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Research article

SCREENING OF THE ANTIBACTERIAL ACTIVITY OF *AMORPHOPHALLUS CAMPANULATUS* FROM BASTAR REGION OF CHHATTISGARH, INDIA

Dhananjay Pandey* and A.K. Gupta

School of Studies in Life Sciences, Pt. Ravishankar Shukla University, Raipur- 492010 (Chhattisgarh), India
pandey.dhananjay333@gmail.com; akguptarsu@gmail.com
09200179463 (M); 0771-2262583 (Fax)
*Correspondence author

ABSTRACT: The present study was aimed to evaluate the antibacterial activity of *Amorphophallus campanulatus* (family: Araceae) commonly known as jangli suran. The root, stem and leaf of *A. campanulatus*, extracted successively with polar (aqueous, methanol), dipolar (acetone) and non polar (chloroform) solvents, yielded more phyto compounds in case of root followed by stem and leaf. The extracts were assessed for their antibacterial activity against both gram positive and gram negative bacteria *viz*., *Bacillus cerus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoneae*. The gram positive bacteria were found to be more sensitive than gram negative bacteria. The inhibition of both gram positive and gram negative bacteria by the extracts indicate the presence of broad spectrum antibiotic potential. The root extract of the plant was found to be more effective in inhibiting the bacterial growth as compared to stem and leaf. The methanol root extract of the plant showed maximum inhibition against all gram positive and one gram negative *Pseudomonas aeruginosa* bacteria followed by acetone and chloroform. The highest activity index was recorded in methanol extract of root in *Pseudomonas aeruginosa*. The results were promising and supported the use of plants root by traditional healers in curing several ailments.

Key words: *Amorphophallus campanulatus*, polar & non-polar solvents, extracts, percentage yield, antibacterial activity, activity index

INTRODUCTION

Nature is bestowed with a rich wealth of medicinal plants that are widely used in curing several diseases since traditional times. The phyto-compounds possessing antimicrobial properties are of great interest as the antibiotic resistance is becoming a global public health concern (Vattem et al., 2004). Plants produce a vast variety of organic compounds having antimicrobial activity that are found in different parts such as root, stem, leaves, bark, flowers, fruits or seeds (Cutter, 2000; Jalalpure et al., 2004). The biological and pharmacological activities in the isolated constituents from various part of plants species showed antimicrobial, anti-tumour, antiviral, anti-inflammatory, cardiotonic, contraceptive, anti-platelet, wound healing and prostaglandin inhibitory properties (Sharma et al., 2009). Medicinal plant are the best source of newer drugs as phytochemicals are more specific, biodegradable and are supposed to have fewer side effects. They also possess biological functionality and structural diversity which is crucial for drug discovery (Verpoorte, 2002). Antibiotics are one of the most powerful weapons in combating bacterial infections but the extensive use of antibiotics to combat diseases have led to the emergence of multidrug resistance (Westh et al., 2004). The bacterial species have developed the genetic potentiality to acquire and transmit resistance against currently available antibacterial substances (Nascimento et al., 2000; Sakagami and Kajimura, 2002). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms has led to the screening of several medicinal plants for their potential antimicrobial activity. *Amorphophallus campanulatus* is a perennial herb with rounded tuberous root that is widely distributed in India, Bangladesh and Africa. The tuberous roots of the plants are used traditionally which serves as an excellent anodyne, aphrodisiac, anti-inflammatory, antihemorrhoidal, emmenagogue, expectorant, hemostatic, carminative, digestive, stomachic, anthelmintic and tonic and is quite effective in the treatment of piles and dysentery (Kirtikar and Basu., 1994).

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The antimicrobial activities of this plant have not yet been explored. In this context present study was carried out to screen the antibacterial potentiality of *Amorphophallus campanulatus* against both gram positive and gram negative bacteria *viz*., *Bacillus cerus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiella pneumoneae.*

MATERIALS AND METHODS

Selection of medicinal plant

Medicinal plant *Amorphophallus campanulatus* was selected based on its usage by the tribal community of Bastar in curing several ailments and its ethno medicinal importance. Healthy and disease free plants were selected for the antibacterial screening.

Collection of the sample

The fresh and healthy root, stem and leaves of *A. campanulatus* were collected and identified at Department of Horticulture, Shahid Gundadhoor College of Agriculture and Research Station, Kumhrawand, Jagdalpur from Bastar region, Chhattisgarh, India. The plant samples were washed under running tap water to remove debris and shade dried for about three weeks to attain a constant weight. The dried samples were mechanically grinded by using a mortar and pestle and finally powdered by laboratory grinder machine and stored in separate air tight bottles till use.

