Turkish Journal of Biology

Volume 31 | Number 3 Article 7

1-1-2007

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Turk J Biol
31 (2007) 167-172

Antibacterial, Antifungal and Cytotoxic Activities of Tuberous Roots of *Amorphophallus campanulatus*

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Received: 08.05.2007

Abstract: Antibacterial, antifungal and cytotoxic activities of ethanol extract of tuberous roots of *Amorphophallus campanulatus* were studied. Disc diffusion technique was used to determine in vitro antibacterial and antifungal activities. Cytotoxicity was determined against brine shrimp nauplii. In addition, minimum inhibitory concentration (MIC) was determined using serial dilution technique to determine antibacterial potency. The extract showed significant antibacterial activities against four gram-positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Streptococcus β-haemolyticus*) and six gram-negative bacteria (*Escherichia coli, Shigella dysenteriae, Shigella sonnei, Shigella flexneri, Pseudomonas aeruginosa, Salmonella typhi*). The MIC values against these bacteria ranged from 16 to 128 µg/ml. The antifungal activity was found weak against the tested fungi. In cytotoxicity determination, LC_{50} of the extract against brine shrimp nauplii was 7.66 µg/ml.

Key Words: *Amorphophallus campanulatus*, ethanol extract, gram-positive, gram-negative, MIC, cytotoxicity, antifungal activity

Introduction

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries (1). Although huge numbers of antimicrobial agents have been discovered, the pathogenic microorganisms are developing resistance against these agents day by day. In third world countries like Bangladesh, Nepal, and Nigeria, irrational use of antimicrobial agents is a major cause of such resistance. In recent years, attempts have been made to investigate the indigenous drugs against infectious diseases (2). Research in the field of indigenous plants is a significant aspect of developing a safer antimicrobial principle through isolation, characterization, identification and biological studies (2).

Amorphophallus campanulatus (Roxb.) Bl. (Fam. Araceae), locally known as Ol Kachu, is a perennial herb with rounded tuberous root stock (corm) that is widely distributed in Bangladesh, India, and Africa (3-5). The tuberous roots of the plant are used traditionally for the treatment of piles, abdominal pain, tumors, enlargement of spleen, asthma and rheumatism (3-5). The tuberous roots of the plant also have tonic, stomachic and appetizer properties (4,5). Some of its traditional uses, such as in the treatment of tumors and enlargement of spleen, have indicated that the tuberous roots of the plant might possess antimicrobial, antifungal or cytotoxic activities; however, its antimicrobial, antifungal or cytotoxic potential has not yet been explored. Hence, the present study was designed to determine antibacterial, antifungal and cytotoxic activities of the ethanol extract of tuberous roots of *Amorphophallus campanulatus*.

Materials and Methods

Plant materials

The tuberous roots of *Amorphophallus campanulatus* were collected during January 2004 from the Katakhali area of Rajshahi district of Bangladesh and identified by Prof. A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh, where its voucher specimen (No. AC9642) was deposited. The tuberous roots were cut, air-dried and ground into powder.

Plant material extraction and fractionation

Powdered dried roots (600 g) of the plant were extracted (cold) with ethanol (2.5 L) in flat bottom glass

containers, through occasional shaking and stirring for 10 days (6,7). The whole extract was filtered and the solvent was evaporated (8) to dryness *in vacuo* with rotary evaporator at 40°-50 °C to afford a blackish green mass (34 g).

Organisms

Antibacterial activity and minimum inhibitory concentration (MIC) were determined against four gram-positive bacteria (*Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Streptococcus β-haemolyticus*) and six gram-negative bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi*). These organisms were available in the Microbiology Research Laboratory of the Pharmacy Department, Rajshahi University, Bangladesh. The pure cultures of these bacteria were collected from the Microbiological Laboratory of the Institute of Nutrition and Food Science (INFS) and Department of Microbiology, University of Dhaka, Bangladesh. Antifungal screening was carried out against four fungi (*Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Rhizopus oryzae*). These organisms were available in the Microbiology Research Laboratory of the Pharmacy Department, Rajshahi University, Bangladesh. The pure cultures of these fungi were collected from the Department of Botany, University of Rajshahi, Bangladesh. Cytotoxicity was determined against brine shrimp nauplii. Brine shrimp nauplii were obtained by hatching brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) in artificial sea-water (3.8% sodium chloride solution) for 48 h.

Media

Nutrient agar media (Difco laboratories) pH 7.2, nutrient broth media (Difco laboratories) pH 6.8, Sabouraud dextrose agar media (Biolife Vole Monza) pH 5.6 and artificial sea-water (3.8% sodium chloride solution) pH 8.4 were used for antibacterial screening, MIC determination, antifungal screening and cytotoxicity determination, respectively.

