IN-VITRO ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *ACORUS CALAMUS*

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ABSTRACT: The aqueous and ethanolic extracts of *Acorus calamus* was evaluated for antibacterial activity against clinically important bacteria viz.. *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 443), *Proteus mirabilis* (MTCC 1429), *Pseudomonas aeruginosa* (MTCC 424). The in-vitro antibacterial activity was performed by agar well diffusion method. The ethanolic extracts of *A. calamus* was active against all the investigated bacterial strains while aqueous extract was totally inactive against the studied gram negative bacterial strains (*E. coli*, *P. mirabilis* and *P. aeruginosa*) and showed moderate antibacterial activity against gram positive bacteria *B. subtilis* and *Stap. aureus* only at high concentration (200µl). However, further works are needed to identify the chemical nature of the active substances as well as their modes of actions on the bacterial cells and their roles in disease curing.

Key words: antibacterial activity; aqueous extract; ethanolic extract; *Acorus calamus*

INTRODUCTION

Antibiotic resistance has become a global concern (Westh, et al., 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Bandow, et al., 2003). 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 (Rojas, et al., 1992). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). 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 (Murugaian, et al., 2009; Srikumar, et al., 2007).

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. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials.

Acorus calamus Linn. belongs to the family Acoraceae, commonly known as "sweet flag" or "calamus", is a semiaquatic, perennial, aromatic herb with creeping rhizomes. The plant is found in the northern temperate and subtropical regions of Asia. Many ethnomedicinal and ethnobotanical uses have been ascribed to the rhizomes of the plant. *A. calamus* Linn. has been used as traditional Indian prescriptions for its beneficial effects on antiproliferative (Mehrotra, et al., 2003), antidiarrhoeal (Balentine & Albano, 1999), antioxidant (Manikandan, et al., 2005) and hypolipidemic activity (Parab & Mengi, 2002). The aim of the present study was to screen for the aqueous and ethanolic extracts of *A. calamus* that could be useful for the development of new tools as antibacterial agents for the control of infectious diseases.

MATERIAL AND METHODS

Collection of Plant Material

Acorus calamus

A. calamus was purchased from Tampcol Ltd, Chennai, India. It was identified and authenticated by The Director of Centre for Advanced Studies on Botany, University of Madras, India.

Preparation of aqueous and ethanolic extracts

The shade dried rhizome (100 g) of *A. calamus* Linn was ground to coarse powder, *placed* in a soxhlet extractor containing 70% of ethanol and resulting extract was concentrated in a rotatory evaporator under reduced pressure. Aqueous extract were obtained by maceration for 24 hrs. Extracts were stored in refrigerator (4°C) until further use.

Bacterial strain

Bacterial strains namely *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 443), *Proteus mirabilis* (MTCC 1429), *Pseudomonas aeruginosa* (MTCC 424) were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. Bacterial strains were maintained at 4°C on nutrient agar slants.

Well Diffusion method

Antibacterial activity of the *A. calamus* extracts were tested using Well diffusion method (Gandhiraja, et al., 2009). The organisms were maintained on an agar slope at 4 °C were sub-cultured for 24 h before use. Isolated colonies of the bacteria were placed into individual tubes containing 5 ml of sterile brain-heart infusion broth (BHIB) (Himedia) and incubated at 37 °C, before adjusting the tubes with 0.5 McFarland Units using sterile BHIB. Turbidity was also verified using spectrophotometric comparison with a 0.5 McFarland standard. The dilutions were used within 15 min of preparation and gently vortexed prior to use. The standardized inoculums (1–2 × 10^7 Colony forming unit (cfu)/ml 0.5 McFarland standards) was introduced on the surface of sterile Mueller-Hinton agar (pH 7.2–7.4) plates using sterile cotton swabs (by streak plate method). The inoculations were done along three axes in a rolling motion to ensure uniform bacterial distribution and growth. Wells were made on the agar surface with 5mm cork borer.

Both aqueous and ethanolic extracts were dissolved in sterile distilled water to form dilution such as 50µg, 100µg and 200µg. Then extracts were introduced into the well using sterile syringe. Controls wells were maintained by introducing extract solvents (water and ethanol) into the well. The plates were incubated at 37ºC for 24 hours. The plates were observed for the zone clearance around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm). The experiment was done three times and the mean values were tabulated.

Result & Discussion

Phytonutrients are plant-derived, naturally occurring compounds possessing antimicrobial, activity (Srikumar, et al., 2007). *In vitro* antibacterial activities of aqueous and ethanolic extracts of *A. calamus* are tabulated (Table. 1). The zone of inhibition obtained was dose dependent and ethanolic extract was found to be the most effective antibacterial agent as compared to the aqueous extract. This may be due to the better solubility of the active components in organic solvent (Lin, et al., 1999). These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay. The growth media also seem to play an important role in the determination of the antibacterial activity. Based on the report by Lin et al. Muller-Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study (Lin, et al., 1999). The ethanolic extract of *A. calamus* was active against both gram positive and gram negative bacterial strains. Aqueous extract of *A. calamus* was totally inactive against the studied gram negative bacteria and showed moderate antibacterial activity against gram positive bacteria only at high concentration (200µl). This is in agreement with previous reports that plant extracts are more active against gram-positive bacteria than gram-negative bacteria (Rabe & Staden, 1997). *B. subtilis* was the most susceptible gram-positive bacteria followed by *S. aureus*. *P. aeruginosa* was the most resistant gram-negative bacterial strain. The mechanisms behind the antibacterial activity are complex to understand and could be attributed to either inhibiting the cell division or to damaging the cell walls of bacteria; which however requires to be investigated in detail.

The results of present study supports the traditional usage of the studied plants and suggests that *A. calamus* extracts possess compounds with antibacterial properties that can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active ethanolic extract can be subjected to isolation of the therapeutic antibacterials and carry out further pharmacological evaluation.

Table 1. *In vitro* Antibacterial activity of aqueous and ethanolic extracts of *Acorus calamus*

Bacteria	*A. calamus* (50µl)		*A. calamus* (100µl)		*A. calamus* (200µl)	
	Zone of inhibition (mm)*					
	Ae	Ee	Ae	Ee	Ae	Ee
Bacillus subtilis	-	10	-	18	6	30
Staphylococcus aureus	-	7	-	15	4	28
Escherichia coli	-	5	-	12	-	22
Proteus mirabilis	-	5	-	9	-	20
Pseudomonas aeruginosa	-	3	-	6	-	11

*Values are mean of three replicates; Ae: aqueous extract; Ee: ethanolic extract;'-': no zone of inhibition.

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