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Phytochemical Analysis and *in vitro* Antimicrobial Activity of Extracts from *Amorphophallus paeoniifolius* (Dennst.) Nicolson and *Amorphophallus commutatus* (Schott) Engl. corms

Chetana Anand Salunke and Rajendra A. Satpute

Govt. Institute of Science, Aurangabad 431004, Maharashtra, India.

Corresponding author: Chetana Anand Salunke; email : salunkechetana630@gmail.com

Abstract

The increasing antibiotic resistance of different strains of pathogens is a global issue. To fight against this problem best, solution is to use traditional medicine system i.e. use of medicinal plants of our community for disease management and control. This study is focused on exploring the phytochemical and antimicrobial properties of *Amorphophallus paeoniifolius* and *Amorphophallus commutatus* corms. For extract preparation, solvents used were methanol, hexane and distilled water. Qualitative phytochemical analysis of corm extracts confirmed the presence of glycosides, saponins, tannins, phenols, flavonoids, carbohydrates and alkaloids. The corm extracts were tested against four bacterial and two fungal pathogens by disc diffusion assay by Kirby Bauer method. The corm extracts of both the *Amorphophallus* species exhibited inhibitory activity against all the fungal and bacterial pathogens tested. The experiment confirmed the potential of both the species of *Amorphophallus* studied and hence corms of both the plants can be used for isolation of bioactive compounds and related pharmacological activities.

Key words: *Amorphophallus paeoniifolius*, *Amorhophallus commutatus*, phytochemicals, antimicrobial activity, corm

Introduction

The Genus *Amorphophallus* of family Araceae is a Monocot, annual, tuberous and herbaceous plant. Genus *Amorphophallus* have more than 200 species in the World (Mayo et al., 1997; Jallel et al., 2011) and 18 in India (Jallel et al., 2011). From top of the underground hemispherical corm compound leaf emerges with a trunk like petiole. Different species of *Amorphophallus* are cultivated for its edible tubers (Tindall, 1983). The tribal people of some villages of Rajasthan uses corms of *Amorphophallus* as antidote for snake bite (Jain et al., 2005). Many wild species of *Amorphophallus* are used as fodder, medicine and for wine production (Hetterschied and Ittenbach, 1996). Corms of *Amorphophallus* are widely used in Indian medicine and are recommended as a remedy in all three of the Indian Medicinal systems viz., Siddha, Ayurveda and Unani (Khare, 2007). The corms of *Amorphophallus* are used for acute rheumatism, enlargement of spleen, asthma, boils and abdominal tumors (Yusuf et al., 1994); cysts, tumors, and piles (Kavita et al., 2011; Ravikumar and Ved, 2004). A flavonoid obtained from corm showed antimicrobial activity (Khan et al., 2008).

The aim and objective of this study is to examine the antimicrobial potential of phytochemicals present in the corms of *Amorphophallus paeoniifolius* and *Amorphophallus commutatus*.

Materials and Methods

Collection of plant materials and preparation of samples

The fresh corms of *A. paeoniifolius* were brought from local market of Lonavala, while corms of *A. commutatus* were collected from forest area of Don Bosco High School and Junior College, Lonavala, (18.7557° N, 73.4091°E) of Maharashtra.

Amorphophallus paeoniifolius corm

Amorphophallus commutatus corm

The corms of *A. commutatus* and *A. paeoniifolius* were washed thoroughly with tap water, air dried, sliced into pieces and dried under shade. Dried corm pieces were ground in an electric blender into powdered form. The powdered samples were stored in airtight containers in refrigerator at 4°C until used.

Preparation of plant extracts

The plant extracts were prepared by Ayurvedic Pharmacopeia method. Ten gram of powder was soaked in 100 ml of distilled water, methane and Hexane separately. The mixtures were stirred for 3-4 hrs on a rotary shaker and allowed to stand for 24 hrs. Mixtures were filtered by using Whatman filter paper. The extracts were concentrated on a water bath and dried in a vaccum. (The Ayurvedic Pharmacopeia of India, 2008).

