

Phytochemical analysis and antimicrobial activity of the ethanolic extract of *Acorus calamus* rhizome

S. SENTHIL KUMAR*, A. SOBAN AKRAM,
T.S. FAREED AHMED and M.S. MOHAMED JAABIR

Department of Biotechnology, Jamal Mohamed College (Autonomous),
Tiruchirappalli - 620 020 (India).

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ABSTRACT

The present study is aimed at evaluating the *in vitro* antimicrobial activity of ethanolic extracts of the medicinal plant *Acorus calamus* against three bacterial (*Pseudomonas* sp., *Bacillus* sp., *Staphylococcus aureus*) and three fungal (*Aspergillus niger*, *Aspergillus flavus*, *Trichoderma* sp.) species. The ethanolic extract of *Acorus calamus* exhibited antimicrobial activity moderately on *Pseudomonas* sp., *Staphylococcus aureus*, and *Aspergillus flavus*. The phytochemicals that are present in the rhizomes of *Acorus calamus* was screened by Gas Chromatography and Mass Spectroscopy (GC-MS) method. The GC-MS study revealed about 10 active phytochemicals present in the rhizomes including alkaloids, aromatic, Palmitic and linoleic acid.

Keywords: Antimicrobial activity, phytochemical analysis, *Acorus calamus*, GC-MS.

INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represent a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. The medicinal value of plant lies in some chemical constituents that produce a definite physiological action on the human body¹. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds.

Of the 2, 50,000 higher plant species on earth, more than 80,000 are medicinal. India is one the world's 12 biodiversity centres with the presence of over 45,000 different plant species. Of these, about 15,000-20,000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities.

In India, drugs of herbal origin derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. For the manufacturing of modern drugs important chemical intermediates needed are also obtained from plants². Today many secondary metabolites are commercially important and find use in number of pharmaceutical compounds. In some cases the crude extract of medicinal plants are being used as medicaments. This paper focuses on the phytochemical analysis of *Acorus calamus* and its antimicrobial activity.

MATERIAL AND METHODS

Preparation of plant extract

The rhizomes of *Acorus calamus* were collected, dried and pulverized well. About 20g of the powdered rhizomes were soaked in 60ml of ethanol. It was left for 24 hours so that, alkaloids, terpenoids and other constituents dissolved. The ethanol extract was filtered using the 0.2 micron syringe filter.

Microbial Strains

Pathogenic organisms were selected based on pathogenesis and drug resistance. They were obtained from the Department of Microbiology, Doctor's Diagnostics, Thillainagar, Tiruchirappalli. The test organisms were *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus aureus*, *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma* sp. The bacterial cultures were inoculated into Mueller Hinton Media and the fungal cultures were inoculated into Potato Dextrose Agar (PDA) medium.

Antimicrobial Activity

The ethanolic extract of *Acorus calamus* plant was tested against three bacterial pathogens such as *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus aureus* and three fungal pathogens such as *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma* sp. for their antibacterial and antifungal activity respectively. Both the antibacterial and antifungal activity was demonstrated by well diffusion method at three different concentrations viz. 25mg, 50mg and 100mg per well.

Phytochemical analysis of plant extract by GC-MS technique

The ethanolic extract of *Acorus calamus* was dried and the powder was dissolved in diethyl ether and filtered through 0.2 micron filter for GC-MS analysis. Before analyzing the plant extract using GC-MS, the temperature of the oven was set to 100°C. The flow rate of gas used and energy of the electron gun was programmed initially. The gas used as carrier and as an eluent was Helium. About 2 μ l of the extract of *Acorus calamus* was injected into

the GC-MS using a micro syringe. The setup was allowed to screen for 36 minutes. The chromatogram was obtained with retention time in the abscissa and percentage of the peak in the ordinate. The compounds present in the extract were identified from the mass spectral data.

RESULTS AND DISCUSSION

Antimicrobial Activity

The antimicrobial activity of the rhizomes of *Acorus calamus* was studied against different clinically isolated strains of bacteria and fungi. The effectiveness of rhizome was assayed by the presence or absence of inhibition zones and zone diameter. The results are presented in Table 1. The results reveal a moderate zone of inhibition against *Pseudomonas* sp., *Staphylococcus aureus* and *Aspergillus flavus*.

Phytochemical analysis

The screening of *Acorus calamus* by GC-MS study revealed 10 different active phytocomponents which included Aromatic, Ketone, Alkaloid, fatty acids like Palmitic and Linoleic acids. Among these, alkaloids, Palmitic and Linoleic acids are the three important compounds known for antimicrobial, anti-inflammatory and antioxidant³ activities (Table 2). Shyobunone is a keto compound and α -Asarone and β -Asarone are aromatic compounds found in the rhizome extract. The phytocompound 9, 12-Octadecadienoic acid was identified at an Rt (Retention time) of 24.02 minutes (Fig. 1).

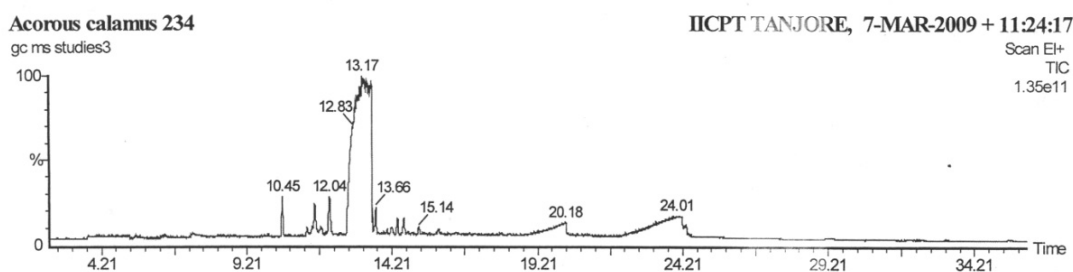


Fig. 1: GC-MS of the methanolic extract of *Acorus calamus* rhizome

Table 1: Antimicrobial Activity of methanolic extract of *Acorus calamus* rhizome

