RESEARCH ARTICLE

Preliminary investigation on the antimicrobial activities of stems of *Arisaema tortuosum*

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ABSTRACT

Arisaema tortuosum, snake plant in English, Birmon in Bengali, family: *Araceae*, a species of considerable stature is widely distributed in Sikkim, Manipur, West Bengal, China and N. Burma. Traditionally, it was used for the treatment of snake bite wound, fractured bone, gastrointestinal problems, cancer, etc. In the present communication, we have investigated the antimicrobial activities of stems of *A. tortuosum*. The antibacterial activities of different extracts of stems of *A. tortuosum* were evaluated by disc-diffusion method using ciprofloxacin as standard. From the results, it was found that ethanol extract showed the highest and petroleum ether extract exhibited lowest antibacterial activity in comparison to other extracts of it. Other extracts showed the moderate type of antibacterial activity. From the preliminary phytochemical screening, it was found that ethanol extract of *A. tortuosum* contains alkaloid, steroid, phenolic compound, and flavonoids. An attempt is being made to characterize this isolated compound. Detailed studies regarding mechanism of action and compound(s) responsible for antimicrobial activities is under process.

Keywords: Antibacterial activity, *Arisaema tortuosum*, disc-diffusion method, ethanol extracts

Introduction

Nature has bestowed our country with an enormous wealth of medicinal plants and India has often been referred to as the medicinal garden of the world. *Arisaema tortuosum* commonly called whipcord cobra lily and by many other names in the Indian subcontinent such as Snake plant (English), Bagh Jandhra (Hindi), Birmon (Bengali), family: *Araceae* (Arum family). It is probably the most common species of *Arisaema* from our part of the world. In its natural habitat, it is found growing in a wide range of climatic zones ranging from the warm foothills right up to cold, winter frost areas. It inhabits lightly shaded woods and also sunny hill slopes growing along with other shrubs and grasses. Found in forests, shrubberies and open slopes to 3000 m. Moist shady places at elevations of 1500-2200 m in Nepal. It ranges entire sub-Himalayan-belt of Northern India to Northeast India, China and Myanmar (Figure 1).

Traditionally, the plant has following applications:

- Paste of the tuber is applied over the wound caused by snake-bite to check poisonous effect
- Dried powdered tubers are applied to snakebites
- In case of abscess in the neck, dried powder of tuber is applied over the neck. It helps in early healing
- Wounds are washed with decoction of root. Paste of root is applied on affected part for faster healing
- Decoction of tuber is given to annuals for early recovery of fractured bone
- Juice of the tubers is applied to the wounds of the cattle to kill any parasites
- Seeds have been mixed with salt and used to treat colic in sheep.

It contains cholesterol, stigmasterol, β-sitosterol, campesterol, lectin, choline chloride, etc.

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On preliminary screening, it was found that this plant has some antimicrobial properties. However, no detailed study had yet been done regarding these activities. Keeping this in view, this study has been undertaken to investigate antibacterial activities against some Gram-positive and Gram-negative strain to substantiate the folklore claim.[1-14]

Experimental

Plant materials

A. tortuosum was collected during the flowering session in September 2010 from the Ranikhet, Uttarkhand, India. The botanical identification was done by Dr. Tariq Husain, Scientist, National Botanical Research Institute, Lucknow, India. Voucher specimen has been preserved for further verification.

Extraction

Shade-dried, powdered, sieved (40 mesh size) plant materials were exhaustively extracted successively with petroleum ether (40-60°C), chloroform and ethanol using a soxhlet extractor. The extracts were concentrated to dryness in vacuum.

Phytochemical screening

Small amount of each dried extract of stem of *A. tortuosum* was appropriately treated to prepare sample solution and then subjected to the phytochemical tests. The phytochemical screening of dried extract was performed using the following reagents and chemicals: Alkaloids with Dragendorffs reagent; flavonoids with the use of Mg and HCl; tannins and phenolic compounds with ferric chloride and potassium dichromate solutions; steroids with Libermann Burchard reagent; terpenoids with tin and thionyl chloride; amino acids with ninhydrin solution and saponins with ability to produce suds. Glycosides with chloroform and concentrated sulfuric acid. These were identified by the characteristic color changes as per standard procedures.[15-26]

Determination of antibacterial activity[27-35]

Accurately, weighed 25 mg of each extract was transferred to different 100 ml volumetric flasks. These extracts were dissolved into dimethyl sulfoxide (DMSO), and volume in each flask was made up to 100 ml with DMSO. These solutions (each having concentrated 250 µg/ml) were used as stock solution. 2 ml and 4 ml of these solutions were transferred to two 10 ml volumetric flask, and further dilution was made to 10 ml mark with DMSO. The final solution contained 50 µg and 100 µg of each plant extract per ml of the solution.

The investigated microorganism consists of three Gram-positive bacteria: *Staphylococcus aureus* (NCIM-2079), *Bacillus subtilis* (NCIM-2439), *Streptococcus pyogenes* (NCIM-2608) and three Gram-negative bacteria: *Escherichia coli* (NCIM-2831); *Agrobacterium tumefacient* (NCIM-2942) *Pseudomonas aeruginosa* (NCIM-2863). Hi Medias marketed preparation ciprofloxacin 30 mcg/disc was used as standard drug.

