



***In vitro* phytochemical screening and antibacterial activity of *Amorphophallus paeoniifolius* (Dennst. Nicolson) against some human pathogens**

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ABSTRACT

Amorphophallus paeoniifolius is indigenous medicinal plants cultivated most part of Asia-pacific region and used in the field of traditional medicine. Main aim of this study is to investigate presence of phytochemical such as alkaloids, phenol, glycosides, saponins, phytosterols, flavonoids, tannins, steroids, and terpenoids and antibacterial potential of various extract of *A. paeoniifolius* using human bacterial pathogens by agar-well diffusion assay. In antibacterial activity, among four extracts, ethyl acetate extract showed maximum activity for *Bacillus subtilis* - MTCC 121 (7.5 mm zone of inhibition) and *Staphylococcus aureus* - MTCC 737 (7 mm of zone of inhibition). Aqueous extract showed 2.75 mm zone of inhibition against *Klebsilla pneumonia* - MTCC 109. Ethanolic extracts showed the zone of inhibition of 3.75 mm for *Staphylococcus aureus* - MTCC 737 and methanolic extract showed 4 mm of inhibition for *Pseudomonas aeruginosa* - MTCC 424. Thus the above studies give basic idea about importance of bioactive compound from this medicinal plants and we can go for further purification and isolation of biologically active molecules from the *Amorphophallus paeoniifolius*.

Key words: *Amorphophallus paeoniifolius*, tuber, phytochemicals, agar well diffusion, antibacterial

INTRODUCTION

Amorphophallus paeoniifolius (Dennst) Nicolson (Araceae) a tuberous, stout indigenous herbaceous medicinal plants commonly known as elephant foot yam, in general grown as vegetable and wildly available tuber plants [1]. This plant commonly grown in various tropical and subtropical regions, particularly in South-east Asia. It is commercially cultivated in India, Sri Lanka, China, Malaysia, Thailand, Indonesia and the Philippines and in tropical regions of Africa. In India, its found to be distributed in the regions of Karnataka, Kerala, Maharashtra, Andra Pradesh, Orissa and Gujarat. It is commonly known as sherla, ujomut, vajramuth, and is one of the red listed medicinal plants of the South India. It has a great export potential since its possess good economical value and commercial cultivation is not in other countries [2-3].

In the field of Ayurvedic medicine tubers are highly valued in vitiated conditions of Vata, Kapha in treatment of piles, haemophilic conditions, skin diseases, intestinal worms, obesity, restorative in dyspepsia, debility. The tubers are used as appetizer, tonic and in stomachache reliever [4-7]. This plant is reported to be used for treatment of cysts, tumors and piles [8]. Apart from this clinical applications, this medicinal plants widely used in folk medicine for treatment of acute rheumatism, tumors, lung swelling, asthma, vomiting, and abdominal pain [9]. Fresh yam acts as an acrid stimulant and expectorant [10]. Moreover, fermented juice from petioles from elephant yam is used to treat diarrhea whereas seeds are used to treat rheumatic swelling [11]. The roots of the plant also possess tonic,

stomachache relieving and appetizer properties [1]. The tuber has been reported to have anti-protease activity [12] anti-bacterial, anti-fungal and cytotoxic as well as analgesic activities [13]. Hence, the present study was performed to investigate the phytochemical screening and antibacterial potential of various extract of *A. paeoniifolius* using four different human bacterial pathogens by agar-well diffusion assay.

EXPERIMENTAL SECTION

Chemicals used

Aqueous, methanol, ethanol, ethyl acetone; DMSO (Dimethyl sulfoxide); obtained from Merck, Darmstadt, Germany. Streptomycin - bacterial antibiotic; were used for analyzing the antibacterial activity of *Amorphophallus paeoniifolius* tuber.

Collection of plant material

For preparation of the solvent extract, disease free fresh tuber were collected from local market of Coimbatore district, Tamil Nadu. Tubers initially washed twice with water, and cut into small pieces (1-2 cm long) and shade dried. Shade dried samples were powdered and samples were stored in air tight container.

Test organisms

Bacterial cultures such as *Bacillus subtilis* – MTCC 121, *Klebsiella pneumoniae* – MTCC 109, *Pseudomonas aeruginosa* – MTCC 424, *Staphylococcus aureus* – MTCC 727 collected from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. These bacterial strains were preserved at 4°C in the recommended broth (IMTECH) as stock cultures and were sub-cultured for 24 h at 37°C prior to use.

Preparation of extracts

About 10 g of powdered sample was extracted with 100 mL of water, methanol, ethanol and ethyl acetate in a soxhlet for 20 cycles. Extracts were then concentrated using flash evaporator. Each sample was made to the concentration of 200µg/mL using DMSO [14].

