Research Article

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STUDIES ON ANTIBACTERIAL EFFECT OF APAMARGA (*ACHYRANTHES ASPERA*) ON MULTI-DRUG RESISTANT CLINICAL ISOLATES

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ABSTRACT
Recent reports on emergence of multidrug resistant bacteria are cause of concern in medical world. Several ayurvedic drugs have been proved to contain the antimicrobial activity. Literature on effect of ayurvedic drugs on multidrug resistant bacterial pathogens is limited. Present study reports the antimicrobial effect of *Achyranthes aspera* (Apamarga) crude extracts on the clinical isolates of multidrug resistant bacteria. The drug was evaluated by using phytochemical tests. Crude extracts of aqueous, methanol, ethanol and chloroform was prepared. Antibacterial activity against clinically isolated multidrug resistant bacteria belonging to groups of bacillus, citrobacter, *E.coli*, klebsiella, proteus and salmonella was tested. The drug showed highest efficacy against Bacillus organism while least effectiveness on Proteus spp bacteria. Results of the study conclude that the medicinal plant *A. aspera* might be useful against multidrug resistance in pathogens of clinical importance.
Key words: Apamarga, *Achyranthes aspera*, anti-bacterial activity, multidrug resistant pathogens.

INTRODUCTION

Infectious diseases are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries like India, due to the relative unavailability of medicines and the emergence of widespread drug resistance. Research on new antimicrobial substances must therefore be continued and all possible strategies should be explored. India is country with rich source of medicinal plants and wealthy knowledge of their usage for various infections mentioned in the many texts of Ayurveda. Medicinal plants mentioned in ayurvedic texts need to be evaluated using modern *in vitro* antimicrobial assays.

Although the introduction and successful development of this therapeutic class of antibacterial agents represents a significant medical achievement, this success has also led to complacency within both greater society and the scientific community with regard to the development of bacterial resistance. In spite of the increased understanding of the factors contributing to the development of resistance over the last 60 years, the extent of this problem has not decreased with time and is currently among the strongest global threats to the treatment of infectious disease. The degree to which this problem has progressed is demonstrated by the fact that resistance has developed against all available classes of antibiotics. Some important examples include penicillin-resistant *Streptococcus pneumoniae,* vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus,* multi-resistant salmonellae, and multi-resistant *Mycobacterium tuberculosis.*

Drugs acting on microbial agents have been mentioned in Ayurvedic texts as Krimighna Dravyas. Evaluation of medicinal plants as an alternative to the antibiotics has raised great interest in scientific community worldwide. Recently several reports have been published regarding screening of medicinal plants for their antibacterial activity against standard bacterial strains.

Achyranthes aspera L., has been reported to have anti-fertility[1], anti-diabetic[2], protyroidic[3], anti-inflammatory and anti-arthritic activity[4], immunomodulator[5], spermicidal[6] and antiparastic[7] effects. In Ayurvedic textual references several dravyas have been mentioned as having antimicrobial activity like, Apamarga (*Achyranthes aspera*) (Raja Nighantu; Bhava Prakash Nighantu).

Reports are scanty with studies on screening against pathogenic bacteria isolated from clinical infections of humans and animals showing resistance to commonly used antibacterial agents. In view of the failures in the therapeutic usage of antimicrobials the present research was planned to evaluate efficacy of (*Achyranthes aspera*) apamarga, a plant referred in Ayurvedic texts as 'krimighna' on clinically isolated multi-drug resistant bacteria.

MATERIALS AND METHODS

Collection and identification of plant material

Based on the textual references in Ayurveda and the available recent literatures, Apamarga (*A.aspera*) was considered for antimicrobial activity in the present study. Their authenticity was identified and confirmed using morphological and anatomical features at Department of Botany, College of Basic Sciences, Punjab Agriculture University, Ludhiana, Punjab. A voucher specimen was deposited at the Herbarium of the Babe Ke Ayurvedic College and Hospital, Daudhar, Moga, Punjab (Herbarium Voucher No.BKACHD/DG/UP/2007/1).