Table 1: Percentage yield of extract(s) of *Arisaema tortuosum*

Extract	Color of extractive	Percent of extractive (w/w)
Petroleum ether	Light green	10.23
Chloroform	Green	16.42
Ethyl acetate	Green	22.14
Ethanol	Brown	35.52
Aqueous	Brownish black	37.02

Table 2: Preliminary phytochemical screening of stem of *Arisaema tortousum*

Constituents/chemical tests	Observation				
	PE	CE	EAE	EE	AE
Alkaloids	-	-	+	+	+
Carbohydrates	-	-	-	+	+
Proteins	-	-	+	+	+
Amino acids	-	-	-	+	-
Steroids	+	+	+	-	-
Glycoside	-	+	+	+	-
Phenolic compounds	-	-	-	+	-
Fixed oils and fats	+	-	-	-	-
Flavonoids	-	-	+	+	+
Saponins	-	-	-	-	+
Coumarin	-	-	-	-	-
Mucilage					

PE: Petroleum ether extract, CE: Chloroform extract, EAE: Ethyl acetate extract, EE: Ethanol extract, AE: Aqueous extract

Figure 1: *Arisaema tortuosum* flowering plant

The antibacterial activity was evaluated by disc-diffusion method (Murillo-Alvarez, 2001; I.P., 1996). Laminar airflow bench was swapped with 70% alcohol, and ultraviolet (UV) lamp was switched on. After 30 min, the UV lamp was switched off. All the reagents, media, inoculums, and glassware were placed in Laminar Air Flow Bench observing all aseptic conditions.

Nutrient agar media was taken in a presterilized Petri-dish. After that, the microorganisms were spreaded over the cooled nutrient agar media with the help of L-shaped glass rod. The sterilized

Table 3: Antibacterial activity of extracts of *Arisaema tortuosum*

Test organism	Zone of inhibition in mm								
	Different extracts (µg/disc)								
	PE		CE		EAE		EE		CPX
	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	30 µg/disc
Staphylococcus aureus (NCIM-2079)	13	15	15	18	16	20	20	24	28
Bacillus subtilis (NCIM-2439)	18	20	16	19	19	23	22	26	33
Streptococcus pyogenes (NCIM-2608)	15	18	15	18	16	21	23	28	34
Escherichia coli (NCIM-2831)	18	20	17	21	18	23	28	30	38
Agrobacterium tumifaciens (NCIM-2942)	13	15	15	17	16	20	22	28	35
Pseudomonas aeruginosa (NCIM-2863)	12	14	17	20	15	19	19	24	28

PE: Petroleum ether extract, CE: Chloroform extract, EAE: Ethyl acetate extract, EE: Ethanol extract. CPX: Ciprofloxacin

Table 4: Antifungal activity of extracts of *Arisaema tortuosum*

Test organism	Zone of inhibition in mm								Fluconazole (25 µg/disc)
	Different extracts (µg/disc)								
	PE		CE		EAE		EE		
	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg	
Aspergillus niger (NCIM-618)	15	18	17	20	20	25	19	28	35
Candida albicans (NCIM-3557)	16	19	24	22	19	24	18	25	32
Cryptococcus neoformans (NCIM-3378)	15	20	16	21	18	22	20	30	37

PE: Petroleum ether extract, CE: Chloroform extract, EAE: Ethyl acetate extract, EE: Ethanol extract

empty disc (7 mm) was impregnated with plant extracts dissolved in DMSO at concentration 50 and 100 µg per ml was used. Disc impregnated with DMSO was used as solvent control for antibacterial activity because of free solubility of test extracts. Then, the disc impregnated with the solution was placed on the surface of the media inoculated with the bacterial strains. Similarly, disc of ciprofloxacin (30 mg/disc) was placed on the seeded agar plate and incubated at 37°C for 24 h. The diameters of zone of inhibition (mm) were recorded, and the experiment was done three times and the mean values are presented and compared with standard drug ciprofloxacin.

Results and Discussion

Results are exhibited in Tables 1-4. From the tables, it is found that the percentage yield of ethanol extract is 35.52% and it contains alkaloid, amino acid, protein, glycoside, flavonoids, and phenolic compounds. The antibacterial and antifungal activity of ethanol extracts on the pathogenic bacteria and fungi was noted at concentration of 50 and 100 µg/ml at 25 ± 2°C for 48 h of incubation. Petroleum ether, chloroform, and ethyl acetate extracts were found to have less potent than ethanol extract of stem of *A. tortuosum*. The antibacterial and antifungal activity of ethanol extracts were comparable to standard drugs used in individual experiment (Tables 3-4).

Conclusion

Preliminary investigation on the antimicrobial activities of stems of *A. tortuosum* reveals that ethanol extract of it has most potent antibacterial and antifungal activity. The compound(s) present in the ethanol extract may be responsible for such activities.

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