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In-Vitro Anti-Bacterial Activities of *Vitex Negundo*

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Abstract – Medicinal plants are commonly used for treating of various diseases, among these plants one is *vitex negundo*. It has antifungal and antibacterial activity. Traditionally, this plant was used in intermittent fever, ulcer, opthalmia, pleurisy etc. The purpose of this study is to evaluate the medicinal value of *vitex negundo*. The extraction was carried out in ethanol and portioned into five sub-fractions in increasing order of polarity from hexane, chloroform, ethylacetate, n-butanol and water, and all are subjected to antimicrobial study. Eight bacterial strains were used in this study in which the ethyl acetate and butanol sub fractions shows highest activity, chloroform fraction shows less activity while aqueous fraction nearly show no activity against the tested species. This results show that some interesting bioactive secondary metabolites can be purified from the active sub-fraction of ethyl acetate and butanol.

Keywords – In-Vitro, Anti-bacterial, *Vitex Negundo*

1. Introduction

Plants were used in different countries for curing various diseases since the dawn of modern civilization. Medicinal plants are widely used in the treatment of different diseases in among human beings which are available in the form of leaf powder, pastes, decoction, infusion and pills etc. [1]. Bioactive compounds are present in medicinal plants offering a rich source of structural biodiversity and played an important role in the innovation of new medicine [2]. Traditionally plant were used in health sectors for a long time, and it advanced in much traditional drug system in various areas of India and Pakistan i.e. Ayurvedic, Siddaha and Tibeetian the world like the Ayurvedic, Siddaha and Tibeetian. In Japan the Kampo system, in Indonesia the Jammu system, in Chinese the medicine system and in Islamic world Hikmat. Medicine in these traditional systems mostly relies on natural resources for the preparation of different drugs and plants are the major constituents which are used in it [3]. Respiratory and anti-inflammatory diseases have been cured by Viola odorata [4] and for the treatment of toothache Syzygium aromaticum (clove) has been used for the purpose of anesthesia [5]. *Vitex negundo* is a wild and small tree [6]. It commonly bears tri or penta-folliate leaves on quadrangular branches. Flowers are bluish-purple in colour. It grows in humid places or along water courses in wastelands and mixed open forests and has been reported to occur in Afghanistan, India, Pakistan, Srilanka, Thailand and Malaysia. The roots are used in rheumatism, dyspepsia, dysentery, and piles and considered as tonic, febrifuge, expectorant, anthelmintic and diuretic. Other compounds reported from the leaves include alkaloids, aromatic acids, flavonoids, iridoids and terpenoids [7]. The extraction of the leaves showed anti-cancer activity against Ehrlich ascites tumor cells [8]. The present study recommended that *vitex negundo* is important plant from medicinal point of view and antimicrobial study of this plant will help in the synthesis of different antibiotics.

2. Materials and Methods

2.1. Collection of Plant Materials

Vitex negundo plant was collected from district sawabi in June 2015, in flowering season. The plant was identified by the faculty of Botany, Hazara University Mansehra, K.P.K, Pakistan.

2.2. Extraction of Crude

The air dried plant material was crushed and soaked in ethanol for one week. The ethanolic extract was combined and evaporated under reduced pressures to afford a gummy residue.

2.3. *Fractionation of Crude Extracts*

The concentrated residue was suspended in a mixture of water and n-butanol and defatted with n-hexane to afford fraction. It was again fractioned with chloroform to afford fraction.

2.4. *Preparation of Stock Solution*

The compound 30 mg were dissolved in Dimethyl sulfoxide (DMSO). It was used as a solvent because it does not show any activity against bacteria therefore DMSO was selected for the present study. Antimicrobial activities are better to perform in winter season because in cool weather the growth of these microorganisms are very slow so there is less chance of any disease or harm from these microorganisms.

2.5. *Preparation of Media*

Antibacterial activity of crude extract and fractions were determined by using well assay methods. Eight bacterial strains *E.coli, pseudomonas aeruginosa, Klesbsiella pneumonia, E.arotovora, B. substilis, A. tunifiction, Salmonella* and one fungal strain *C. albican* were used in antibacterial assay. Muller Hinton agar (Oxoid, UK) media was prepared in conical flask according to directions of manufacturer. The media along with petri dishes, pipette and metallic borer were sterilized in autoclave for 15 minutes at 121◦C and 15 psi pressure. The media was poured into petri dishes under aseptic conditions and allowed to solidify.

2.6. *Well Assay Method*

The modified method of Perez et al. 1990 was followed. Bacterial culture corresponding to 106 µl was inoculated on the surface of the solidified media. Then 6 mm wells were dug in the medium by using sterile metallic borer. Stock solutions of crude extract and fractions in DMSO at concentrations of 1 mg/6mL were prepared and 200 mL from each stock solution was added into respective wells. The zones of inhibition were measured after 24 hours of incubation at 37 ^{0}C. Doxycycline was used as standard. The zones of inhibition of water, Ethyl acetate, Chloroform, Ethanol and n-hexane fractions were compared with zones of inhibition of standard drug (Doxycycline). The amount of growth in each well was measured after 24 hours.

