Research Article

Comparative Analysis on Antibacterial Activity of Commercially Available Antibiotics and Extracts of *Acorus calamus* (Linn) on Wound Infection Causing Pathogens

V.Vishnupriya, Dr.B.Bharathi*, K.Aswini
Department of Microbiology, MMM College of Health Sciences, Chennai, India.
***Corresponding author's E-mail:** bharathikarnan@gmail.com

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ABSTRACT

The antibacterial drugs are said to be costlier and have more side effects. Moreover multiple drug resistant strains are on the raise in this era and thus complicating treatment. On the other hand, herbal preparations are comparatively cheaper and have lesser side effects. So, herbal preparations can be used as medicine for the treatment of infections caused by bacteria and fungi. Medicinal plants have been a major source of therapeutic agents for alleviation and cure diseases. In the present investigation comparative analysis of antibacterial activity of *Acorus calamus* and commercially available antibacterial antibiotics Ciprofloxacin (5µg), erythromycin (15µg), penicillin (5µg), tetracycline (30µg) and ampicillin (10µg) on wound infection causing pathogens was carried out. The antimicrobial activity of different extracts of *Acorus calamus* was analyzed by using agar well diffusion method and antibiotic sensitivity by paper disk diffusion method. The results revealed that the *Acorus calamus* produce a significant reduction of bacterial pathogens compared to the antibacterial antibiotics.

Keywords: *Acorus calamus*, agar well diffusion, multiple drug resistant strains.

INTRODUCTION

The development of resistance to existing antibiotics and increasing public concern over environmental pollution and toxicity generated a continuing need for new antibiotic agents. The interest to study the plants is because of their medicinally and pharmacological important active ingredients such as alkaloids, flavonoid, glycoside, steroid, saponin, and resin etc.[1] WHO indicates the major need of drug discovery for infectious pathogenic and non-pathogenic infectious diseases (CVD, cancer, Alzheimer etc).[2] Large numbers of medicinal plants are used for the treatment of various diseases as compared to allopathic medicine[1]. Plant extract has been used traditionally to treat a number of infectious diseases including those caused by bacteria and fungi. Using herbs as antimicrobials played an important role in nearly every culture on earth including Asia, Africa, Europe, and America. The primary benefits of using plants derived medicines are that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment.[3] Phytoconstitutens are the natural bioactive compounds found in plants. Phytochemicals have antioxidant or hormone-like effect which helps in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues.[4]

Acorus calamus Linn (family Araceae) commonly known as sweet flag is an important folk medicine in India, a semi aquatic, perennial herb with creeping rhizome. It is widely used for the formulation of medicine in Chinese and Indian ayurveda. It is one such herb claimed to posses antimicrobial activity. [5]

MATERIALS AND METHODS

Organisms used in the Study

The following bacterial pathogens namely *Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa* were procured from the American Type Culture Collection (MTCC), Chandigarh, India. All the cultures were grown on nutrient agar plates and maintained in the nutrient agar slants at 4°C. Overnight culture in the nutrient broth was used for the present experimental study.

Antibiotic sensitivity of the bacteria

Antibiotic sensitivity test[9]

The organisms were tested for antimicrobial drug susceptibility against 5 commonly used antibiotics by disc diffusion method by Kirby-bauer method. The commercially available antibiotics used were Ciprofloxacin (5µg), erythromycin (15µg), penicillin(5µg), tetracycline(30µg) and ampicillin(10µg). Based on the inhibition, the results are interpreted using the criteria recommended for by (CLSI 2012), isolates were classified as either sensitive (S), intermediate (I) or resistant(R)

Collection of plant material

The study was conducted in the Microbiology Laboratory, MMM College of Health Sciences, Mogappair, Chennai. *Acorus calamus* plant rhizomes were collected from Herbal shop and sun-dried for 7 days and powdered using blender and stored in a sterile container for future testing[8].