Extraction procedure

15 g powdered material was extracted with 150 ml of different solvents according to their increasing polarity successively for 8-10 hours in the soxhlet apparatus at a temperature not exceeding the boiling point of the respective solvents. After extraction excess solvent was removed by distillation and the concentrated extracts so obtained were further dried in incubator at 40^{o}C. The percentage yield and other physical properties were observed. The residual extracts after drying were dissolved in 50% DMSO and stored in refrigerator at 4^{o}C in small and sterile glass tubes.

Microorganisms used for the test

The present study was carried out with the bacterial strains procured from IMTECH, Chandigarh, India. The bacterial strains used for antibacterial screening were *Bacillus cereus* (MTCC-430), *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-96), *Staphylococcus epidermidis* (MTCC-435), *Escherichia coli* (MTCC-1687), *Proteus vulgaris* (MTCC-744), *Pseudomonas aeruginosa* (MTCC-741) and *Klebsiella Pneumoniae* (MTCC-3384). The bacterial strains were maintained on nutrient agar slants, sub cultured regularly and stored at 4^{o}C for further use.

Inoculum preparation

One loop full of overnight grown bacterial culture was inoculated in 25 ml nutrient broth at 37^{o}C on a rotary shaker incubator for 16-18 h. The inoculum size of each bacterial strains were standardized by adjusting the optical density of the culture broth to a turbidity corresponding to 0.08 at 620 nm using a spectrophotometer which is equivalent to 10^{8} cfu/ ml (Basri and Fan, 2005).

Screening of antibacterial activity

The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method (Irobi *et al*., 1994). The bacterial strains were first grown in a nutrient broth for 18 h before use and standardized to 10^{8} cfu/ ml. 200 µl of the standardized cell suspension were spread on Muller Hinton Agar (Hi-media) plate using a sterile swab and air dried to remove the surface moisture. Wells were then bored into the agar using a sterile 6 mm diameter cork borer. The crude extract was introduced into the well at a concentration of 2 mg/ 20 µl, allowed to stand at room temperature for about 1h as a period of pre incubation diffusion to minimize the effect of variation in time between the application of different solutions and later the plates were incubated at 37^{o}C for 24 h. Controls were also set up in parallel and the effects were compared with streptomycin at a concentration of 10 µg/ 20 µl. The plates were observed for the zone of inhibition after 24 h. The experiment was conducted in triplicates and the results are expressed as mean ± SE.

RESULT AND DISCUSSION

The physical properties of the root extracts were almost viscous in nature with reddish brown in colour and the percentage yield was found to be highest in case of polar solvents (5.73%) followed by dipolar and non-polar solvents (Table 1).

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Table-1: Physical characteristics of the extract of *Amorphophallus campanulatus* in different solvents

Characteristics	Chloroform	Acetone	Methanol	Aqueous
ROOT				
% Yield	1.86	2.13	3.86	5.73
Colour	Yellowish Brown	Brown	Reddish	Reddish Brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Consistency	Sticky	Viscous Pasty	Viscous	Highly Viscous
STEM				
% Yield	3.06	0.66	3.53	3.46
Colour	Dark Greenish	Dark Brown	Yellowish Red	Reddish Brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Consistency	Less viscous	Viscous	Viscous pasty	Less viscous
LEAF				
% Yield	3.60	1.06	3.40	2.33
Colour	Dark Green	Greenish brown	Brown	Dark Brown
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Consistency	Sticky	Viscous pasty	viscous	viscous