Qualitative phytochemical analysis

The crude powder, aqueous and methanol extracts were subjected to phytochemical analysis to confirm the presence of alkaloids, tannins, phenols, carbohydrates, proteins, amino acids, lipids, glycosides, steroids, flavonoids and saponins using standard techniques.

Thin layer chromatography (TLC)

The phytocompounds of the Methanol and Hexane extracts were analysed by Thin Layer Chromatography. About 10 µl of each extract was applied on preloaded aluminium Silica gel plate (TLC Silica Gel – 60 F_{254}, Merk). The plates were developed in an air tight chamber containing Toluene : Ethyl acetate (7 : 3) as a solvent. The developed plates were air dried, observed under visible light, in UV chamber at 254 nm,365 nm, by keeping in Iodine chamber and finally by spraying with reagent Methanol Sulphuric acid (5%). The Rf values of different spots were calculated (Table 2 and 3).

Antimicrobial activity

Procurement of culture

The pure cultures of the microorganisms were obtained from the National Collection of Industrial Microorganisms (NSIM), NCL, Pune. The bacterial cultures of *Streptomyces albus*, *Escherichia coli*, *Proteus mirabilis*, *Streptococcus faecalis* and the fungi used for testing were *Candida albicans* and *Aspergillus niger*.

Disc diffusion assay by Kirby Bauer method

All the extracts were subjected to antimicrobial assay using disc diffusion assay by Kirby Bauer technique. The nutrient plates were prepared by pouring 20 ml Mueller Hinton agar and Potato Dextrose agar in sterile petri plates for bacteria and fungi respectively and allowed to solidify. Twenty four hours old bacterial cultures and 48 hrs fungal cultures were inoculated by using cotton swabs. The cotton swabs were dragged across the agar surface in a zigzag pattern for uniform inoculation. The sterilized filter paper discs of 5 mm were dipped into individual extracts and placed on the swabbed agar plates before incubation period of 48 hours at 37° C for bacteria and 72 hrs at 30° C for fungi. The plates were observed for zones of growth inhibition, and the diameter of these zones was measured in mm. The effect of the extract was compared with the standard antibiotics viz.; Fluconazole and Streptomycin for fungi and bacteria respectively.

Results and Discussion

Phytochemical analysis

The phytochemical composition of corm extracts (Table 1) indicates the presence of alkaloids, tannins, phenols, carbohydrates, glycosides, flavonoids and saponins. The plant derived compounds have a potential for many biological activities including antimicrobial activity (Savoia, 2012) and hence the plant secondary metabolites serve as defence mechanisms against many microorganisms (Vaghasiya et al., 2011). Thus, presence of various phytochemicals confirms the use of both the species of *Amorphophallus* as a potent source of phytomedicine.

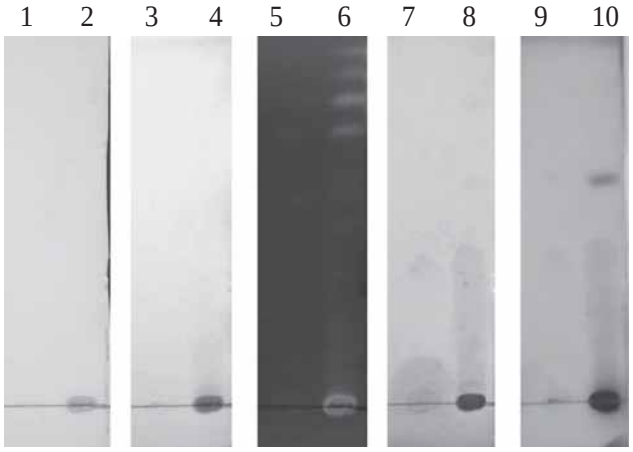
Antimicrobial activity

The methanol, hexane and aqueous extracts of both the species of *Amorphophallus* were tested against the pathogens viz., *E. coli*, a most common causative agent of gastroenteritis, *P. mirabilis* which is causative organism of urinary tract infection and kidney stones, *S. faecalis* which can cause urinary tract infection, meningitis, wound infection, *S. albus*, a causative agent of wound infection and acne vulgaris, *C. albicans* which causes

