Antimicrobial efficacies of methanol extract of Asteracantha longifolia, Ipomoea aquatica and Enhydra fluctuans against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Micrococcus luteus

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Citation

J Bhakta, P Majumdar, Y Munekage. *Antimicrobial efficacies of methanol extract of Asteracantha longifolia, Ipomoea aquatica and Enhydra fluctuans against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Micrococcus luteus*. The Internet Journal of Alternative Medicine. 2008 Volume 7 Number 2.

Abstract

Present experiment was executed to investigate the antimicrobial efficacy of Asteracantha longifolia, Ipomoea aquatica and Enhydra fluctuans leaf extracts on four pathogenic bacterial strains, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Micrococcus luteus. Methanol extract of herbs were used at four different concentrations for examining the antibacterial efficacy on bacterial strains using agar-cup-diffusion assay and measuring the diameter (mm) of the clear zone around the cup. The mean values of the clear zone in S. aureus were 1.17 to 3.85, 1.02 to 1.45 and 1.14 to 2.58 times greater in A. longifolia, I. aquatica and E. fluctuans, respectively compared to that of the remaining bacteria. This results obviously implied that the methanol extract of three types of herbs have a highest antimicrobial efficacy against the S. aureus over P. aeruginosa, E. coli and M. luteus. Though, no clear zone was found in the methanol extract of the A. longifolia and E. fluctuans againstP. aeruginosa, but the mean values of the clear zone of the I. aquatica extract exhibited 7.4 mm in P. aeruginosa and showed 28 to 118%, 12 to 96% and 200 to 250% higher than that of the remaining two herb extracts. Therefore, it may be concluded that A. longifolia andE. fluctuans herbs have no activity against the P. aeruginosa, whereas I. aquatica exerted a higher magnitude of antimicrobial activity against the tested four types of bacterial species than that of the rest two herb extracts due to compositional variation in the active biomolecules of three herbs.

INTRODUCTION

Herb is an immeasurable wealth of nature not only from the global environmental perspective but also from the medicinal point of view. It plays a significant role ameliorating the disease resistant ability and combating against various unfavourable metabolic activities within the living system. Numerous infectious diseases have been known to be controlled by herbal remedies that have been proved variously since primitive to present history of the mankind. Since time immemorial, man has used various parts of plants in treatment and prevention of various ailments ([1]). Unimaginably unrevealed and unmatched varieties of compounds are present in the diversified herbs on earth. From these points of view, it is obvious that natural products, either in the form of pure compounds or as standardized plant extracts, provide unlimited opportunities to develop a variety of new drugs.

Antibiotic resistance has become a global concern ([2]). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens ([3]). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases ([4]). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections ([5]). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity ([67]). Recent, studies have suggested that several plants species exhibit promising antimicrobial

effects ([8]). Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials ([7]). In recent years, it has been proposed that the herbal extracts may be used as natural antifungal agents to inhibit the growth of foodbrone pathogen ([9]) and as a source of various medicinal agents ([10]).

From the above understanding, the present study has been focused on three wild herbs, Asteracantha longifolia (Fam: Acanthaceae English name: Hygrophila, Local name: kulekhara), Ipomoea aquatica (Fam: Convolvulaceae, English name: water spinach, Local name: Kalmi) and Enhydra fluctuans (Fam: Convolvulaceae, English name: Enhydra, Local name: Helencha), usually found in moist places on the banks of tanks, ditches, paddy fields, etc., throughout India and commonly used as green vegetable. Asteracantha longifolia is a source of the Ayurvedic drug Kokilaaksha and the Unani drug Talimakhana and its seeds are acrid, bitter, aphrodisiac, tonic, sedative, and useful in diseases of the blood ([11]). Enhydra fluctuans has been used in Indian medicine in treatment of nervous ailments, skin diseases and as a laxative ([12]).

However, from the above account, it is obvious that there is no information available about the antimicrobial activity of these herbs. The present investigation was designated to explore the antibacterial activity of methanol extract of the above mentioned herbs.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

Fresh leaf materials of Asteracantha longifolia, Ipomoea aquatica and Enhydra fluctuans were collected from different field of Kalyani, West Bengal State, India. The materials were identified from the taxonomy laboratory, Department of Botany, Kalyani University.

PREPARATION OF PLANT EXTRACT

The shade and air-dried plant material was ground into fine powder (less than 20 mesh) using a stainless-steel grinder machine and stored in pill vials at room temperature (30°C). An aliquot (2 g) of the powdered plant material was extracted with 60 ml (20 ml x 3 times) 80% methanol for 24 h. After centrifugation at 3800g for 30 min, the supernatants were collected and allowed for evaporation to dryness [9]. The dried methanol extract was then dissolved in ethanol (EtOH) to get different concentration.

