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## Anti-microbial activities of various extracts of *Acorus calamus L.*

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### ABSTRACT

To examine the chloroform, ethyl acetate and 50% aqueous – ethanol extracts of the rhizomes of the plant of *Acorus calamus L.* for their anti-bacterial and anti-fungal properties against a wide range of Micro-organisms. The anti-bacterial activity was studied by agar dilution method using Muller Hinton (MH) agar media. The results were compared with standard ciprofloxacin (5 µg/disc). The anti-fungal activity of the extracts was investigated by tube dilution method using Sabouraud Dextrose Agar (SDA) medium and the results compared with standard Clotrimazole (125 µg/ml). All studied extracts showed potential anti-bacterial and anti-fungal properties. The MIC (Minimum Inhibitory Concentration) was found to be <200 µg/ml for chloroform extract and ethyl acetate extract and less than 133.33 µg/ml for 50% aqueous-ethanol extract. Hence the MIC was of varying range (133.33-200 µg/ml) for all the tested extracts, with different micro-organisms. The anti-fungal properties of all the studied extracts were comparable with that of standard clotrimazole. The present study brought to light the scientific data on the anti-infective property of the plant.

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### KEYWORDS

*Acorus calamus L.*;  
Anti-microbial activity;  
Anti-bacterial activity;  
Anti-fungal property.

### 1. INTRODUCTION

*Acorus* is a genus of monocot flowering plants belonging to the family Acoraceae. Common names include Calamus and Sweet Flag. It is known as *vasambu* in Tamil language. The name 'acorus' is derived from the Greek word 'acoron', a name used by Dioscorides, which in turn was derived from 'coreon', meaning 'pupil', because it was used in herbal medicine as a treatment for inflammation of the eye. The genus *acorus* includes as many as six species: *Acorus americanus*, *Acorus calamus L.*, *Acorus gramineus*, *Acorus triqueter*, *Acorus latifolius*, *Acorus xiangyeus*, of which Calamus has been an item of trade in many cultures for thousands of years. *Calamus* has been used

medicinally for a wide variety of ailments. *Acorus calamus* was often added to wine, and the root is also one of the possible ingredients of absinthe. Among the northern Native Americans, it is used both medicinally and as a stimulant; in addition, the root is thought to have been used as an entheogen among the northern Native Americans. In high doses, it is hallucinogenic; *Calamus* has been used as a "street drug alternative". The earlier reported studies on the plant *Acorus calamus* revealed the presence of anti-epileptic, inhibition of tyrosine L-DOPA oxidation in melanin synthesis, choline esterase inhibitor, anti-diabetic, prevention of hyperproliferation response in kidneys, insecticidal, anti-spasmodic, neuroprotective, anti-stress, anti-oxidant, anti-cancer, hypolipidemic, anti-diarrheal, nematocidal,

TABLE 1: Anti-bacterial activity of various extracts of *Acorus calamus L.* (MIC)

Description	Micro-organisms (MIC in µg/ml)								
	<i>E.Coli</i>	<i>K.pneumonia</i>	<i>P.aeruginosa</i>	<i>Vibrio cholerae</i>	<i>S.aureus</i>	CONS	<i>S.typhii</i>	<i>S.paratyphii A</i>	<i>S.paratyphii B</i>
Control	+	+	+	+	+	+	+	+	+
Ciprofloxacin	5	5	5	5	5	5	5	5	5
Chloroform extract	>200	>133.33 <200	>66.66 <133.33	>133.33 <200	>200	>200	>66.66 <133.33	>66.66 <133.33	>133.33 <200
Ethyl acetate extract	>66.66 <133.33	>200	>66.66 <133.33	<33.33	>200	>133.33 <200	>66.66 <133.33	<33.33	<33.33
50% Aqueous ethanol extract	>200	>133.33 <200	>66.66 <133.33	>200	>133.33 <200	>133.33 <200	>66.66 <133.33	<33.33	>200

and sedative and hypnotic properties of the plant<sup>[1-15]</sup>. But there exist a lacunae in the microbial properties of the plant. On careful literature review it was found to possess strong anti-microbial activities even among many resistant pathogens, which was not explored fully as the screening was restricted to only methanolic extract in case of anti-bacterial property and hexane extract in case of anti-fungal activity<sup>[16,17]</sup>. Hence in the present study an attempt was made to study the anti-microbial properties of the plant *Acorus calamus*, which may through light on the minds of the researches to come out with a potent template with potent anti-microbial property.

## 2. MATERIAL

The plant specimen for the proposed study were procured from crude drug merchant, Chennai, Tamil Nadu and its authenticity was confirmed by survey of Medical plants unit, Siddha C.C.R.A.S., Govt of India, Palayamkottai, Thirunelveli-627 002, Tamil Nadu, India and PARC, Tambaram, Chennai, 600 018, India. Voucher specimen of *A. calamus L.* (NO.12008) deposited in the herbarium of the Department of Pharmacognosy, Mohamed Sathak A.J. College of Pharmacy, Sholinganallur, Chennai-119. The rhizomes were used for the study. The following micro-organisms were procured from standard laboratory maintained in the Institute of Microbiology, Madras Medical College, Chennai-600 003 and used for the study.

### Bacteria

*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhii*, *Salmonella paratyphii A* and *B*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Vibrio cholerae* and Coagulase Negative *Staphylococcus* (CONS).

The medium MH agar, ciprofloxacin discs (5 µg/

disc) were obtained from Hi-media Laboratories Limited, Mumbai-400 086, India.