Preliminary phytochemical analysis

Preliminary phytochemical analysis was done for qualitative assessment of phytoconstituents as per the standard protocols mentioned previously[8]. Grounded dried powder was subjected to tests for detection of tannins, alkaloids, saponins, cardiac glycosides, anthraquinone glycosides, steroids: (terpenoids and flavonoids), resins and volatile oils.

Preparation of crude drug extract from plant material

Aqueous extraction of the drug was done by subjecting 20 grams of air dried powder to soxhlet extraction continuously for 12 hrs. The extract was air dried under mild heat of 50^0 C till moisture completely evaporates. Ethanol, methanol and chloroform extractions were accomplished following the procedures reported elsewhere with minor modification suiting to the laboratory conditions. For each extraction the ground powder was weighed 100 gms and immersed in ten times of 80% ethanol or methanol or chloroform and allowed for cold percolation on magnetic stirrer for 24 hrs. The extracts were first filtered through double layer of muslin cloth and then with Whatman No.1 filter paper. The filtrate was air dried under low heat of 50^0 C and stored at -20^0 C till further use.

Bacterial strains

Standard bacterial and fungal strains were procured from Institute of Microbial Technology (IMTEC), Chandigarh, Department of Animal Biotechnology, Guru Anged Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana and Department of Microbiology, Christian Medical College, Ludhiana. Bacterial isolates were *Klebsiella* spp. *Proteus* spp. Salmonella spp. Citrobacter spp. *E.coli* and *Bacillus spp*.

Isolation and characterization of pathogenic bacteria from clinical samples

Pathogenic bacteria were isolated from clinical samples from different systems like, oral cavity, gastrointestinal and respiratory tract, urinary and genital infections. Pathogens were isolated from clinical samples collected at Department of Medical Microbiology, Christian Medical College (CMC), Ludhiana, Punjab. Isolated bacteria were identified based on colony morphology and staining characters as per the standard protocols mentioned in Berge's Manual of Systematic Bacteriology[9].

Selection of multi-drug resistant bacteria

Clinical isolates were subjected to antibiotic sensitivity test following standard disc diffusion method[10]. Isolates having resistance to more than six of the tested antibiotic discs were considered as multi-drug resistant and were selected for further analysis.

Table 1: Biochemical parameters to identify the members of Enterobacteriaceae on selective/indicator media

	Salmonella	*Proteous*	*E.coli*	*Klebsiella*	*Citrobacter*
Lactose	-	-	+	+	+/-
Sucrose	-	-	+/-	+	-
Xylose	+	+	+	+	+
H_2S	+	+	-	-	-
Lysine	+	-	+	+	-
MacConkey agar	Pale colonies	Pale colonies	Bright pink	Pink	Pink
Brilliant green agar	Red colonies	Red colonies	Yellow green	Yellow green	Green
XLD agar	Red/black centre	Yellow/black centre	Yellow	Yellow	Yellow

Table 2: Yield of crude extract of *Achyranthes aspera*

Solvent used for drug extract	Yield of crude extract (%)
Aqueous	7.9
Ethanol	5.5
Methanol	4.8
Chloroform	3.5

Table 3: Antibacterial activity (percent inhibition) of crude plant extract of *Achyranthes aspera* against selected bacterial isolates

Plant material	Bacillus	Citrobacter	*E.coli*	Klebsiella	Proteous	Salmonella
Aqueous	50%	0%	10%	0%	0%	10%
Ethanol	100%	0%	10%	0%	0%	10%
Methanol	100%	75%	50%	10%	0%	25%
Chloroform	100%	50%	50%	10%	0%	25%
Bacteria control	0%	0%	0%	0%	0%	0%
DMSO control	0%	0%	0%	0%	0%	0%

