounds extracted from neem and Tulasi against HAMRSA.

Keywords: *Azadiracta indica*; Ocimum santum; AZA; Eugenol; ZOI

Introduction

Antibiotic resistance by microbial pathogens is considered a major threat to mankind [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for causing life-threatening diseases in nosocomial infections. The increase in drug resistance worldwide has prompted researchers to find novel antibiotics from natural sources for controlling the spread of infection. Plant-based products are cheaper and can be used as an alternative to synthetic drugs. Screening of medicinal plants for biologically active compounds gives clues to develop newer antimicrobial agents, after which possible chemical manipulation provides new drugs to treat infectious diseases [2].

Neem (*Azadiracta indica*) used in traditional medicine for the treatment of various diseases. Neem has diverse therapeutic applications such as antiviral, antimicrobial, antibactericidal, antipyretic, anticancer, antidiabetic, and immune modulatory activities [3]. The bioactive compounds obtained from neem leaf extract being effective in the treatment of anorexia and skin problems. Azadiractin (AZA) is the most abundant and relevant compound present in *Azadiracta indica* [4].

Ocimum sanctum L. (syn. *O. tenuiflorum* L.) has been extensively used in the Ayurvedic system of medicine for various ailments. The extracts from leaves of ocimum (Tulasi) have to inhibit the growth of gram-positive bacteria and gram-negative bacteria showing antibacterial activity. Tulasi leaves possess antifungal, antiviral activity and anti-tubercular activity and inhibits in-vitro growth of *Mycobacterium tuberculosis* [5].

The bio-active compound of leaves of the *Ocimum sanctum* L. is Eugenol (l-hydroxy-2-methoxy-4-allylbenzene) which is mainly responsible for the therapeutic potentials of cardiovascular system, urinary system, reproductive system, immune system, gastric system, blood biochemistry, central nervous system [6]. The volatile oil of *O. gratissimum* contains mostly thymol and eugenol as bio-active compounds and shows antimicrobial activity [7].

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Materials & Methods

Collection of plant material

Fresh plant material were collected *Azadirachta indica* (Neem), *Ocimum sanctum* L (Tulasi), tree located at Hyderabad, Telangana. The identification was done by using a plant sample from the herbarium (Figure 1).

The moisture content determined by using equation - 1

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100\%$$

Where W_1 = weight of plant leaves before drying, W_2 = weight of plant leaves after drying [8].

The dried leaves were pulverized to coarse powder. The leaves powder was stored in air tight container in dark at room temperature to prevent oxidation and contamination. This was done separately for both Neem leaves and Tulasi leaves.

Extraction of bioactive plant products from Neem and Tulasi leaves

10 grams of leaves powder was placed carefully into the top of the thimble subsequently 150ml of pre-heated solvent was poured into the Soxhlet apparatus and extraction was carried out under n-hexane to ethanol ratio (50:50 v/v) at 70°C for 3 hrs. The obtained extracts were filtered using wattman filter paper and evaporated using rotary evaporator. The extracts were stored in air tight container at 4°C separately for future use.

Azadiractin and Eugenol

These compounds were obtained from commercial suppliers.

Isolation of test organism

Pus samples were collected from Research units, hospitals and cultured on Muller-Hinton agar medium (Figure 2). The presumptive isolates were sub-cultured on MHA medium for another 24hrs. The suspected colonies were used for Identification of *Staphylococcus aureus* on the basis of microscopic examination, cultural characteristics and bio chemical reactions based on Bergey's manual of systemic bacteriology.

Preparation of stock solution

To carry out the antimicrobial activities against selected organism (HAMRSA), the stock solution was prepared by adding 0.5 grams of leaf extracts (Neem & Tulasi) dissolved in 10 ml DMSO (100mg/ml), 0.5 grams of the bioactive compounds (AZA & EUG) dissolved in 10 ml DMSO (100 mg/ml) [9]. The solution was then centrifuged and supernatant was collected in separate tube. These tubes were covered with paraffin wax and stored at 4°C for further work.

Figure 1: Collection of plant materials. A) *Azadiracta indica*; B) *Ocimun sanctum*

Determination of antimicrobial activity by AWD method:

The bio-active plant products (Neem leaves, AZA & Tulasi leaves, Eugenol) were tested for antimicrobial activity against the test organism (HAMRSA) by agar well diffusion method (AWD) (Figure 3). The bacterial strain was grown in nutrient broth for 4 - 8 hrs and the turbidity of the broth culture was adjusted to 0.5 McFarland units ($2X10^6$). Mueller Hinton agar plates were prepared and about 0.1 ml of bacterial cultures was spread uniformly. Agar surface was cut with the help of sterile cork borer having a diameter of 6mm size. After this, 50μl of original stock solution was added by using micropipette in each well. The final concentration of the compounds in the well was 1 mg/ml. The extract was allowed to diffuse in the medium; the prepared plates were left at room temperature for 30 minutes and then incubated at 37°C for 24 hrs. Vancomycin antibiotic (100mg/ml) was used as positive control and Dimethyl Sulfoxide (DMSO) as a negative control [10].