Preparation of plant extract

25g of each plant material was soaked in 100 ml of ethanol, ethyl acetate, chloroform and water separately and allowed to stand for 72 hrs followed by filtration. The extracts were then collected in a container and stored at 4^0C in a refrigerator.

Phytochemical tests

Freshly prepared plant extracts were subjected to standard phytochemical analyses using standard procedure.[10] In order to find out the presence of various phytoconstituents such as alkaloids, terpenoids, flavonoids, tannins, steroids, anthroquinones, saponins, resins, glycosides and phenols.[6]

Assay to evaluate antibacterial activity of plant extracts

The antibacterial activity of plant extracts was evaluated using agar well diffusion method. Pure cultures of each bacterial strain were sub cultured in nutrient broth on a rotary shaker at 200 rpm for 24 hours at 37°C. For preparing Mueller Hinton agar (MHA) plates, the MHA medium was boiled to dissolve completely and sterilized by autoclaving at 15 lbs pressure (121°C) for 30 minutes. After sterilization, 20 ml of MHA media was poured into the sterile petri plates and kept at room temperature for solidification. Then, each strain was swabbed uniformly onto the individual Mueller Hinton agar plates using sterile cotton swabs. Wells of 6 mm diameter were made on Mueller Hinton agar plates using sterile cork borer and 50 µl of plant extracts were poured into each well on all plates. The plates were incubated overnight at 37°C and the results were observed by the presence of bacterial growth inhibition zone around the sample loaded well and their diameters (mm) were measured using measuring scale. The assay was performed in triplicates[11]

Commercially available antibiotic discs namely Ciprofloxacin, Amoxicillin, Erythromycin, Tetracycline and Penicillin were used to determine the drug sensitivity pattern of tested bacteria[12,13].

Antibacterial activity of *A. calamus* .[13]

Antibacterial activity was performed by agar well diffusion method. The bacterial samples were inoculated using spread plate technique on the surface of sterile Mueller Hinton agar plates. A well of about 2.5mm diameter with sterile cork borer was aseptically punched on each agar plates. Antimicrobial activity of different extracts (ethanol, ethyl acetate, aqueous and chloroform) of *A.calamus* introduced into the well in the plates with different concentrations (10µl, 25 µl and 40 µl). A negative control well was too made with no extract on the agar plates. Plates were kept in laminar air flow for 30 minutes and then incubated at 37^0C for 24 hours. Resulting zone of inhibition was measured in millimeter.

RESULTS AND DISCUSSION

The antimicrobial activity of different extracts of *Acorus calamus* and antibiotic sensitivity of five commercially available antibiotics was performed against five different wound infection causing pathogens *like Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis.*

Antibiotic sensitivity pattern of bacterial spp

Antibiotic susceptibility testing was performed for three gram negative and two gram positive organisms. All five organisms were resistant to ampicillin & penicillin and all were sensitive to ciprofloxacin. *S.aureus* highly sensitive to ciprofloxacin (15mm) followed by erythromycin (12mm) and tetracycline (11mm). *S.pyogenes* and *P. mirabilis* were nearly equally sensitive to ciprofloxacin (13mm and 14mm) and erythromycin followed by tetracycline. *P.aeruginosa* was more sensitive to ciprofloxacin (12mm) followed by erythromycin (11mm) and tetracycline (7mm). *K. pneumonia* was resistant to all antibiotics except ciprofloxacin (12mm (Table 1).