Phytochemical analyses

The phytochemicals like alkaloids, phenol, glycosides, saponins, phytosterols, flavonoids, tannins, steroids, and terpenoids present in the different solvent extracts of *Amorphophallus paeoniifolius* were estimated [15-16].

ANTIBACTERIAL ACTIVITY

Preparation of inoculums

Bacterial cultures of *Bacillus subtilis* – MTCC121, *Klebsiella pneumoniae* – MTCC109, *Pseudomonas aeruginosa* – MTCC424, and *Staphylococcus aureus* – MTCC 727 were used as test organisms for evaluating the antibacterial potential of tuber extracts of *Amorphophallus paeoniifolius*. A loop full of each culture was suspended in 500 µl of sterilized media broth respectively taken in eppendorfs and was used as inoculums for testing.

Antibacterial activity testing by Agar-Diffusion method

Antibacterial activity of the extracts were performed with *Streptococcus aureus* – MTCC 737, *Klebsiella pneumoniae* - MTCC 109, *Pseudomonas aeruginosa* - MTCC 424 and *Bacillus subtilis* - MTCC 121 were used as test organism. Mueller Hinton agar was prepared, sterilized, poured over the plates and allowed to solidify. The microbes were sub-cultured in the nutrient broth for 24 h and were streaked over the plates. In solidified plates, well created and were poured with 20 µL of the extracts of concentration 200 µg/mL in DMSO. 20 µL of Streptomycin (20 µg/mL in DMSO) was used as a positive control and 20 µL of DMSO was used as a negative control and plates were incubated for 24 h at 37°C.

RESULTS AND DISCUSSION

Phytochemical extraction and analysis

Figure 1 shows four different solvent extractive values (%) of phytochemicals from *Amorphophallus paeoniifolius*. Similarly, In Phytochemical analysis of various solvent extracts of *Amorphophallus paeoniifolius* were given in table 1. Similarly, presence of various phytochemicals extracted from *Amorphophallus commutatus* var. *Wynadensis* reported [17-18].

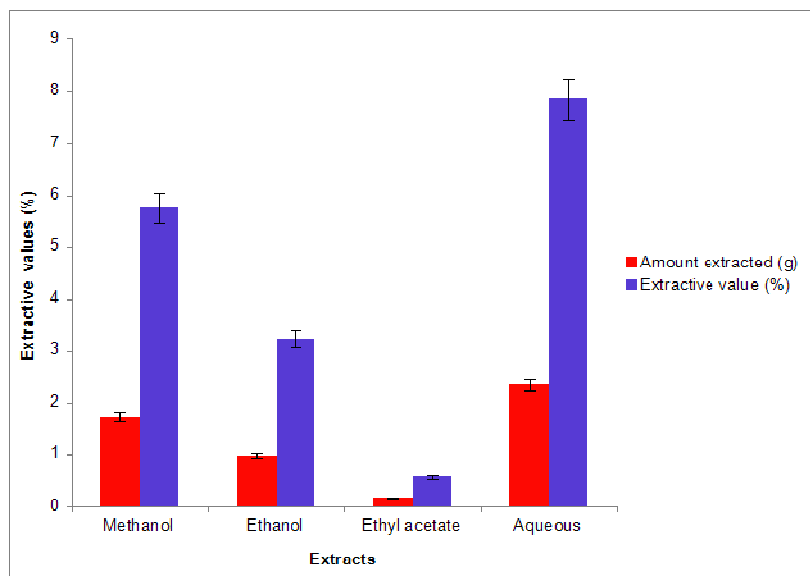


Figure 1. Extractive value of solvent extracts from *Amorphophallus paeonifolius*

Table 1. Preliminary phytochemical screening of *Amorphophallus paeonifolius*

| Tests | Methanol | Ethanol | Ethyl acetate | Aqueous |
|-------------------------|----------|---------|---------------|---------|
| Alkaloids | | | | |
| Mayer's test | - | - | - | - |
| Hager's test | - | - | - | - |
| Wagner's test | - | - | - | - |
| Glycosides | | | | |
| Bontanger's test | - | - | - | - |
| Saponins | + | - | - | + |
| Polysterols | | | | |
| Libermann- Buchard test | + | + | + | + |
| Phenols | | | | |
| Ferric chloride test | + | + | + | + |
| Lead acetate | + | + | + | + |
| Gelatin test | + | + | + | + |
| Flavonoids | | | | |
| Alkaline reagent test | + | + | + | + |
| Tannins | | | | |
| | + | + | + | + |
| Terepenoids | | | | |
| | + | + | + | + |
| Steroids | | | | |
| | + | + | + | + |
| Gum and Mucilage | | | | |
| | + | + | + | + |
| Carbohydrates | | | | |
| Molish test | + | + | + | + |
| Benedict's test | + | + | + | + |