BACTERIAL STRAINS

On the basis of pathogenic importance, four pathogenic bacterial strains (Escherichia coli, Fam: Enterobacteriaceae, gram-negative; Pseudomonas aeruginosa, Fam: Pseudomonadaceae, gram-negative; Staphylococcus aureus, Fam: Staphylococcaceae, gram-positive; and Micrococcus luteus, Fam: Micrococcaceae, gram-positive) were obtained from the laboratory stock of Department of Botany, Kalyani University. All the Bacterial strains were maintained at 4°C on nutrient agar (Hi-Media, India) slants and cultured at 37°C using same agar media ([9]).

PREPARATION OF BACTERIAL CULTURE

Aseptically, a single colony was transferred to a 100 ml sterilized nutrient broth by a loop, cotton plugged and placed in incubator overnight at 37 °C. After 12 to 18 h of incubation, the cultures were diluted with sterile normal saline to bring the final inoculum size approximately 10^5 to 10^6 cfu/ml ([913]). One ml of the standard bacterial culture was used as inoculation in a nutrient agar petri dish.

ANTIMICROBIAL ACTIVITY ASSAY

One milliliter of cultured bacteria was inoculated on agar plate for preparation of loan of bacteria used for the test of antimicrobial activity following standard methods of agar-cup-diffusion assay. Serial two-fold dilutions (7.5 – 60 mg/ml) of the plant extract were prepared and dispense in each cup (5 mm diameter) of the bacterium seeded/inoculated agar plate @ 30µl/cup. EtOH was used in dissolving the extract; therefore, it is used as control in the assay. Each inoculums and control were employed in triplicate and were incubated at 37 °C for 24 h. antimicrobial activity was assessed based on measurement of the diameter (mm) of the clear zone around the cup.

STATISTICAL ANALYSIS

The triplicate data were expressed as mean ± S.E.M. The results are subjected to an analysis of variance (ANOVA) using the SPSS 10.05 statistical package. If the main effects were disclosed significant at a probability level of $P < 0.05$, the ANOVA was followed by a least significance difference (LSD) test using statistical package EASE and M-STAT.

RESULTS

ANTIMICROBIAL ACTIVITY OF

In vitro, agar well diffusion assay of extract activity against four bacterial strains pronounced a significant species dependant response ($P < 0.05$, ANOVA). No clear zone of the extract was observed in P. aeruginosa and activity was maximum in S. aureus (10.7 0.25 mm in 60 mg/ml) followed by E. coli (10 0.05 mm in 60 mg/ml) and M. luteu (7 0.12 mm in 60 mg/ml). Clear zone of the extract was started at the concentration of 15 mg/ml in both E. coli and S. aureus and increased with increasing concentration, whereas clear zone was found only at extract concentration 60 mg/ml in M. luteu (Figure 1a).

ANTIMICROBIAL ACTIVITY OF

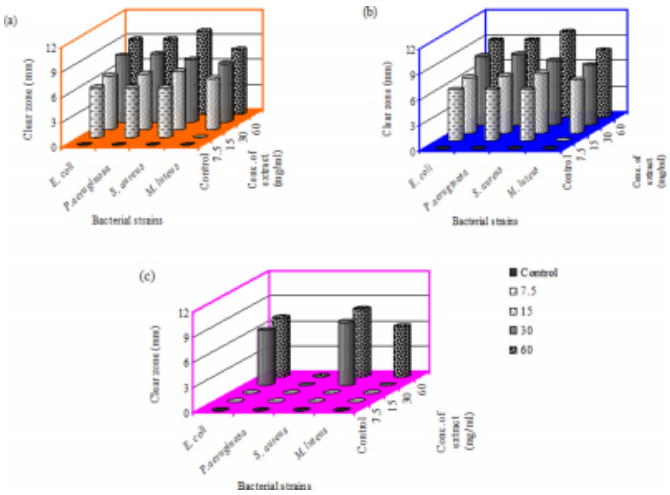
A remarkable and significant antimicrobial activity of I. aquatica extract against all employed bacterial strains was also registered ($P < 0.05$, ANOVA) measuring the clear zone (0 – 10mm) around the extract content well of agar. Mean clear zone was maximum (7.62 0.17 mm) in S. aureus and showed the following order of variation: S. aureus > P. aeruginosa > E. coli > M. luteu. Though clear zone was increased with increasing concentration of extract appearing from lowest concentration (7.5 0.3 mg/ml) in E. coli, P. aeruginosa and S. aureus but in M. luteu the clear zone started at 15 mg/ml concentration (Figure 1b).