3. Results and Discussion

Our study showed that vitex negundo possesses antibacterial and antifungal activities. The antibacterial study was performed against eight bacterial strains i.e. *E.coli, A. tumeifecian, P. aeruginosa, Salmonella typhi, E. carotovora, B.atropeous, Klebsella* and *B.substilis* and one fungal *C.albican* strain which is shown in Figure 1.

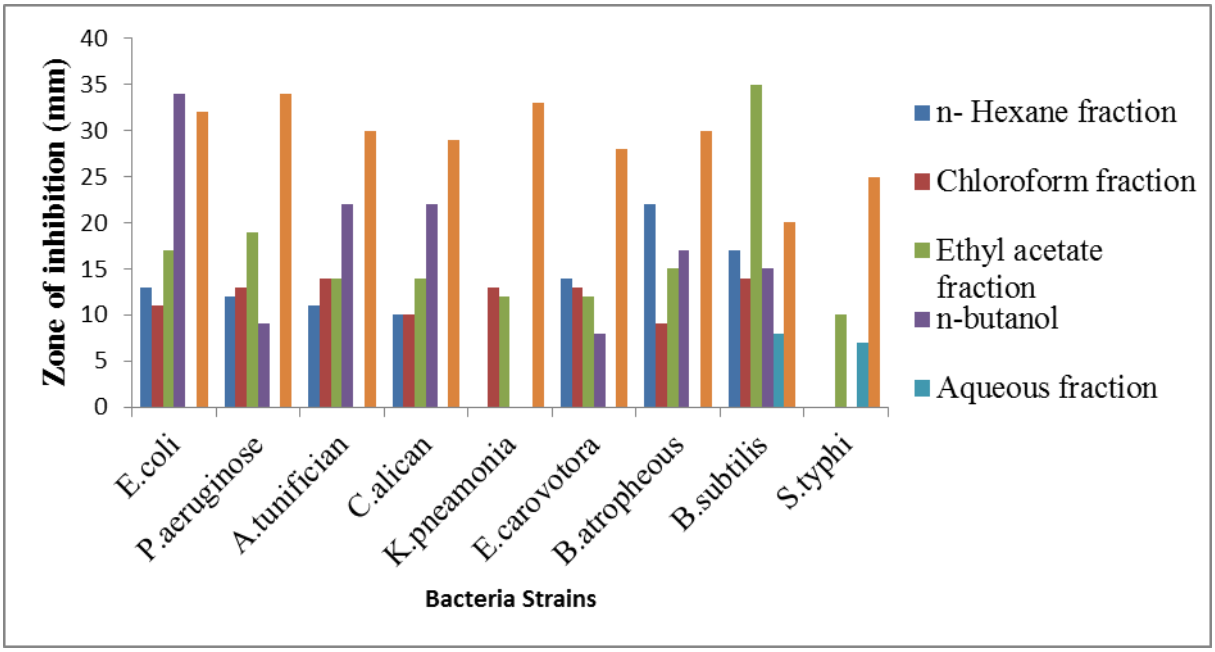

Figure 1. Antimicrobial Activity of different fractions of *vitex negundo*

The highest zone of inhibition against *E.coli* was shown by n-butanol fraction (34 mm) which was even high then the used standard. Some interesting compounds antibacterial agents can be purified from the tested butanol subtraction. Aqueous fraction shows no activity. Ethyl acetate fraction also gives good zone of inhibition and was 17 mm while n-hexane and chloroform fraction almost gave equal result i.e. 13 and 11 mm respectively. Ethyl acetate fraction gives highest zone of inhibition against P. aeruginosa while aqueous fraction shows no activity. N-butanol fraction shows highest zone of inhibition against *A. tunifician* and was 22 mm while aqueous fraction was again inactive. Chloroform and ethyl acetate fractions give 14, 14 mm zone of inhibition. The highest zone of inhibition against *C. albican* was shown by n-butanol (22 mm) while aqueous fraction shows no activity. Ethyl acetate fraction also show good zone of inhibition (10,10 mm) against *K. pneumonia* only chloroform and ethyl acetate fraction were active and gave 13 and 12 mm zone of inhibition respectively while remaining three fractions were inactive against *E.carotovara*, n-hexane, Chloroform and ethyl acetate fractions almost show equal zone of inhibition (14, 13 and 12 mm) respectively. Aqueous fraction was completely inactive against *B. arophoeus*. N-hexane fraction shows good activity and gives good 22 mm zone of inhibition. Ethyl acetate and n-butanol

fraction also give good activity and its zone of inhibition was 15 and 17 mm. Aqueous fraction was again inactive. Ethyl acetate fraction gives the highest zone of inhibition against *B. subtilis* 35 mm which was very high then the standard. It shows that ethyl acetate fraction can be used for the synthesis of more active and bioactive compounds against *S. typhi* only ethyl acetate and aqueous fraction are active while remaining three fractions were completely inactive. The zone of inhibition against *E. coli*, *A. tumeifecian*, *P. aeruginosa*, *Salmonella typhi*, *E. carotovora*, *B. atropeous*, *Klebsella* and *B. subtilis* and C. *albican* are given in Figure 1.

4. Conclusion

Our present study shows that *vitex negundo* is a medicinal plant having antibacterial and antifungal constituents

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