The highest percentage yield in aqueous solvent might be due to the fact that water is a universal solvent and extracts most of the compounds. Similar findings were also reported by Bae (2004) and Agrawal et al., (2010). Stem and leaf extracts were almost viscous to sticky in nature with almost greenish brown in colour and the percentage yield of the stem and leaf extracts were found to be maximum in case of polar organic solvents (3.53%) and non polar solvents (3.60%) respectively. The study illustrates the presence of various phytochemical in different parts of the plants and their affinity towards different solvents according to their polarity. The antibacterial activity in the crude extract of root, stem and leaf of *Amorphophallus campanulatus* in four different solvents based on their polarity as chloroform, acetone methanol and aqueous were assessed against both gram positive and gram negative bacteria *viz. Bacillus cerus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Proteus vulgaris Pseudomonas aeruginosa* and *Klebsiella pneumoneae*. The root extracts were found to be more effective to these bacteria followed by stem and leaf. The potential sensitivity of the extracts against microorganisms and the zone of inhibition were recorded and are presented in (Table 2) which shows that the methanolic root extract exhibited significant zone of inhibition against all the four gram positive bacteria with maximum against *Bacillus cerus* followed by *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis*. Amongst gram negative bacteria, *Pseudomonas aerugenosa* showed the highest inhibition whereas *Escherichia coli, Proteus vulgaris* and *Klebsiella pneumoneae* were found to be resistant against all the extracts (Fig. 1). The antibacterial activity of the methanolic extracts of stem and leaf showed the highest antibacterial activity of 16.06±0.06 and 15.20±0.20 against *Bacillus cerus* in case of stem and leaf respectively followed by acetone and chloroform, whereas no significant activity was observed in case of their aqueous extract. These results clearly indicate that organic solvents were more suitable for the extraction of the active principles responsible for antibacterial activity as also reported by Das et al., (2010). The decline in activity of aqueous extract might be due to the excessive heating of the aqueous soluble active constituents during the extraction process which often affect biologically active substances such as flavonoids, essential oils and other heterogeneous phytoconstituents present in the extract as also reported by Ekwenye and Edeha (2010). The phytochemical analysis of *A. campanulatus* showed the presence of alkaloids, flavonoids, tannins, polyphenolics, terpenoids, phytosterols, resins and saponin which might be responsible for the antimicrobial activity of plant extracts. In the present study the gram positive bacterial strains were found to be more susceptible as compared from gram negative bacteria against the plant extracts tested. The higher resistance of gram-negative bacteria to plant extracts is due to thick murein layer in their outer membrane, which prevents the entry of inhibitory substances into the cell and have outer phospholipid membrane carrying the structural lipopolysaccharides components, this makes the cell wall impermeable to antimicrobial substances whereas, gram positive bacteria have single layered cell wall with peptidoglycan constituting the outer layer, which is not an effective permeability barrier (Kala and Senthilkumar 2010).

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Table-2: Antibacterial activity (Zone of inhibition in mm, Mean ± SE) of *Amorphophallus campanulatus*

Bacteria	Chloroform	Acetone	Methanol	Aqueous	Streptomycin
ROOT					
B. cerus	14.16±0.16	18.16±0.16	19.13±0.13	ND	27.13±0.13
B. subtilis	11.13±0.13	12.16± 0.16	14.20±0.20	ND	23.06±0.06
S. aureus	12.26±0.26	14.13± 0.13	16.13±0.13	ND	28.03±0.03
S. epidermidis	12.20±0.20	13.06± 0.06	14.26±0.26	ND	26.16±0.16
E. coli	ND	ND	ND	ND	21.13±0.13
P. vulgaris	ND	ND	ND	ND	18.16±0.16
P. aeruginosa	9.13±0.13	13.20±0.20	28.06±0.06	ND	15.10±0.10
K. pneumoneae	ND	ND	ND	ND	20.06±0.13
STEM					
B. cerus	13.13±0.13	14.20±0.20	16.06 ±0.06	ND	27.13±0.13
B. subtilis	10.06±0.06	11.16±0.16	14.10±0.10	ND	23.06±0.06
S. aureus	10.16±0.16	12.10±0.10	14.06±0.06	ND	28.03±0.03
S. epidermidis	11.06±0.06	12.13±0.13	13.20±0.20	ND	26.16±0.16
E. coli	ND	ND	ND	ND	21.13±0.13
P. vulgaris	ND	ND	ND	ND	18.16±0.16
P. aeruginosa	7.06±0.06	10.10±0.10	12.16±0.16	ND	15.10±0.10
K. pneumoneae	ND	ND	ND	ND	20.06±0.13
LEAF					
B. cerus	9.16±0.16	10.10±0.10	15.20±0.20	ND	27.13±0.13
B. subtilis	8.20±0.20	10.26±0.26	12.13±0.13	ND	23.06±0.06
S. aureus	8.10±0.10	14.20±0.20	12.16±0.16	ND	28.03±0.03
S. epidermidis	9.16±0.16	10.13±0.13	11.26±0.26	ND	26.16±0.16
E. coli	ND	ND	ND	ND	21.13±0.13
P. vulgaris	ND	ND	ND	ND	18.16±0.16
P. aeruginosa	ND	ND	ND	ND	15.10±0.10
K. pneumoneae	ND	ND	ND	ND	20.06±0.13