### Fungi

*Aspergillus flavus*, *Penicillium chrysogenum*, *Mentographytes spp.* and *Candida albicans*. Clotrimazole were obtained from Hi-media Laboratory Ltd, Mumbai-400 086, India.

## 2. METHOD

### 3.1 Preparation of plant extract

Freshly collected plant material rhizomes were dried in shade, then coarsely powdered and 1 Kg of powder was extracted in an aspirated bottle with chloroform, ethyl acetate and 50% aqueous-ethanol, by cold maceration for 3-7 days. All extracts was filtered through whattman filter paper no.41 and evaporated on a water bath and finally dried in vacuum. The residue of all extracts was suitably diluted with DMF (Di-methyl formamide) to get a final concentration of 1000 µg/ml and used for the study.

### 3.2 Anti-bacterial activity<sup>[19]</sup>

The plates were prepared by using MH agar and the extracts of various dilutions allowed to solidify and dry. Then a loopful of bacterial cultures was inoculated at 37°C for 24 hours. The results were read by the presence or absence of growth of organisms (TABLE 1) and the MIC was determined. The same procedure was followed for the investigation of all extracts.

### 3.3 Anti-fungal activity<sup>[18-20]</sup>

For anti-fungal activity a stock solution of extract was serially diluted suitably with Dimethyl formamide (DMF) to get the final concentration of 1000µg/ml and used for the study. A volume of 0.5 ml of micro-organism suspensions containing approximately  $4 \times 10^6$  cells

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**TABLE 2: Anti-bacterial activity of various extracts of *Acorus calamus L.* (Zone of inhibition)**

Description	Zone of inhibition in mm.								
	<i>E.Coli</i>	<i>K.pneumonia</i>	<i>P.aeruginosa</i>	<i>Vibrio cholerae</i>	<i>S.aureus</i>	CONS	<i>S.typhii</i>	<i>S.paratyphiiA</i>	<i>S.paratyphiiB</i>
Ciprofloxacin (5µg/disc)Standard	26	32	24	20	20	24	22	18	16
Chloroform extract	06	14	06	14	18	18	06	12	08
Ethyl acetate extract	14	10	12	10	15	14	12	04	10
50% Aqueous ethanol extract	10	09	06	11	14	08	10	04	12

**TABLE 3: Anti-fungal activity of various extracts of *Acorus calamus L.***

Fungi	MIC in µg/ml			
	Clotrimazole	Benzene extract	Chloroform extract	50% Aqueous-ethanol extract
<i>Candida albicans</i>	125	>50<125	>50<125	>50<125
<i>Aspergillus flavus</i>	125	>50<125	>125<200	>50<125
<i>Mentoglyphytespp.</i>	125	>50<125	>125<200	>50<125
<i>Penicillium chrysogenum</i>	125	>50<125	>50<125	>50<125

Clotrimazole (125µg/ml) from Hi-media Laboratory Ltd, Mumbai-00 086, India, Values are an average of triplicate.

were used to inoculate the surface of the solidified media (slants) prepared by using Sabourauds Dextrose Agar (SDA) medium and allowed to set and then incubated at 37°C for 1-4 weeks. The results were read by noting the presence or absence of growth of the organisms and compared with standard Clotrimazole (125µg/ml) (TABLE 2).

### 4. RESULTS

All extracts demonstrated anti-bacterial activity as shown in TABLE 1 and 2 against the various bacteria tested. The results of all extracts were comparable with that of the standard ciprofloxacin (5 µg/disc). All the extracts tested inhibited the growth of fungi *Aspergillus flavus*, *Penicillium chrysogenum*, *Mentoglyphytes spp.* and *Candida albicans*. In concentrations >50<125 µg/ml. In lower concentration (50µg/ml) all extracts were found to be ineffective against the fungi tested. The results of the extracts are comparable with that of standard Clotrimazole (125µg/ml) (TABLE 3).

### 5. DISCUSSION

The results of the present study clearly indicated the anti-bacterial and anti-fungal properties of the various extracts of the plant *Acorus calamus*. The Anti-bacterial activity was comparable with that of the stan-

dard anti-bacterial agent Ciprofloxacin (5 µg/ml) against the organisms tested. The anti-fungal activity was comparable with the standard Clotrimazole. All extracts showed activity against the tested fungi in concentration > 50<125 µg/ml and anti-bacterial activity against the tested organisms in concentration > 133.33<200 µg/ml and their Zone of inhibition was also as comparable with that of the standard Ciprofloxacin. The presence of phyto-constituents like triterpenes, flavones, glycosides, sugar, alkaloids, tannin, saponins and volatile oils in *Acorus calamus* has been reported earlier<sup>[21,22]</sup> and these phyto-constituents in other plants possess anti-microbial and anti-fungal properties. The TLC of all extracts showed the presence of all the above phytoconstituents which may be possibly responsible for the anti-bacterial property of the extracts. And possibly either or all of these constituents may be contributing to the anti-bacterial property to various extracts of the plant *Acorus calamus L.* However, further studies in the role of these phyto-constituents in the anti-bacterial and anti-fungal properties of chloroform, ethyl acetate and 50% aqueous-ethanol extracts may bring out a unique template to cure infections of multiple drug resistant pathogens in future.

These findings support the beneficial effects of the extracts against the pathogenic organisms. Further studies on the isolation and characterization of the phyto-constituents from chloroform, ethyl acetate and 50% aqueous-ethanol extracts of the plant *Acorus calamus L.* and elucidation of constituents responsible for activity should throw light for the future development of phyto-medicine with anti-bacterial and anti-fungal properties.

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