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IN VITRO EVALUATION OF THE ANTIBACTERIAL EFFICACY OF *ACORUS CALAMUS* LINN. ROOT EXTRACT AGAINST SELECTED GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIAL PATHOGENS

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ABSTRACT

The *in vitro* antibacterial efficacy of the aqueous root extract of *Acorus calamus* Linn. was evaluated against four bacterial strains, namely *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, and *Staphylococcus aureus*, at concentrations ranging from 10% to 100%. The extract exhibited the highest antibacterial activity against *S. aureus*, producing an inhibition zone of 34.0 mm at 100% concentration, followed by *P. vulgaris* (32.0 mm at 100%), *B. subtilis* (28.0 mm at 100%), and *E. coli* which showed the lowest inhibition (23.0 mm at 60%). When compared with standard antibiotics, tetracycline (25 mg/mL) exhibited inhibition zones of 30.0 mm for *E. coli*, 18.0 mm for *P. vulgaris*, 26.0 mm for *S. aureus*, and 23.0 mm for *B. subtilis*. Similarly, chloramphenicol (25 mg/mL) showed maximum inhibition against *P. vulgaris* (31.0 mm), followed by *E. coli* (30.0 mm), while both *S. aureus* and *B. subtilis* recorded inhibition zones of 28.0 mm each. These results indicate that the aqueous root extract of *Acorus calamus* possesses significant antibacterial potential, particularly against *Staphylococcus aureus* and *Proteus vulgaris*, suggesting its possible use as a natural antimicrobial agent.

KEYWORDS: *Acorus calamus Linn.*, bacteria, antibacterial activity, tetracyclin and chloramphenicol.

INTRODUCTION

Plants are recognized as major sources for the development of antimicrobial agents and have been utilized in the treatment of human and animal diseases for centuries (Mon *et al*., 2008). Infectious diseases remain a leading global health concern, accounting for nearly half of all deaths in tropical regions. Notably, deaths due to infectious diseases, which ranked fifth in 1981, rose to the third leading cause by 1992—an alarming increase of 58% (Venkataswamy *et al*., 2010).

Among pathogenic microorganisms, *Staphylococcus aureus* has gained particular attention due to the emergence of multiple drug-resistant strains, which pose serious challenges to clinical treatment. In addition to causing a variety of infections, *S. aureus* is also a major etiological agent of food poisoning and exhibits remarkable resistance to heat, desiccation, and radiation. According to the World Health Organization (2002), approximately 62–80% of the world's population still depends on traditional medicines for the treatment of common ailments. The continuous rise in multidrug-resistant microbial strains and the occurrence of pathogens with reduced susceptibility to conventional antibiotics have created an urgent need for novel antimicrobial agents (Rajesh *et al*., 2007). Moreover, in developing countries, synthetic drugs are often costly, limited in availability, and sometimes associated with adulteration and adverse effects. Consequently, there is an increasing interest in identifying new infection-control strategies based on natural products (Sieradzki *et al*., 1999).

Medicinal plants have long been a vital source of bioactive compounds contributing to human health, with their therapeutic use traceable to the earliest stages of civilization (Saroj, 2019). Natural products derived from plants exhibit a wide range of biological activities and play significant roles in drug discovery (Galal *et al*., 1991; Elhoussine *et al*., 2010). Higher plants synthesize hundreds to thousands of diverse secondary metabolites with various biological and pharmacological properties (Hamburger and Hostettmann, 1991). Many of these phytochemicals possess antimicrobial properties effective against both plant and human pathogens (Lee *et al*., 1998). The increasing prevalence of antibiotic resistance has renewed scientific interest in exploring plants as potential sources of antibacterial compounds (Nagendra *et al*., 2010; Kiran *et al*., 2011). In this context, the present study aims to evaluate

the *in vitro* antibacterial activity of the aqueous root extract of *Acorus calamus* Linn. Belongs to Family Acoraceae against four bacterial species.

MATERIALS AND METHODS

Plant Material: Healthy and disease-free roots of *Acorus calamus* Linn. were collected from Mysore, Karnataka, India. The collected roots were thoroughly washed two to three times with running tap water, followed by a final rinse with sterile distilled water to remove adhering soil and debris. The cleaned root materials were air-dried under shade on sterile blotting paper at room temperature. The dried samples were subsequently used for extraction.

Aqueous extraction: Fifty grams of thoroughly washed roots of *Acorus calamus* Linn. were macerated with 50 mL of sterile distilled water using a Waring blender (Waring International, New Hartford, CT, USA) for 10 minutes. The resulting macerate was filtered through a double layer of sterile muslin cloth to remove coarse debris, followed by centrifugation at 4,000 × *g* for 30 minutes. The supernatant was then filtered through Whatman No. 1 filter paper and sterilized by autoclaving at 121°C for 15 minutes. The sterile aqueous extract was stored aseptically in a brown glass bottle at 5°C until further use (Lalitha *et al.*, 2011).