ANTIMICROBIAL ACTIVITY OF

There was also a clear variation of antimicrobial activity of E. fluctuans extract in all strains of bacteria tested ($P < 0.05$, ANOVA). Likewise the response of A. longifolia extract, no clear zone was created in P. aeruginosa and a very small clear zone (0 – 8 mm) was also found in the remaining bacterial strains (Figure 1c). Mean value of the clear zone was maximum in S. aureus and followed by E. coli and M. luteu. In E. coli and S. aureus, the clear zone was started from concentration of extract 30 mg/ml, whereas the clear zone appeared in the highest concentration (60 mg/ml) in M. luteu.

Figure 1

Figure 1. Antimicrobial activity of (a), (b) and (c) on four pathogenic bacterial species examined.



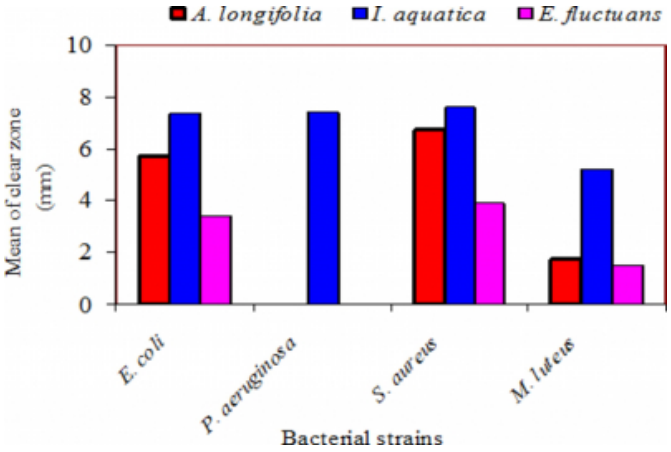
DISCUSSION

Agar well diffusion assay of extract activity showed a variable clear zones, 0 to 10.7 mm, 0 to 10 mm and 0 to 8 mm in different extract concentrations of A. longifolia, I. aquatica and E. fluctuans, respectively. Above obtained results of the present study clearly demonstrated that though there were variations but methanol extract of A. longifolia, I. aquatica and E. fluctuans have an antimicrobial activity against pathogenic bacteria, S. aureus, P. aeruginosa, E. coli and M. luteu. According to Pawar, A. longifolia is variously used in ayurvedic drug (Kokilaaksha), Unani drug (Talimakhana), tonic and sedative preparation as well as it is also used for the treatment of the blood disease ([11]).

The mean values of the clear zone in S. aureus were1.17 to 3.85, 1.02 to 1.45 and 1.14 to 2.58 times greater compared to that of the remaining bacteria in A. longifolia, I. aquatica and E. fluctuans, respectively (Figure 2). Present results obviously implied that the methanol extract of three types of herbs have a higher antimicrobial efficacy against the S. aureus over P. aeruginosa, E. coli and M. luteu.

Figure 2

Figure 2. Mean of clear zone for antimicrobial activity of three herbs against four bacterial species tested.



Though no clear zone was found in the methanol extract of the A. longifolia and E. fluctuans against P. aeruginosa, but the mean values of the clear zone of the I. aquatica extract exhibited 7.4 mm in P. aeruginosa. I. aquatica showed 28 to 118% (in E. coli), 12 to 96% (in S. aureus) and 200 to 250% (in M. luteu) higher antimicrobial efficacy than that of the remaining two herb extracts. Therefore, it may be concluded that I. aquatica exerted a higher magnitude of antimicrobial activity against the tested four types of bacterial species compared to that of the rest two herb extracts. In this context, furthermore it is obvious that A. longifolia and E. fluctuans herbs have no activity against the P. aeruginosa as no clear zone was found.

On account of the above critical appraisal of data, therefore, it could be concluded that (i) I. aquatica has potentially higher antimicrobial efficacy when three herbs species is considered and all tested herbs have a promising antimicrobial efficacy against S. aureus than that of the remaining bacteria examined, (ii) Differential antimicrobial activity of one herb against different bacteria only due to present of different active phytocompounds of an herbs and (iii) Compositional variation of herb active biomolecules in different plants is responsible for exertion of differential antimicrobial activity. It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections ([14]). Secondary plant metabolites (phytochemicals compounds have been extensively investigated as a source of medicinal agents ([10]). Among those antimicrobial compounds, phenolic compounds, terpenoids, and alkaloids' are very important compounds in antimicrobial or antioxidant effects ([15,16,17,18,19,20]). Further research is necessary to determine the different antibacterial compounds from these herbs and their full spectrum of efficacy. However, the present study of in vitro antimicrobial evaluation of these herbs forms a primary platform for further phytochemical and pharmacological studies. These promissory extracts open the possibility of finding new clinically effective antimicrobial compounds.

ACKNOWLEDGEMENTS

Authors are grateful to Prof. P. K. Bandyopadhyay for providing his laboratory facility and also thankful to Prof. A. Kaviraj, Head, Department.of Zoology for his kind support in the experiment. We are also thankful to Professors of the Department of Botany for extending his cooperation in identification of the herbs.

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