Table 1: Antibiotic sensitivity of bacterial spp

Sl	Bacteria	Zone of inhibition (mm)				
		Ampicillin (AMP)	Penicillin (P)	Erythromycin (E)	Tetracycline (TE)	Ciprofloxacin (CIP)
1	*Staphylococcus aureus*	R	R	12	11	15
2	*Streptococcus pyogenes*	R	R	1 3	6	13
3	*Klebsiella pneumoniae*	R	R	R	R	12
4	*Pseudomonas aeruginosa*	R	R	11	7	12
5	*Proteus mirabilis*	R	R	14	5	14

R- Resistant-No inhibition zone

The antibiotic sensitivity of the isolated organisms were analysed statistically by mean and standard deviation. The results of antibiotic sensitivity was done with three replicates and showed (Table: 2). Among the 5 antibiotics the highest mean values were observed for ciprofloxacin upon *S.aureus* followed by erythromycin and ciprofloxacin upon *P. mirabilis.* The other antibiotics showed intermediary values and for penicillin, erythromycin and tetracycline nil mean values obtained upon *S.aureus, K.pneumoniae* respectively. The antibacterial effect of ethanolic extract of *A.calamus* exhibited maximum inhibition zone (18 mm) against *Salmonella gallinarum*.

Whereas the minimum inhibitory zone (7 mm) was noticed against *Stphylococcus aureus*. Regarding antifungal effect of ethanolic extract showed maximum inhibition zone (14 mm) and minimum inhibitory zone (8 mm) was observed against *Trichoderma viridae* and *Aspergillus flavus* respectively[14].

Table 2: Statistical analysis of antibiotic sensitivity of bacterial pathogens

Organism	Zone of inhibition				
	AMP	P	E	TE	CIP
S.aureus	5.7±0.1	-	12.2±1.11	11.16±0.96	15.2±1.168
S.pyogenes	5.9±0.15	5.7±0.1	13.3±1.13	6±1	13.3±1.137
K.pneumoniae	5.3±0.21	6±0.1	_	_	12.2±1.11
P.aeruginosa	6±0.1	5.5±0.3	11.1±0.96	7.5±1.07	12.2±1.11
P.mirabilis	5±0.22	6±0.2	14.4±1.74	5.3±1.29	14.4±1.16

Phytochemical screening

Acorus calamus was subjected to systemic phytochemical screening by aqueous extraction. *Acorus calamus* aqueous extract was found to contain carbohydrate, proteins, aminoacids,glycosides,alkaloids, saponins, tannins, fat and oils and phytosterols (Table: 3). Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consist of alkaloids, terpenoids and phenolic compounds[13]. The presence of glycosides has been used for over two centuries as stimulants in cases of cardiac failure[14].

Table 3: Phytochemical screening of *Acorus calamus*

Sl No	Phytochemical Tests	Aqueous extract of *Acorus calamus*
1)	Carbohydrates a) Molisch's test	+
	b) Fehling's test	+
	c) Barfoed's test	-
2)	Proteins and Amino acids Million's test	+
	Ninhydrin test	+
	Biuret test	-
3)	Glycosides-Legal test	-
	Keller-killiani test	+
	Modified Borntrager test	-
4)	Alkaloids Mayer's test	+
5)	Flavonoids a) Shinoda test	-
	Alkaline Reagent test	-
6)	Tannins	
	a)Ferric chloride test	-
	b)Lead acetate test	-
7)	Saponins a)Foam test	+
8)	Phytosterols a)Salkowski test	+

Comparative analysis of antibiotics and extracts *A.calamus*

Table 4 showed the comparative analysis of antibiotic activity and ethanol extract of *Acorus calamus*. For *Staphylococcus aureus* 24mm inhibition zone was obtained for 40µl chloroform extracts followed by 40µl of ethyl acetate extracts of *A.calamus* respectively. For *S.pyogenes* 19mm inhibition was obtained for 40µl chloroform extracts followed by the same extract. For *K.pneumoniae* 40µl ethanol extracts gave inhibition about 18mm followed by 15mm for 40µl of chloroform extracts. For *P.aeruginosa* 40µl chloroform extracts showed about 21mm zone and aqueous extracts showed 17mm zone. For *P.mirabilis* ethyl acetate extracts showed 26mm zone followed by 14mm zone by ciprofloxacin antibiotic.When compared to antibiotic sensitivity, the antibacterial activity of the plant extracts showed better control over the pathogens.