Results & Discussion

The emergence of microbial drug resistant pathogens has become a huge global threat all over the world. The development of effective control measures for the treatment and management of drug-resistance bacteria is the need of the hour.

The antibacterial activity of methanol extracts of bio-active plant products was investigated using agar well diffusion method against HAMRSA (Figure 4). The antibacterial activity of bio-active plant products (neem leaves, AZA & Tulasi leaves, Eugenol) compared with the zone of inhibition of vancomycin. The zone of inhibition obtained by the vancomycin antibiotic was 12mm in diameter, & with neem

Figure 2: Pure culture of HAMRSA.

Figure 3: Bioactive plant products (AZA &EUG).

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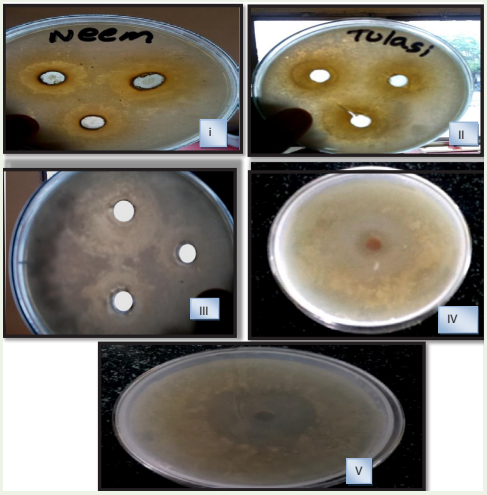

Figure 4: Antibaterial activity of Bioactive plant products by AWD method. **I.** Neem leaves extract; **II.** Tulasi leaves extract; **III.** Bio active plant product – Azadiractin; **IV.** Vancomycin antibiotic; **V.** Bio active plant product - Eugenol

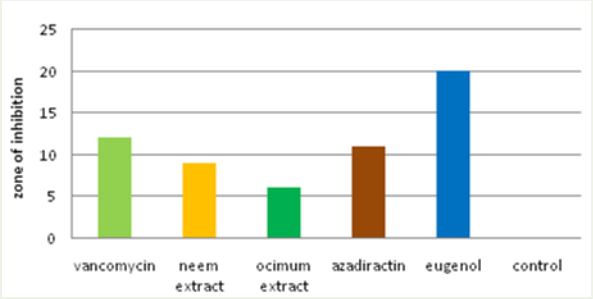

Figure 5: Antibacterial activity of Bio-active plant products against HAMRSA.

leaves extract, it was 9 mm, with Tulasi leaves extract, it was 6 mm, with Azadiractin 11 mm diameter, and with Eugenol 20mm diameter was obtained (Figure 5). This pattern of antimicrobial resistance was very diverse. In similar studies conducted by Venugopal (2021) [9], the zone of inhibition obtained with eugenol against MRSA was 15mm, whereas Coralie P. et al (2018) [11] obtained the zone of inhibition of 3.8 mm with eugenol against *S.aureus.* Maragathavalli et al. (2012) conducted experiments using neem extracts in methanol and ethanol solvents and obtained the same zone of inhibition of 12 mm in both cases [12]. Sivaramakrishnan (2015) used *Ocimum sanctum* extract against *S.aureus* and obtained the zone of inhibition of 25mm [13].

The medicinal plants are rich in secondary metabolites (potential source of drugs) and essential oils of the therapeutic importance, used in traditional system of medicine [14]. *A.indica* showed good antibacterial activity with greater potential of bioactive compounds like Azadiractin shows antimalarial and anticancer activities. Azadiractin has the ability to induce anti- proliferative effect in proteins and involved in cell cycle, transduction and apoptosis [15].

Ocimum sanctum L used as traditional medicine such as analgesic, antimicrobial, anticancer, antidiabetic, antifertility, antiasthamatic and anti stress agents. Several studies have been carried out by Indian scientists and researchers suggest the role of essential oil and Eugenol which is phenolic compound (1 –hydroxy-2 methoxy – 4- methylbenzene) in therapeutic importance. Eugenol possesses membrane stabilizing properties on synaptosomes, Erythrocytes and mast cells, in the treatment of rheumatoid arthirities, antiulcerogenic action.

Conclusion

The traditional medicine is safe when compared to synthetic drugs. The bio active plant products like neem extract, Tulasi extract, Azadiractin and eugenol have antibacterial activity against HAMRSA. Many of the existing synthetic drugs cause numerous side effects; hence plant-based newer drug compounds with minimal side effects can be used for therapeutic applications.

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