Test pathogens: Four bacterial species, namely *Escherichia coli* (Gram-negative), *Proteus vulgaris* (Gram-negative), *Bacillus subtilis* (Gram-positive), and *Staphylococcus aureus* (Gram-positive), were obtained from the Research Centre, Pooja Bhagavat Memorial Mahajana Postgraduate Centre, K.R.S. Road, Metagalli, Mysore, India. The bacterial cultures were sub-cultured on nutrient agar medium and incubated at 37°C for 24 hours to obtain fresh growth. Following incubation, the cultures were preserved aseptically at low temperature until further use.

Preparation of Inoculum

Preparation of standard culture inoculums of test organism: All the test bacterial species were inoculated individually into 2 mL of sterile nutrient broth and incubated at 37°C for 24 hours to obtain actively growing cultures. The turbidity of each bacterial suspension was then adjusted to match the 0.5 McFarland standard, corresponding to an approximate cell density of 1×10^8 CFU/mL, as recommended by the World Health Organization (Bole *et al.*, 2010). The standardized inocula were used immediately for evaluating the antibacterial activity of the aqueous root extract of *Acorus calamus* Linn.

Antibacterial assay

Agar cup diffusion method: The antibacterial activity of the aqueous root extract of *Acorus calamus* Linn. was evaluated using the agar well diffusion method as described by Joshi *et al.* (2009) with slight modifications. Overnight cultures of *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, and *Staphylococcus aureus* were used as test organisms. Each bacterial suspension was evenly spread on sterile Petri plates containing solidified nutrient agar medium using a sterile cotton swab to ensure uniform lawn growth.

After the medium had set, wells of 5.0 mm diameter were aseptically bored into the agar using a sterile cork borer. Five wells were prepared in each plate—one at the center and four along the periphery. The agar plugs were carefully removed using a flamed and cooled wire loop to prevent contamination.

Different concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) of the aqueous root extract were prepared using sterile distilled water, and 50 µL of each concentration was carefully dispensed into the respective wells using a micropipette. The plates were then incubated at 37°C for 24 hours. Following incubation, the antibacterial activity was assessed by measuring the diameter of the inhibition zones (in millimeters) around each well.

Each treatment was performed in ten replicates to ensure statistical reliability. Standard antibiotics, tetracycline (25 mg/mL) and chloramphenicol (25 mg/mL), were included as positive controls to compare the efficacy of the plant extract against the test organisms. The minimum inhibitory concentration (MIC) of the extract was also determined for all bacterial species tested.

Statistical Analysis: The experimental data were subjected to statistical analysis using analysis of variance (ANOVA). Percentage data were arcsine-transformed prior to analysis to normalize the distribution. Mean comparisons were performed using Tukey's Honestly Significant Difference (HSD) test at a 5% probability level ($P \leq 0.05$) to determine significant differences among treatments.

RESULT

The antibacterial activity of the aqueous root extract of *Acorus calamus* Linn. was evaluated against four bacterial species — *Staphylococcus aureus*, *Proteus vulgaris*, *Bacillus subtilis*, and *Escherichia coli* — at concentrations ranging from 10% to 100%.

Among the tested organisms, *S. aureus* exhibited the highest susceptibility to the extract, showing a maximum inhibition zone of 34.0 mm at 100% concentration, followed by 31.0 mm at 90%, 29.0 mm at 80%, and 26.0 mm at 70% concentration. The lowest inhibition was observed at 10% (13.0 mm) and 20% (16.0 mm) concentrations.

In *P. vulgaris*, the maximum inhibition zone measured 32.0 mm at 100% concentration, followed by 28.0 mm at 90%, 23.0 mm at 80%, and 22.0 mm at 70%. Moderate inhibition was observed at lower concentrations (10–50%), indicating a dose-dependent antibacterial effect.

B. subtilis exhibited moderate antibacterial activity, with inhibition zones of 28.0 mm, 25.0 mm, 21.0 mm, and 17.0 mm at 100%, 90%, 80%, and 70% concentrations, respectively.

The least activity was recorded against *E. coli*, which showed inhibition zones of 23.0 mm across 50–100% concentrations. At lower concentrations, the inhibition zones were 13.0 mm (10%), 15.0 mm (20%), 17.0 mm (30%), and 20.0 mm (40%), respectively.

When compared with standard antibiotics, tetracycline (25 mg/mL) produced inhibition zones of 30.0 mm (*E. coli*), 21.0 mm (*P. vulgaris*), 26.0 mm (*S. aureus*), and 23.0 mm (*B. subtilis*). Similarly, chloramphenicol (25 mg/mL) exhibited inhibition zones of 30.0 mm (*E. coli*), 31.0 mm (*P. vulgaris*), 28.0 mm (*S. aureus*), and 28.0 mm (*B. subtilis*).

The minimum inhibitory concentration (MIC) of the aqueous root extract was determined to be 80% for *E. coli* and 100% for *P. vulgaris*, *S. aureus*, and *B. subtilis* (Table 1).