+ indicates the presence of the compound, - indicates the absence of the compound

Antibacterial assay

Antibacterial activity of the tuber (200 µg) was performed against 4 different human bacterial pathogen and results were given in figure 2. The antibacterial activity of all four extracts, ethyl acetate extract showed maximum activity for *Bacillus subtilis* - MTCC 121, *Staphylococcus aureus* - MTCC 737 (7.5 and 7 mm of zone of inhibition respectively). Aqueous extract showed maximum inhibition for *Klebsilla pneumonia* - MTCC 109 (2.75 mm). Ethanolic extracts showed the zone of inhibition of 3.75 mm for *Staphylococcus aureus* - MTCC 737 and methanolic extract showed 4 mm of inhibition for *Pseudomonas aeruginosa* - MTCC 424. Thus all the extracts have a considerable effect on all four bacterial human pathogens. Studies suggest phytochemicals like flavonoids, phenolic compounds, tannins, ascorbic acid, saponins and alkaloids have antibacterial activity. The presence of these phytochemicals in the tuber extracts was responsible for the antibacterial activity.

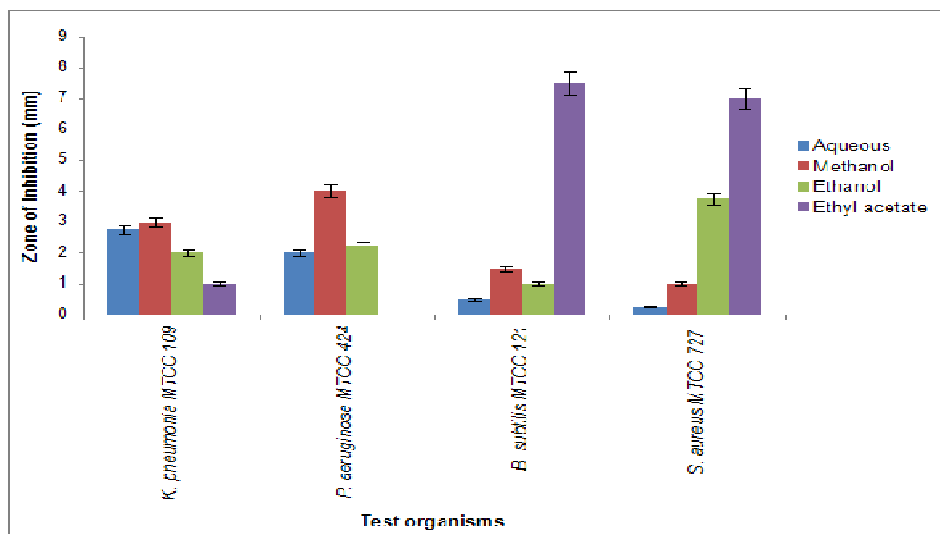


Figure 2. Antibacterial activity of solvent extracts from *Amorphophallus paeoniifolius*

Similarly, three different extracts, like petroleum ether, methanol and water, all three shows maximum anti-bacterial activity against the human pathogens, especially *Salmonella typhi* (34.7 mm) and *E. coli* (27.13 mm) of zone of inhibition [17]. Another report, both aqueous and organic extracts of *Amorphophallus sp.* Shows good antimicrobial properties against grampositive pathogenic bacteria (*Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Streptococcus β-haemolyticus*) and six gram-negative pathogenic bacteria (*Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi*) [19]. Generally, the antimicrobial activity of any medicinal plants is attributed to the presence of secondary metabolites like terpenoids, glycosides etc., [20-21].

CONCLUSION

From these studies, four different extracts of *Amorphophallus paeoniifolius* shows presence of various photochemical and it possess good antibacterial activity against some human bacterial pathogens. Thus, the present study concluded that most of solvent extracts shows presence of Phenols, Glycosides, Polyesterols, Flavonoids, Tannins, Terpenoids, Steroids, Gum and Mucilage and Carbohydrates. In case of antibacterial activity, methanolic extracts shows maximum inhibition for most of the bacterial isolates, similarly, ethyl acetate shows maximum inhibition for *Bacillus subtilis* - MTCC 121, *Staphylococcus aureus* - MTCC 737 (7.5 and 7 mm of zone of inhibition respectively). Further studies are in progress to isolation and purification of biologically active molecules from the *Amorphophallus paeoniifolius*.

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