Testing the antibacterial activity of plant extracts

Antibacterial activity of the plant extracts was done following the dilution method mentioned elsewhere. Bacterial strains were grown to exponential phase in Muller-Hinton broth at 37^0C for 18 hrs and adjusted to a final density of 10^8 CFU/ml by diluting fresh cultures and comparing with McFarland density. Antimicrobial activity of the different plant extracts was determined by a viable colony count. Nine hundred microliter of a suspension of 10^8 bacteria/ml was added to 100 µl of plant extract and incubated at 37^0C in shaker incubator for 60 min. Similarly, positive bacteria control was kept in

each experiment in which isolates were diluted in DMSO. Following incubation serial tenfold dilution was made in Nutrient Broth (10^{-1} through 10^{-4}) and suspension from the last dilution was spread on to MHA plates and incubated at 37^0 C 24 hrs. Following incubation number of colonies in each plate was counted as colony forming unit (CFU) per plate. The effectiveness of the plant at killing bacterial isolates was expressed as percentage inhibition of colony growth (i.e. percentage of bacteria killed) compared to the control.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis for tannins alkaloids, saponins, cardiac glycosides, steroids: terpenoids and flavonoids were conducted to confirm the authenticity of the drugs collected for the present study. All the results were in accordance with the previously published standard observations. Characteristics of bacterial species isolated in the study showed standard biochemical reactions (Table 1). Yield of crude extract after extraction with various solvents are presented in Table 2.

Aqueous extract of apamarga showed moderate inhibition on Bacillus, negligible inhibition on *E.coli* and no inhibition on citrobacter, klebsiella and proteus organisms. Ethanol extract showed complete inhibition on Bacillus, negligible inhibition on *E.coli* and no inhibition on citrobacter, klebsiella and proteus organism. Methanol extract showed complete inhibition on bacillus, significant inhibition on citrobacter, moderate inhibition on *E.coli*, negligible inhibition on klebsiella and no inhibition on proteus organism. Chloroform extract showed complete inhibition on bacillus, moderate inhibition on citrobacter and *E.coli*, negligible on klebsiella and no inhibition on Proteus organisms. Results are presented in Table 3.

Current research on natural molecules and products primarily focuses on plants since they have been extensively described in Ayurvedic literatures and practiced routinely in Indian system of medicine. Ancient ayurvedic textual references of apamarga are on its sahasraveerya, krimighna, rakshoghna, rasayana, harshoghna, vishaghna and rojopravarthaka. It has been included in charaka samhimta as krimighna mahakashaya. In the present study ethanol, methanol and chloroform extract of apamarga showed the 100% inhibition on Bacillus organism where as aqueous extract showed 50% inhibition which is in partial agreement with the previous observations that ethanol extract has antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Shigella dysentriae*[11]. In contrast to the present observations one of the previous report could detect the antibacterial activity of crude methanol extract against *E.coli, B.subtilis, Yersinia enterocolitica* and *Candida albicans*[12]. Interestingly, none of the apamarga extracts showed any inhibitory activity on proteus bacteria while showed negligible inhibition on salmonella and klebsiella. This is the first report on effect of apamarga on Salmonella, proteus and klebsiella bacteria. In a similar study chloroform and methanol root and shoot extracts of *A. aspera* were shown to have antibacterial activity against Klebsiella sp and ether extract of root against *B.subtilis*[13]. Results suggest that extract has significant antibacterial and antifungal activity. The results of the present study confirm the explanations of ayurvedic claims as krimighna.

Results of the study conclude that the medicinal plant *Achyranthes aspera* mentioned in Ayurvedic literature is having huge potential for use as antibacterial agents especially in a situation where available drugs are not effective following development of multidrug resistance in pathogens of clinical importance. However it should be noted that these *in vitro* results may not translate into clinical effectiveness. Further, studies are needed to understand the phytoconstituents responsible for the observed antibacterial activity and to standardize the dosage and administration schedules for successful clinical applications in medical practice.

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