S. No.	Name of Organism	Zone of inhibition (mm)		
		25mg	50 mg	100 mg
1	<i>Pseudomonas</i> sp.	17	20	23
2	<i>Bacillus</i> sp.	-	-	-
3	<i>Staphylococcus aureus</i>	15	25	29
4	<i>Aspergillus niger</i>	-	-	-
5	<i>Aspergillus flavus</i>	10	12	18
6	<i>Trichoderma</i> sp.	-	-	-

Table 2: Phyto-components identified in the ethanolic extract of the rhizome of *A. calamus*

NO	RT	Name of the Compound	Molecular Formula	MW	Peak Area %
1	10.45	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	C ₁₁ H ₁₄ O ₂	178	1.15
2	11.54	Shyobunone	C ₁₅ H ₂₄ O	220	2.11
3	12.04	A Asarone	C ₁₂ H ₁₆ O ₃	208	1.83
4	13.17	B Asarone	C ₁₂ H ₁₆ O ₃	208	71.51
5	14.39	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	C ₁₅ H ₂₄ O	220	0.65
6	14.62	Trimethoxyamphetamine,2,3,5-	C ₁₂ H ₁₉ NO ₃	225	0.91
7	15.14	Pyrimidin-2-one, 4-[N-methylureido]-1-[4-methylaminocarbonyloxymethyl]	C ₁₃ H ₁₉ N ₅ O ₅	325	0.33
8	15.80	4a,7-Methano-4Ah-naphth[1,8a-b]oxirene,octahydro-4,4,8,8-tetramethyl-	C ₁₅ H ₂₄ O	220	0.28
9	20.18	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	5.23
10	24.01	9,12-Octadecadienoicacid	C ₁₈ H ₃₂ O ₂	280	16.00

Acorus calamus also called as 'Vasambu' in Tamil and belongs to the family Acoraceae. It is a tall perennial wetland monocot with scented leaves and rhizomes which have been used medicinally, for its odor, and as a psychotropic drug⁴. It also possesses antibacterial⁵ antifungal⁶ and antispasmodic activity as reported by others⁷. The plant is a native of Europe. It is distributed throughout the tropics and subtropics, especially in India and Sri Lanka. Plenty of sunlight is necessary for growth as well as after harvest for drying the rhizomes. The Sweet Flag is capable of improving memory power and intellect. It is also reported for its useful property in infantile fever, cough, bronchitis

and asthma. The drug is reported to cure hysteria, insanity and chronic rheumatic complaints. *Acorus calamus* extract is used as anti-hypersensitive drug and possess vascular modulating effect which is mediated through multiple mechanisms⁸. The antimicrobial effect of the methanolic extract of the rhizome of *Acorus calamus* is probably due to its chemical composition as revealed by the GC-MS study. Shyobunone, α -Asarone and β -Asarone, fatty acids like 9, 12-Octadecadienoic acid have already been reported for antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic and nematocidal activities (Table 3).

Table 3: Phyto-components identified in the ethanolic extract of *Acorus calamus* rhizome

NO	RT	Name of the Compound	Molecular Formula	MW	Peak Area %	Compound Nature	**Activity
1	10.45	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	C ₁₁ H ₁₄ O ₂	178	1.15	Aromatic compound	No Activity reported
2	11.54	Shyobunone	C ₁₅ H ₂₄ O	220	2.11	Ketone compound	No Activity reported
3	12.04	A Asarone	C ₁₂ H ₁₆ O ₃	208	1.83	Aromatic compound	Antipyretic, Antispasmodic, Emetic Fungicide, Mutagenic, Sedative Myorelaxant, Tranquilizer, Pesticide, Cardio depressant, Psychoactive, Anticonvulsant
4	13.17	B Asarone	C ₁₂ H ₁₆ O ₃	208	71.51	Aromatic compound	-do-
5	14.39	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecanol, 4,4,11,11-tetramethyl-	C ₁₅ H ₂₄ O	220	0.65	Aromatic compound	No Activity reported
6	14.62	Trimethoxyamphetamine,2,3,5-	C ₁₂ H ₁₉ NO ₃	225	0.91	Alkaloid	Antimicrobial, Anti-inflammatory
7	15.14	Pyrimidin-2-one, 4-[N-methylureido]-1-[4methylaminocarbonyloxymethyl]	C ₁₃ H ₁₉ N ₅ O ₅	325	0.33	Alkaloid	Antimicrobial, Anti-inflammatory
8	15.80	4a,7-Methano-4Ah-naphth[1,8a-b]oxirene,octahydro-4,4,8,8-tetramethyl-	C ₁₅ H ₂₄ O	220	0.28	Aromatic compound	No Activity reported
9	20.18	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	5.23	Palmitic Acid	Antioxidant, Hypo cholesterolemic, Nematicide, Pesticide, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor
10	24.02	9,12-Octadecadienoicacid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280	16.00	Linoleic Acid	Hypo cholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Antiandrogenic, 5-Alpha reductase inhibitor Antihistaminic, Anticoronary, Insecticide, Antieczemic, Antiacne

**Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database

CONCLUSION

The results indicate that this shrub should be studied more extensively to explore its potential in the treatment of infectious diseases. Recently, there has been enormous interest in the medicinal

plants for their antimicrobial activities in controlling pathogens^{9, 10}. In that way, the methanolic extract of the *Acorus calamus* rhizome could be a potential source of novel antibacterial agent because of the significant antimicrobial effect seen in the clinical isolates tested in this study.

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