Antibacterial screening

In vitro antibacterial screening was carried out by disc diffusion method (9-11), which is a qualitative to semi-quantitative test. Briefly, 20 ml quantities of nutrient agar were plated in petri dish with 0.1 ml of a 10^{-2} dilution of each bacterial culture (18 h old). Filter paper discs (6 mm in diameter) impregnated with various concentrations of plant extract were placed on test organism- seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent methanol followed by drying off was used as negative control. The activity was determined after 18 h of incubation at 37^0 C. The diameters of zone of inhibition produced by the extract were then compared with the standard antibiotic kanamycin 30 µg/disc. Each sample was used in triplicate for the determination of antibacterial activity.

Minimum inhibitory concentration (MIC) determination

Serial tube dilution technique (12,13) was used to determine MIC of the extract against these bacteria. The plant extract (1.024 mg) was dissolved in 2 ml distilled water (3 drops Tween 80 was added to facilitate dissolution) to obtain stock solution having concentration 512 µg/ml. In serial dilution technique, 1 ml prepared stock solution was transferred to test tube containing 1 ml nutrient broth medium to give concentration of 256 µg/ml, from which 1 ml was transferred to another test tube containing 1 ml nutrient broth medium to give concentration of 128 µg/ml and so on to concentration of 2 µg/ml. After preparation of suspensions of test organisms (10^7 organism per ml), 1 drop of suspension (0.02 ml) was added to each broth dilution. After 18 h incubation at 37 °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 was 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 3 drops of Tween 80 and kanamycin were used as negative and positive control, respectively.

Antifungal screening

In vitro antifungal screening was carried out by disc diffusion method (10,11). Here, 20 ml quantities of Sabouraud dextrose were plated in petri dish with 0.2 ml of a 10^{-2} dilution of each fungal culture (10 h old). Filter paper discs (6 mm in diameter) impregnated with various concentrations of the extract were placed on test organism-seeded plates. Methanol was used to dissolve the extract and was completely evaporated before application on test organism-seeded plates. Blank disc

impregnated with solvent methanol followed by drying off was used as negative control. The activity was determined after 72 h of incubation at 30 °C. The diameter of zone of inhibition produced by the extract was then compared with the standard antibiotic nystatin 30 µg/disc. Each sample was used in triplicate for the determination of antifungal activity.

Cytotoxicity assay

The cytotoxicity assay was performed on brine shrimp nauplii using Mayer method (14,15). Brine shrimp nauplii were obtained by hatching brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) in artificial sea-water (3.8% sodium chloride solution) for 48 h. Dissolution of extract was performed in artificial sea-water using DMSO. Each 5 ml solution of different concentrations (0.5, 1, 2, 5, 10, 20 and 40 µg/ml) of the extract was taken in different vials where brine shrimp nauplii were given and observed for mortality for 24 h. The resulting data were transformed to probit analysis (16,17) for the determination of LC_{50} values of the extract. Artificial sea-water medium containing DMSO that was used for the analysis was used as control. Gallic acid and vincristine sulfate were used as standards in this assay.

Acute toxicity study

Acute toxicity study was carried out by using graded doses of each fraction in albino mice. The extract was administered intraperitoneally in graded doses (200 to 1000 mg/kg body weight). They were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality (22).

Results

In the acute toxicity study, the extract was found to be safe and no mortality was observed to a dose as high as 800 mg/kg. The results of antibacterial activity of ethanol extract of tuberous roots of *Amorphophallus campanulatus* against the test bacteria are presented in Table 1. In comparison to reference standard kanamycin (30 mg/disc), the extract exhibited significant antibacterial activity at 300 mg/disc. The plant extract showed highest activity against *Bacillus megaterium* and lowest against *Salmonella typhi*. The MIC values against these gram-positive bacteria ranged from 16 to 32 mg/ml and against gram-negative bacteria from 16 to 128 µg/ml (Table 2).

Table 1. In vitro antibacterial activities of ethanol extract of *Amorphophallus campanulatus* tuberous roots.