Table 5 showed the statistical analysis of antibacterial activity of *Acorus calamus* extracts. Highest mean value observed for 40µl of chloroform extract followed by 40µl of ethyl acetate and 25µl of chloroform extract against *Staphylococcus aureus*. 40µl of chloroform extract shown highest mean value for *Streptococcus pyogenes* followed by 25µl of chloroform extract. Highest mean value observed for 40µ of ethanol extract against *Klebsiella pneumoniae* followed by 25µl of chloroform extract. 40µl of chloroform extract shown the highest mean value for *Pseudomonas aeruginosa* followed by 25µl of chloroform extract. Highest mean value showed for ethyl acetate 40µl extract against *Proteus mirabilis* followed by 25µ of ethyl acetate extract.

Table 4: Comparative analysis of antibacterial activity of antibiotics and extracts of *Acorus calamus*

S.no	Antibiotic used (Zone of clearance)						*Acorus calamus* extracts											
							Ethanol extract			Ethyl acetate			Aqueous extract			Chloroform		
	Name	AMP	P	E	TE	CIP	10 µl	25 µl	40 µl	10 µl	25 µl	40 µl	10 µl	25 µl	40 µl	10 µl	25 µl	40 µl
1	*S.aureus*	R	R	12	11	15	5	5	10	11	17	21	9	12	14	12	21	24
2	*S.pyogenes*	R	R	13	6	13	R	R	R	R	8	10	7	9	11	11	14	19
3	*K.pneumoniae*	R	R	R	R	12	8	13	18	R	10	13	11	12	13	R	15	R
4	*P.aeruginosa*	R	R	11	7	12	R	R	R	7	11	16	8	11	15	13	17	21
5	*P.mirabilis*	R	R	14	5	14	R	5	13	8	16	26	7	8	10	9	11	15

Statistical analysis of antibacterial activity of *Acorus calamus*

Table 5: P value and Correlation value of *Acorus calamus*

Organisms	*A.Calamus* extracts	Correlation value	P value
S.aureus	Ethanol	**0.999**	**0.03**
	Ethyl acetate	**0.997**	**0.04**
	Aqueous	0.964	0.17
	Chloroform	0.989	0.09
S.pyogenes	Ethyl acetate	**0.999**	**0.02**
	Aqueous	**1.000**	**0.01**
	Chloroform	**1.000**	**0.00**
K.pneumoniae	Ethanol	0.989	0.09
	Ethyl acetate	0.978	0.13
	Aqueous	0.978	0.13
	Chloroform	**1.000**	**0.01**
P.aeruginosa	Ethyl acetate	0.988	0.09
	Aqueous	1.000	0.1
	Chloroform	0.995	0.60
P.mirabilis	Ethanol	0.990	0.09
	Ethyl acetate	**0.997**	**0.04**
	Aqueous	0.994	0.06
	Chloroform	**0.998**	**0.03**

Statistical table 5 showed the correlation among the extract of *Acorus calamus* against the pathogenic wound infection causing organism. Among the extract of *Acorus calamus* ethanol and ethyl acetate extracts showed a significant antibacterial effect on *Staphylococcus aureus*. The ethyl acetate, aqueous and chloroform extracts showed a significant antibacterial activity on *Streptococcus pyogenes*. Chloroform extract showed a significant effect on *Klebsiella pneumonia*e. The ethyl acetate and chloroform extract showed a significant antibacterial effect on *Proteus mirabilis*.

CONCLUSION

The study concludes that the various bioactive compounds of *Acorus calamus* extracts could be used as an alternate to antibiotics, considering the side effects and escalating levels of antibiotic resistance among microorganisms. This study also stated that the various extracts of *A.calamus* are having proven antibacterial activity and they can be used as potent antimicrobial formulation in pharmaceutical and medicine industries in near future. We hope that this study would direct to the establishment of the phytochemical compounds that could be used to invent new and more potent antimicrobial drugs from natural origin. Further work will emphasize the isolation and characterization of active principles responsible for bioefficacy and bioactivity.

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