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ANTIBACTERIAL SCREENING OF *AMORPHOPHALLUS CAMPANULATUS ROXB.* AGAINST SELECTED HUMAN PATHOGENIC BACTERIA

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ABSTRACT

Ethyl acetate, ethanol, acetone, chloroform and petroleum ether extracts of the dried tuber *Amorphophallus campaulatus* were tested for their antibacterial efficacy by the disc diffusion method against four gram-positive bacterial species, *viz.*, *Staphylococcus haemolyticus*, *Staphylococcus lentus*, *Staphylococcus aureus* and *Bacillus cereus* six gram-negative bacterial species *viz.*, *Escherichia coli*, *Serratia marcescens*, *Enterobacter amnigenus*, *Klebsiella pneumonia*, *Klebsiella oxytoca* and *Brevibacterium paucivorans.* Among the various solvent extracts studied ethanol, acetone and chloroform tuber extracts showed a highest antibacterial activity followed by ethyl acetate and petroleum ether.

KEYWORDS: *Amorphophallus campanulatus*, Antibacterial, Disc diffusion method.

INTRODUCTION

Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the three levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific aliments has been in vogue since ancient times. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries.[1]

Infection diseases are one of the main reasons which cause the death, killing almost 50.000 people every day.[2] Medicinal plants are used by 80% of the world population as the only

available medicines especially in developing countries[3], the use of medicinal plants is very wide spread in many parts of the world because it is commonly considered that herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics.[4] The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds.[5]

Resistance to antimicrobial agents is emerging in a wide variety of pathogens and multiple drug, resistance is becoming common in diverse organisms such as *Staphylococcus* sp.[6],[7]

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% of species restricted with the tropics. The family contains several well-known cultivated foliage and flowering plants.[9] Elephant foot yam, botanically equated to *Amorphophallus campanulatus* (Araceae). The corms are dry, acrid, pungent; increases both appetite and taste; digestive, anthelmintic and aphrodisiac; useful in vitiated conditions of vata and kapha, elephantiasis, inflammations, haemorrhoids, haemorrhages, abdominal pain, asthma, piles, dysentery, splenopathy, amenorrhoea, seminal weakness, fatigue, anaemia and general debility.[10,11] There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross infections. The development of new antibiotics should be continued as these are of primary importance to maintain for the effectiveness of antimicrobial treatment.[12] Hence, the present investigation was carried out to analyze the antibacterial activity against selected bacterial pathogens from *Amorphophallus campanulatus.*

MATERIAL AND METHODS

PLANT MATERIAL

The tuber part of plant of *Amorphophallus campanulatus* was collected from Kalrayan Hills located at Viluppuram district.

PREPARATION OF PLANT POWDER

The plant parts were carefully examined and old insect damaged, fungus infected leaves, stems and roots were removed. The selected healthy plant parts were spread out and shade dried in the laboratory at room temperature for 5-8 days or until they broke easily by hand. The dried plant parts were ground to a fine powder by using an electronic blender and the powders were stored in a closed container at room temperature for further uses.

PLANT EXTRACTION

SOLVENT EXTRACTS

Fifty grams of the powdered tuber material was boiled separately with 300 ml of each of the solvents viz. methanol, ethanol, acetone, chloroform and petroleum ether in a soxhlet apparatus for 48 h at different temperatures (depends on the boiling point of the respective solvents). At the end of 48 h each extract was filtered through Whatman No.1 filter paper and filtrates were concentrated at room temperature. The paste like extracts were stored in pre-weighed screw cap bottles and the yield of extracts was calculated based on initial and final weight of the container. These screw cap bottles with the extracts were kept in refrigerator at 4℃. Each of the extract was individually reconstituted by using minimal amount of the extracting solvent prior to use.

ANTIBACTERIAL ACTIVITY TEST (Disc diffusion method)

DISC PREPARATION

The filter paper discs of uniform size are impregnated with the compound (plant extract) usually consisting of absorbent paper. It is most convenient to use Whatman No.1 filter paper for preparing the discs. Dried discs of 6 mm diameter were prepared from Whatman No.1 filter paper and sterilized in an autoclave. These dried discs were used for the test.

TESTED MICROORGANISMS

Antibacterial activity of *Amorphophallus campanulatus* Roxb. Powder extracts was investigated against ten bacterial species such as *Staphylococcus haemolyticus, Staphylococcus lentus, Staphylococcus aureus, Bacillus cereus, Escherichia coli, Serratia marcescens, Enterobacter amnigenous, Klebsiella pneumoniae, Klebsiella oxytoca and Brevibacterium paucivorans.* The species that were purchased from Department of Microbiology, K.AP Viswanatham medical college, Tiruchirappalli, Tamil Nadu.

