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Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF *ALOCASIA DECIPIENS* SCHOTT

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ABSTRACT

Objective- In the present work, we made an attempt to assess *in vitro* antibacterial activity of methanol extract of rhizome of *Alocasia decipiens* Schott of family Araceae against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsiella sp.*

Method- Different concentrations of the methanolic extract (100%, 50%, 25% and 10%) were prepared and their antibacterial screening was carried out by modified filter paper disc method. The MIC of the extract was determined by the serial tube dilution method. The selected microorganisms were also tested against different antibiotics (10mcg/disc).

Results- The present experiment showed that methanol extract at 100% concentration showed significant result against the test organisms where *Staphylococcus aureus* was observed as the most sensitive (16mm) and *Klebsiella sp*. was found to be the resistant one. Zone of inhibition observed, against different test organisms were also supported by the results obtained from MIC.

Conclusion- Our experiment proposes *Alocasia decipiens* as one of the important medicinal plant of Araceae, as its methanol extract of rhizome showed good results against four different bacterial strains.

Keywords: Antibacterial, Methanol extract, *Alocasia decipiens*

INTRODUCTION

Plants, the sources of bioactive constituents have been used traditionally to cure various ailments in Ayurveda, Unani & Siddhi. Though, during last few years, synthetic drugs occupy the position for curing various diseases, but, due to their side effects, scientists are now focusing to explore the potentiality of traditional medicines [1]. Numerous research works have been done aiming to know the different antimicrobial and phytochemical constituents of medicinal plants and in using them for the treatment of microbial infection, as possible alternatives to chemically prepared synthetic drugs to which many infectious micro organisms have become resistant [2]. This multidrug-resistance in pathogenic microbes against antimicrobial agents and development of numerous defense mechanisms thus, led a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action [3]. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. In the last few decades, researchers have turned their attention to explore the potential of traditional medicinal plants for their therapeutic properties. Initial screenings of plants for their possible antimicrobial activities is generally carried out by using crude aqueous or alcohol extractions [4]. There are numerous illustrations of plant derived drugs.

In the present article, we have selected *Alocasia decipiens* Schott to evaluate for its *in vitro* antibacterial screening. The selected plant belongs to the family Araceae. The Araceae is a large family comprising about 105 genera and approximately 3000 species of herbaceous monocotyledons. These are predominantly tropical in distribution with 90% of genera and 95% 0f species restricted to the tropics. The family contains several well known cultivated foliage and flowering plants like *Philodendron, Monstera, Spathiphyllum*, *Anthurium*, etc. A number of food crops also belong to Araceae, notably *Xanthosoma, Colocasia*, *Amorphophallus,* etc. One of the important character of the family is the inflorescence structure; small flowers born on fleshy axis (spadix) subtended by a modified leaf (spathe) [5]. Majority of the members of Araceae also contain crystals of calcium oxalate which are often cited as causing intense irritation when handling or consuming raw. However, this supposition is contradicted by the fact that although irritation generally is not produced by properly cooked plants, the crystals remain after heating. Other compound must therefore be involved with causing this reaction. Studies on *Dieffenbachia* demonstrated that a proteolytic enzyme and other compounds are responsible for the severe irritation caused by this plant and the raphides of calcium oxalate do not play major role [6].

MATERIALS AND METHODS

Plant Material

The plant, *Alocasia decipiens* was identified in Central National Herbarium, Botanical Survey of India, Shibpur, Howrah-711103, West Bengal, India. The rhizome of the plant was selected for carrying out the experiment.

Preparation of extract

The coarsely powdered shade dried rhizome of *Alocasia decipiens* was extracted in a Soxhlet apparatus with Petroleum ether followed by ethyl acetate and methanol. All the extracts were then concentrated separately by using a rotary evaporator. The methanolic extract was used to screen the antibacterial activity. Different concentrations of the extract were prepared (100%, 50%, 25% and 10%) from the concentrated extract by diluting with appropriate volume of methanol.

Microorganisms used

The bacterial species used for the antibacterial activity were *Bacillus subtilis, Staphylococcus aureus, Klebsiella sp.* and *Escherichia coli.* All the bacterial strains were collected from Silchar Medical College, Assam, India.

Preparation of the media

13 gram of the powdered media (HIMEDIA) was dissolved in 1000ml sterile distilled water in a conical flask. The weighed amount was mixed properly and allowed to dissolve by heating over a water bath. The conical flask was then plugged with cotton wool and wrapped with aluminium foil. The flask was then autoclaved at 120°C at 15lb pressure for 15 minutes. The sterilized medium was then poured over the sterilized glass petriplates (Borosil) and allowed to cool and solidify.

Antibacterial activity

The antibacterial screening was carried out following modified filter paper disc method [7]. 6mm discs of Whatman No. 1 filter paper were prepared and immersed in each of the different concentrations of the extract (100%, 50%, 25% and 10%) and kept for overnight

[8]. The discs were dried at room temperature and kept in dry condition. The collected microbial cultures were aseptically swabbed on the surface of sterile nutrient agar plates using sterile cotton swabs.

Using an ethanol dipped and flamed forceps, the dried paper discs were aseptically placed over the seeded agar plates sufficiently separated from each other to avoid overlapping of inhibition zones. The plates were incubated at 37^{0}C for 24 hours and the diameter of the zone of inhibition was measured in mm.