Test organism	Strain No.	Diameter of zone of inhibition (mm)		
		Ethanol Ext 150 µg/disc (Mean ± SEM)	Ethanol Ext 300 µg/disc (Mean ± SEM)	Kanamycin 30 µg/disc (Mean ± SEM)
Gram-positive				
Bacillus subtilis	QL 40	18 ± 0.9	23 ± 1.5	31 ± 1.5
Bacillus megaterium	QL 38	17.5 ± 1.3	25 ± 1.6	26 ± 2.1
Staphylococcus aureus	ATCC 259233	19.5 ± 1.1	22 ± 0.9	29 ± 2.3
Streptococcus-β-haemolyticus	CRL	20 ± 1.6	23 ± 1.5	32 ± 2.2
Gram-negative				
Escherichia coli	FPFC 1407	16 ± 1.2	19 ± 1.6	27 ± 1.9
Shigella dysenteriae	AL 35587	15 ± 0.7	21 ± 0.8	32 ± 2.1
Shigella sonnei	AJ 8992	14 ± 1.2	19 ± 1.6	31 ± 2.2
Shigella flexneri	AL 30372	18 ± 1.2	18 ± 1.3	32 ± 1.4
Pseudomonas aeruginosa	CRL	15 ± 1.2	20.5 ± 0.8	30 ± 18
Salmonella typhi	B 56	13.5 ± 1.3	17 ± 1.1	27 ± 1.7

The control disc used for solvent had no zone of inhibition, so their data was omitted from the above data. Data are represented in the form of mean of three tests ± SEM of the standard group.

Table 2. Minimum inhibitory concentrations (MIC) of ethanol extract of *Amorphophallus campanulatus* tuberous roots.

Bacteria	MIC values of ethanol extract (μg/ml)	MIC values of kanamycin (μg/ml)
Bacillus subtilis	32	4
Bacillus megaterium	16	4
Staphylococcus aureus	16	4
Streptococcus β-haemolyticus	32	8
Escherichia coli	32	8
Shigella dysenteriae	32	2
Shigella sonnei	32	4
Shigella flexneri	16	16
Pseudomonas aeruginosa	64	8
Salmonella typhi	128	4

The plant extract showed weak antifungal activity against a number of test fungi (Table 3).

In cytotoxicity assay with brine shrimp nauplii, the LC_{50} value of the ethanol extract of tuberous roots of the plant was 7.66 μg/ml. The cytotoxicity of the plant extract was compared with cytotoxicity of standard gallic acid and vincristine sulfate, LC_{50} values of which were 5.03 and 0.79 μg/ml, respectively (Table 4). No mortality was found in the control group. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted on graph paper.

Discussion

The acute toxicity result reveals that *Amorphophallus campanulatus* might be considered as a broad non-toxic plant. Though it showed activity against all tested bacteria, activity against gram-positive bacteria was better than against gram-negative bacteria. Antibacterial potency of the plant extract against these bacteria expressed in MIC as presented in Table 2 also indicated the plant extract is effective against gram-positive bacteria at lower concentration than that against gram-negative bacteria. Overall, this extract showed significant antibacterial activity against both gram-positive and

Table 3. In vitro antifungal activity of ethanol extract of *Amorphophallus campanulatus* tuberous roots.

Test organism	Diameter of zone of inhibition (mm)		
	Ethanol Ext 150 μg/disc	Ethanol Ext 300 μg/disc	Kanamycin 30 μg/disc
Aspergillus flavus	10 ± 1.4	10 ± 1.1	20 ± 2.1
Aspergillus niger	9 ± 1.2	11 ± 1.3	21 ± 1.8
Candida albicans	8 ± 1.4	9 ± 1.4	19 ± 1.3
Rhizopus oryzae	7 ± 0.9	10 ± 1.5	22 ± 1.0

The control disc used for solvent had no zone of inhibition, so their data was omitted from the above data. Data are represented in the form of mean of three tests ± SEM of the standard group.

Table 4. Cytotoxicity of ethanol extract of *Amorphophallus campanulatus* tuberous roots.

Sample	LC_{50} (µg/ml)	95% confidence limits (µg/ml)	Regression equation	x2 value
Plant extract	7.66	5.77 - 10.17	Y = 2.57 + 2.74 X	3.40
Gallic acid	5.03	3.20 - 7.89	Y = 3.17 + 2.59 X	0.32
Vincristine sulfate	0.79	0.56 - 1.12	Y = 2.60 + 2.65 X	1.71

gram-negative bacteria. The result of antifungal screening indicated that its antifungal application is clinically insignificant. Moderate cytotoxicity of the plant extract indicates that it can be selected for further cell line assay, since many scientists have shown a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts or isolated compounds from terrestrial plants (18-21).

These antibacterial, antifungal and cytotoxic studies are the first reported for this plant. Significant antibacterial and moderate cytotoxic activities found by the experiment support the claims of traditional medicine practitioners for its use as a remedy against tumors and enlargement of spleen. However, to know the exact mechanism of action of *Amorphophallus campanulatus* tuberous root extract, further studies with purified fractions/bioactive compounds are warranted.

Acknowledgement

The authors wish to thank Professor A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi, Rajshahi, Bangladesh for identification of the plant.

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