Table 1: Antibacterial activity of aqueous extract of *Acorus calamus Linn.* L. (Root) against four important species of bacteria.

Bacteria	Inhibition(mm) Concentration of the Aqueous extract											Standard Antibiotics	
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	MIC	Tetracycline 25mg	Chloramphenicol 25mg
E. coli	13.0^{a} ±0.0	15.0^{b} ±0.0	17.0^{c} ±0.0	20.0^{d} ±0.0	23.0^{e} ±0.0	23.0^{e} ±0.0	23.0^{e} ±0.0	23.0^{e} ±0.0	23.0^{e} ±0.1	23.0^{e} ±0.0	50%	30.0^{f} ±0.0	30.0^{f} ±0.0
P. vulgaris	0.0^{a} ±0.0	6.0^{b} ±0.0	13.0^{c} ±0.0	15.0^{d} ±0.0	15.0^{d} ±0.0	17.0^{e} ±0.1	22.0^{f} ±0.0	23.0^{g} ±0.0	28.0^{h} ±0.1	32.0^{j} ±0.0	90%	28.0^{h} ±0.0	31.0^{i} ±0.0
S. aureus	13.0^{a} ±0.0	16.0^{b} ±0.0	18.0^{c} ±0.1	20.0^{d} ±0.0	20.0^{d} ±0.1	22.0^{e} ±0.1	26.0^{f} ±0.1	29.0^{h} ±0.0	31.0^{i} ±0.0	34.0^{j} ±0.1	100%	26.0^{f} ±0.0	28.0^{g} ±0.0
B. subtilis	0.0^{a} ±0.1	8.0^{b} ±0.0	11.0^{c} ±0.0	13.0^{d} ±0.0	13.0^{d} ±0.0	15.0^{e} ±0.0	17.0^{f} ±0.0	21.0^{g} ±0.1	25.0^{i} ±0.0	28.0^{j} ±0.1	100%	23.0^{h} ±0.0	28.0^{j} ±0.0

- Values are the mean of five replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

DISCUSSION

The emergence of multidrug-resistant microbial pathogens has become a critical global health concern in recent decades (Peng *et al.*, 2006). This problem is largely attributed to the indiscriminate and repetitive use of antimicrobial agents, often resulting from inadequate or incomplete treatment of infectious diseases (Shariff, 2001). Microorganisms have evolved several adaptive mechanisms to counteract the effects of antibiotics, including the production of enzymes that inactivate or degrade antimicrobial compounds, thereby rendering them ineffective (Ritch *et al.*, 1999).

Nature has long served as a prolific source of therapeutic agents, with many modern pharmaceuticals being derived directly or indirectly from natural products traditionally used in folk medicine (Abbas *et al.*, 2008). Medicinal plants, in particular, have played a vital role in healthcare systems across the world for centuries, and their use continues to grow due to their proven efficacy and minimal side effects compared to synthetic drugs (Cowan, 1999). In recent years, scientific interest in medicinal plants has increased substantially, driven by the global rise of antibiotic resistance and the need for safer, more effective treatment options.

Plant-derived antimicrobials represent a vast and largely untapped reservoir of bioactive compounds with potential therapeutic applications. The findings of the present study demonstrate that the aqueous root extract of *Acorus calamus* Linn. exhibits significant antibacterial activity against both Gram-positive and Gram-negative bacteria, indicating its potential as a natural source of antimicrobial agents.

Based on these observations, it may be concluded that the roots of *A. calamus* contain potent bioactive compounds responsible for antibacterial activity. Further phytochemical investigations are necessary to isolate, characterize, and identify the specific active constituents responsible for this activity. Additionally, evaluating the efficacy of these compounds against a broader range of human and plant pathogens will be essential to determine their potential for development into novel antimicrobial agents.

CONCLUSION

The results of the present investigation revealed that the aqueous root extract of *Acorus calamus* Linn. exhibited significant antibacterial activity against all four tested bacterial species, indicating the presence of potent bioactive constituents with inhibitory effects on both Gram-positive and Gram-negative bacteria. Among the tested organisms,

Staphylococcus aureus demonstrated the highest susceptibility, followed by *Proteus vulgaris*, *Bacillus subtilis*, and *Escherichia coli*. The observed antibacterial efficacy across different concentrations suggests that *A. calamus* roots possess broad-spectrum antimicrobial potential.

Although the aqueous extract demonstrated promising results, further research is essential to explore the antibacterial properties of extracts prepared using different solvents to identify compounds with higher efficacy. Comprehensive phytochemical investigations should be undertaken to isolate, purify, and characterize the specific bioactive molecules responsible for the observed antibacterial activity. Structural elucidation of these compounds using advanced spectroscopic and chromatographic techniques will contribute to understanding their mechanism of action.

Standardization of extraction protocols and evaluation of the pharmacological safety and stability of these compounds will be necessary for potential therapeutic applications. Overall, *Acorus calamus* Linn. represents a valuable natural resource for the development of novel antimicrobial agents to combat multidrug-resistant bacterial pathogens.

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