PROCEDURE

Sterile liquid Muller Hinton Agar medium (pH 7.4 ± 2) was poured (10-15 ml) into each sterile petriplates. The growth media also seem to play an important role in the determination of the antibacterial activity. Based on the report by Lin *et al*. Muller- Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study.[8] After solidification, 100 µl of suspension containing 108 CFU/ml of each test bacteria was spread over Muller Hinton Agar plates. The sterile filter paper discs (6 mm in diameter) were impregnated with 10µl of the 3 mg/ml extracts (30µg/disc) placed on the inoculated agar. Negative controls were prepared in using the same solvents employed to dissolve the plant extract. Chloramphenicol (30µg/disc) was used as positive reference control to determine the sensitivity of the plant extract on each bacterial species. The inoculated plates were incubated at 37° C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones. Each assay was conducted in triplicate.

IV. Statistical analysis

Agar disc diffusion activity was performed in three replicates under strict aseptic conditions to ensure consistency of all conclusions. Data of all experiments were statistically analyzed and expressed as Mean ± Standard Deviation.

Table 1: Antibacterial Screening of Tuber Extracts of *Amorphophallus campanulatus* Roxb. On Pathogenic Bacteria.

Test bacteria	Inhibition zone diameter in mm (mean ± SD)										
	Ethyl acetate		Ethanol		Acetone		Chloroform		Petroleum ether		Positive Control
	Experiment (30 µg/disc)	Negative control	Experimental (30 µg/disc)	Negative control	Experimental (30 µg/disc)	Negative control	Experimental (30 µg/disc)	Negative control	Experimen (30 µg/disc)	Negative control	Chloramphenicol (30 mcg/disc)
Gram-negative											
Staphylococcus hemilyticus	**8±1**	-	**10.33±0.57**	-	**10.66±1.52**	-	**9±2**	-	-	-	**28±0**
Staphylococcus lentus	**7.33±0.57**	-	**10.66±3.51**	-	**12.33±1.52**	-	**8±1**	-	-	-	**23±0**
Staphylococcus aureus	-	-	**12±1**	-	**15±0**	-	**10.33±3.51**	-	-	-	**26.6±0.5**
Bacillus cereus	-	-	**9.33±2.51**	-	**7±1.73**	-	**9.33±1.15**	-	**8±0**	-	**21±0**
Gram-positive											
E.coli	6±0	-	**8.66**±2.08	-	**12**±2	-	**5**±4.35	-	-	-	**25±0**
S. marcescens	-	-	**10.33**±2.08	-	**6.66**±1.15	-	**8.66**±2.88	-	-	-	**28±0**
Enterobacter aerogens	7.33±1.54	-	**12.33**±3.05	-	**9.33**±1.15	-	**7**±1	-	**6.66**±0.57	-	**24±1**
Klebsiella pneumoniae	6.33±0.57	-	**10.33**±0.57	-	**9.66**±2.08	-	**13.33**±1.52	-	-	-	**12.3±0.5**
Klebsiella oxytoca	6±0	-	**8.66**±2.08	-	**12**±2	-	**5**±4.35	-	-	-	**23.6±0.5**
Brevebacterium paucivorans	-	-	**10.33**±2.08	-	**6.66**±1.15	-	**8.66**±2.88	-	-	-	**30±0**

'–' represents as 'no inhibition.

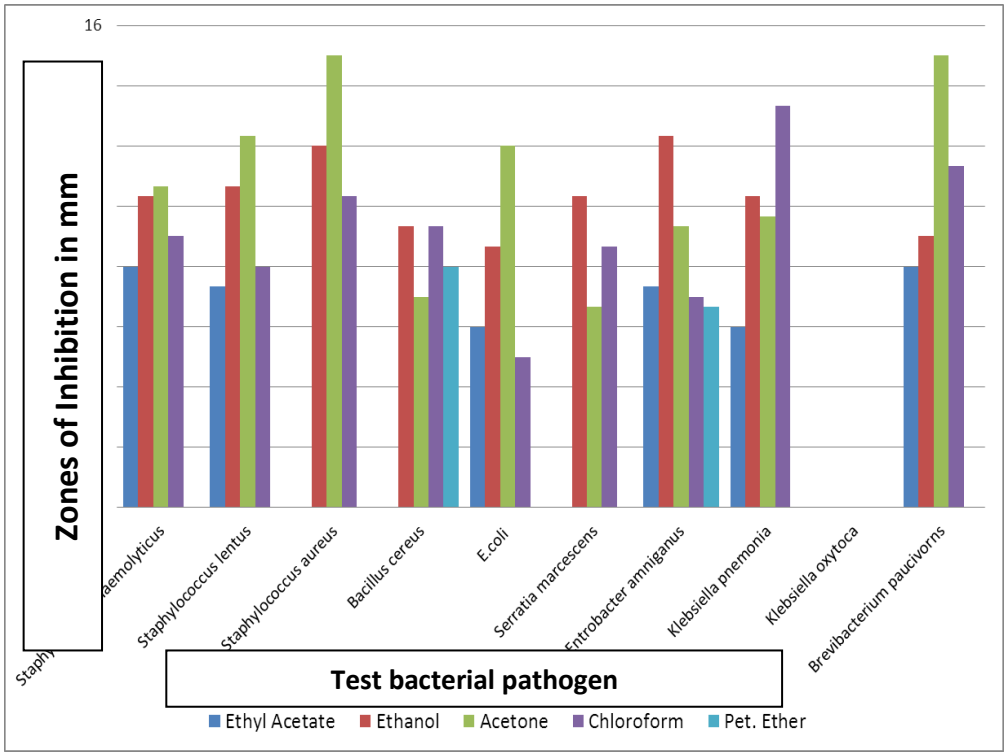

Fig. 1: Antibacterial Screening of Tuber Extracts of *Amorphophallus campanulatus* Roxb. On Pathogenic Bacteria.