ND- Not detected

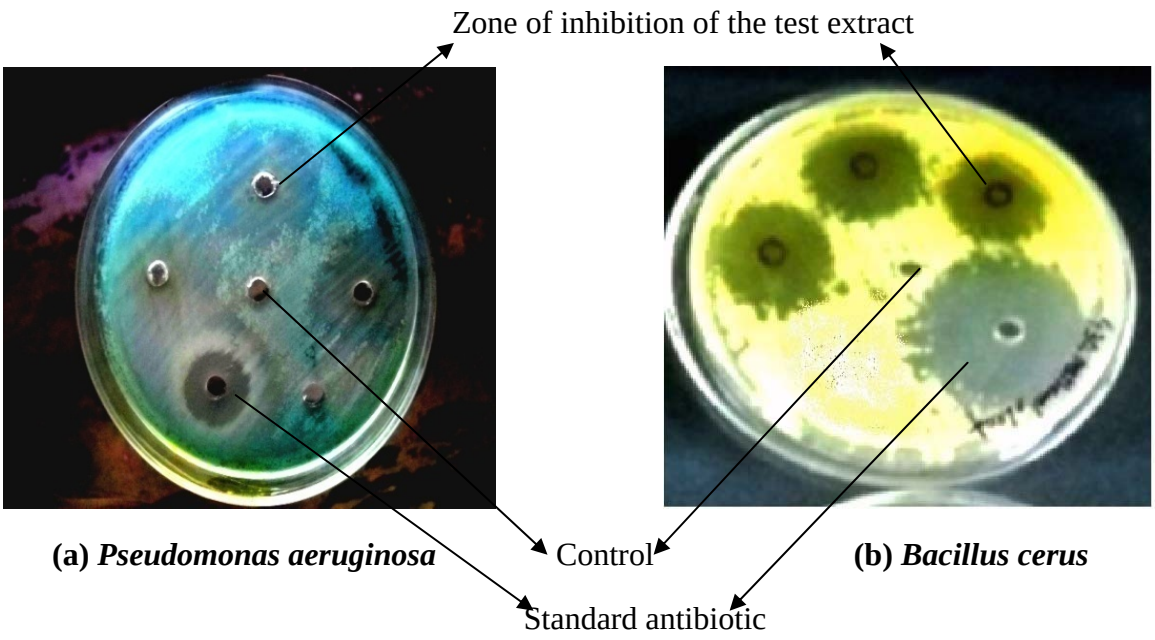

Fig. 1: Antibacterial activity of methanol extracts of root of *A. campanulatus* against *Pseudomonas aeruginosa* and *Bacillus cerus*

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The activity index was calculated to express the relationship between zones of inhibition of the extracts with the standard antibiotics (Usman et al., 2009). Among the root, stem and leaf extracts of *A. companulatus*, the highest activity index of 1.85 was recorded in methanol extract of root in gram negative bacteria as *P. aeruginosa* whereas, activity index of 0.70 was observed for gram positive bacteria as *B. cerus* against streptomycin (Table 3). Higher activity index (>0.5) in the crude extract indicates potential antibacterial activity in the plant. It would be revealing if the extracts are purified and bioactive principles identified. The finding of present study offers a scientific basis for the usage of this plant root by the tribal community as their food and traditional healers in curing bacterial diseases.

Table-3: Activity index of the extract of *Amorphophallus campanulatus* in different solvents

Bacteria	Chloroform			Acetone			Methanol		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
B. cerus	0.52	0.48	0.33	0.66	0.52	0.37	0.70	0.59	0.56
B. subtilis	0.48	0.43	0.35	0.52	0.48	0.44	0.61	0.61	0.52
S. aureus	0.43	0.36	0.28	0.50	0.43	0.50	0.57	0.50	0.43
S. epidermidis	0.46	0.42	0.35	0.49	0.46	0.38	0.54	0.50	0.43
E. coli	-	-	-	-	-	-	-	-	-
P. vulgaris	-	-	-	-	-	-	-	-	-
P. aeruginosa	0.60	0.46	-	0.87	0.66	-	1.85	0.80	-
K. pneumoneae	-	-	-	-	-	-	-	-	-

CONCLUSION

The present investigation was carried out to study the antibacterial activity in the root, stem and leaf extracts of *Amorphophallus campanulatus* in four different solvents against four gram positive and four gram negative bacteria. The results revealed that the methanol root extracts exhibited antibacterial activity against all the four gram positive bacteria and only one gram negative bacteria. The above findings would open an avenue to explore local potential medicinal plants which could be highly useful in combating bacterial diseases with natural, low cost and non-side effect remedies.

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