Serial tube dilution technique

The MIC of the extract was determined by the serial tube dilution technique [9]. Air dried methanol extract (1.024 mg) dissolved in 2ml distilled water (few drops of Tween 80 was added to facilitate dissolution) to obtain stock solution having concentration of 512µg/ml. In serial dilution technique, 1ml prepared stock solution was transferred to test tube containing 1ml nutrient broth medium to give concentration 256µg/ml from which 1ml was transferred to another test tube containing 1ml nutrient broth medium to give concentration 128µg/ml and so on up to concentration 2µg/ml. After preparation of suspension of test organisms (10^7 organisms per ml), 1 drop of suspension (0.02 ml) was added to each broth dilution. After 24h incubation at 37^0 C, the tubes were then examined for the growth. The MIC of the extract was taken as the lowest concentration that showed no growth. Growth observed in those tubes where the concentration of the extract was below the inhibitory level and the broth medium was observed turbid (cloudy). Distilled water with few drops of Tween 80 and kanamycin were used as negative and positive control respectively.

Antibiotic sensitivity testing

The test microorganisms were also tested against different antibiotics (10mcg/disc).The collected microbial cultures were aseptically swabbed on the surface of sterile nutrient agar plates using sterile cotton swabs. Using an ethanol dipped and flamed forceps, the antibiotic discs were aseptically placed over the seeded agar plates sufficiently separated from each other to avoid overlapping of inhibition zones. The plates were incubated at 37^{0}C for 24 hours and the diameter of the zone of inhibition was measured in mm

RESULTS AND DISCUSSION

From time immemorial man has been using various parts of plants against common ailments with varying degree of success. The knowledge of drug has developed together with the evolution of scientific and social progress. The practitioners of traditional and indigenous medicine rely mainly on medicinal plants and herbs for preparation of therapeutic substances. Initial screening for the potential antibacterial compounds from plants may be performed by crude extracts. The two most commonly used methods used to determine antibacterial activity are dilution assay and the disc or agar well diffusion assay [10]. In the present investigation different concentrations of methanolic extract was tested for their inhibitory activity on both Gram positive and Gram negative bacteria.

Table 1: Antibacterial activity of the methanolic extract in different concentration

S. No.	Test organisms	Diameter of inhibition zone (mm) of different concentration			
		10%	25%	50%	100%
1	*Escherichia coli*	Nil	Nil	10	11
2	*Bacillus subtilis*	Nil	10	11	12
3	*Staphylococcus aureus*	10	11	11	16
4	*Klebsiella sp.*	Nil	Nil	10	10

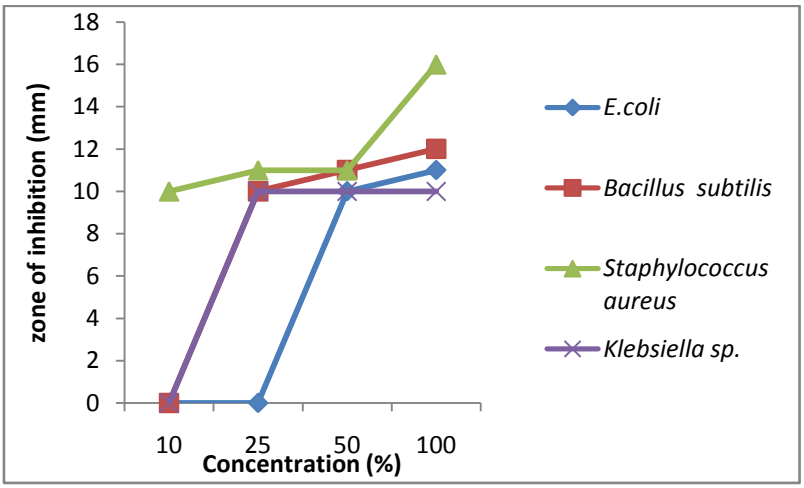

Fig. 1: Antibacterial activity of different concentration of methanolic extract

All test organisms were inhibited by methanolic extract of the plant up to 25%. At 100%, the maximum zone of inhibition was observed against *Staphylococcus aureus*, a Gram positive organism and the minimum was against *Klebsiella sp*., a Gram negative organism. This indicates that methanolic extract has the potential of a broad spectrum activity against Gram positive bacteria. But the variation in the size of the zone of inhibition have been observed among the different group of bacteria. And this may be due to the lipid content of the membranes of the different groups of the microorganisms and the permeability of different constituents of the plant extract.

Table 2: Antibacterial activity of different antibiotics against test organisms

S. No.	Test Organisms	Zone of inhibition (mm) of different antibiotics			
		Amoxycilin	Penicillin	Ciprofloxacin	Amikacin
1	*Escherichia coli*	15	Nil	18	12
2	*Bacillus subtilis*	10	Nil	16	13
3	*Staphylococcus aureus*	18	14	14	13
4	*Klebsiella sp.*	Nil	Nil	20	12

The 100% methanolic extract of the plant showed good zone of inhibition when compared with the activities of commercially used antibiotics (Table- 2). Natural products of higher plants may offer a new source of antibacterial agents and from the present result it is clear that the medicinal value of the selected plant is comparable to the present day antibiotics.

Table 3: MIC determination of methanol extract against the test organisms

S. No.	Test Organisms	MIC (µg/ml)
1	*Escherichia coli*	8
2	*Bacillus subtilis*	2
3	*Staphylococcus aureus*	2
4	*Klebsiella sp.*	16

The MIC of the extract varied from 2µg/ml to 16µg/ml. Table 3 showed the minimum inhibitory concentration of the extract against test organisms. *Staphylococcus aureus* was more sensitive than other bacteria tested.

CONCLUSION

Our experiment proposes *Alocasia decipiens* as important medicinal plant as its methanolic extract of rhizome showed good antibacterial activities against different bacteria (especially Gram positive). As compared to the standard antibiotic, the plant extract showed lower efficacy towards *E. coli* and *Klebsiella sp*. and this may be probably due to the crude nature of the extract or may be the plant extract is active towards Gram positive bacteria. So further study can be done in this connection to isolate the specific antibacterial compound/ compounds.

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