RESULT AND DISCUSSION

The ethanol extract was active against *B. subtilis*. Moreover, they found that the ethanol extract was more effective in cases of *S. aureus* and *E. coli*. It has been reported that different solvents have different extraction capabilities (Majorie, 1999).

The results of antibacterial screening are given in the Table- 1. *In vitro* antibacterial activity of the ethyl acetate, ethanol, acetone, chloroform and petroleum ether tuber extract of *A. campanulatus* was evaluated by disc diffusion assay against clinical pathogenic bacteria the bacteria include both gram-positive and gram-negative. When the five solvent extracts were compared with each other and with that of standard antibiotic (Chloramphenicol). The ethanol, acetone and chloroform tuber of the plant extract observed to have highest potential compared to that of ethyl acetate and petroleum ether extract. (Archana *et al*., 2012) reported that Gram negative bacteria are more resistant to plants extract compared to gram positive bacteria.

The studies on *Adhatoda vasica* have higher antibacterial activity toward gram positive and gram negative bacteria. According to (Mihaela., 2010) gram positive bacteria have lack of

additional permeability barrier compared to gram negative which makes it more susceptible toward the plant extracts.

The ethanol tuber extract showed much greater inhibition on *Enterobacter aminogenos* (12.3±3.0), *staphylococcus aureus* (12±1), *staphylococcus lentus* (10.6±3.5), *serratia marcescens* (10.3+2.0), *Klebsiella pneumonia* ((10.3+0.5), *staphylococcus haemolyticus* (10.3±0.5). modetate tuber extract inhibition zones were observed against *bacillus cereus* (9.3±2.5) and *brevebacterium paucivorans* (9±1). Ethanol tuber extract low degree or inhibition was observed against *Escherisia coli* (8.6±7.0). No zone of inhibition against *Klebsiella oxytoca*.

The ethanol extract showed greater activity against both gram positive and gram negative organism. reported that the acetone and alcohol extracts of *A. paniculata* with higher inhibitory activity against *Bacillus subtilis* and *Staphylococcus aureus*.[18]

Acetone tuber extracted also showed significance activity against tested bacteria such as *Brevebacterium paucivouans* (15±0), *Staphylococcus lentus* (12.3±1.3), *Escheritia coli* (12±2) and Staphylococcus haemolyticus (10.6±1.5). Moderated acetone tuber extract inhibition zones were observed against *Klebsiella pneumonia* (9.6±2.0) and *Entetobacter aminogenus* (9.3±1.1). Low degree zones of inhibition in acetone tuber extract against *Bacillus cereus* (7±1.7) and *Serratious marcescens* (6.6±1.7). No zone of inhibition against *Klebsiella oxytoca* (Table.1).

Observation made from chloroform tuber extract showed a highest activity against *Klebsiella pneumonia* (13.3±1.5), *Brevebacterium paucivorans* (11.3±1.5), *Staphylococcus aureus* (10.3±3.5). Moderate chloroform tuber extract inhibition zones were observed against *Bacillus cereus* (9.3±1.1), *Staphylococcus haemolyticus* (9±2), *Serratious marcescens* (8.6±2.8) and *Staphylococcus lentus* (8±1). The minimal zones were observed in chloroform tuber extract against *Enterbacter aminogenos* (7±1) and *Escheritia coli* (5±4.3). No zone of inhibition against *Klebsiella oxytoca*. The acetone extract showed greater activity against gram-positive organism than against gram-negative organism. Antibacterial activity of the acetone and ethyl acetate extracts may be due to the greater solubility of the extract in these organic solvents.[14]

Few bacteria pathogenic moderate zones of inhibition were observed in ethyl acetate tuber extract such as *Brevebacterium paucivorans* (8±1), *Staphylococcus haemolyticus* (8±1), *Enterobacter aminogenos* (7.3 ±1.5), *Staphylococcus lentus* (7.3±0.5), *Klebsiella pneumonia* (6.3±0.5) and *Escheritia coli* (6±0) and other test bacterial pathogen no more zone found observed.

(Mohanasundari., 2007) reported that investigation highlight the fact that the organic solvent extracts exhibit greater antibacterial activity because the active principles were either polar or non-polar and were extracted only through successive organic solvents.

No results found in petroleum ether tuber extract against all test bacteria (Table.1) except *Bacillus cereus* (8±0) and *Enterobacter aminogenos* (6.6±0.5). The minimum zone of inhibition were determined in petroleum ether extract of *M. elengi* and *A. aspera* against *S. aureus* (Prabhat, Ajaybhan, *et al*., 2010).

CONCULSION

It is important to search out and promote medicines that are plant-based. This work will help to identify active ingredients for the treatment of bacterial diseases. Further studies are needed to assess the effects of the selected plants on other pathogenic organisms.

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