Table 1. Phytochemical screening of *Amorphophallus* species

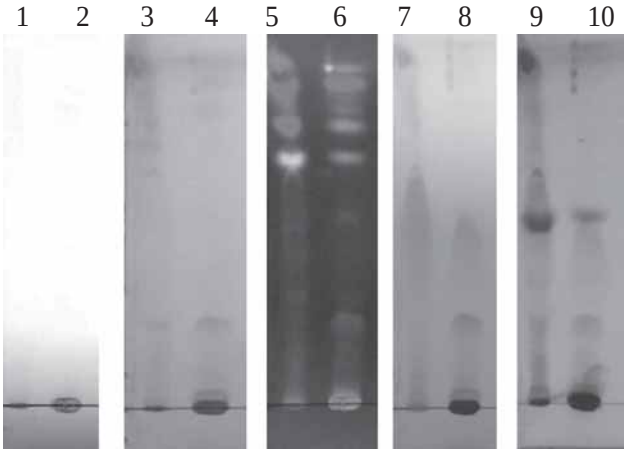
Extract	Name of the compound	*Amorphophallus paeoniifolius*	*Amorphophallus commutatus*
Methanol Extract	Alkaloids	+	+
	Saponins	-	-
	Flavonoids	+	+
	Tannins	+	+
	Phenol	+	+
	Carbohydrates	-	-
	Proteins	-	-
	Amino acids	-	-
	Lipids	-	-
	Glycosides	+	+
	Steroids	-	-
Aqueous Extract	Alkaloids	+	+
	Saponins	-	-
	Flavonoids	+	+
	Tannins	+	+
	Phenol	+	+
	Carbohydrates	-	-
	Proteins	-	-
	Amino acids	-	-
	Lipids	-	-
	Glycosides	+	+
	Steroids	-	-
Crude Powder	Alkaloids	+	+
	Saponins	+	+
	Flavonoids	+	+
	Tannins	-	-
	Phenol	-	-
	Carbohydrates	+	+
	Proteins	-	-
	Amino acids	-	-
	Lipids	-	-
	Glycosides	+	-
	Steroids	-	-

Key : + present, - absent



Lane 1 – Hexane extract under visible light
Lane 2 – Methanol extract under visible light
Lane 3 – Hexane extract under UV 254nm
Lane 4 – Methanol extract under UV 254nm
Lane 5 – Hexane extract under UV 365nm
Lane 6 – Methanol extract under UV 365nm
Lane 7 – Hexane extract in Iodine chamber
Lane 8 – Methanol extract in Iodine chamber
Lane 9 – Hexane extract after derivatization
Lane 10 – Methanol extract after derivatization

Fig. 1. TLC Analysis of *Amorphophallus paeoniifolius*



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Lane 6 – Methanol extract under UV 365nm
Lane 7 – Hexane extract in Iodine chamber
Lane 8 – Methanol extract in Iodine chamber
Lane 9 – Hexane extract after derivatization
Lane 10 – Methanol extract after derivatization

Fig. 2. TLC Analysis of *Amorphophallus commutatus*

Table 2. TLC analysis of *Amorphophallus paeoniifolius*

	Rf values of Major spots	
	Methanol extract	Hexane extract
Visible light	0.05 and 0.21 (Yellow)	Nil
UV 254nm	0.05, 0.10 (Blue)	Nil
UV 365nm	0.05, 0.10, 0.21 (Blue), 0.36 (Yellow), 0.47, 0.57, 0.73, 0.78, 0.84, 0.89 (Blue)	0.57, 0.73, 0.78, 0.84 (Blue)
Iodine	0.05, 0.21, 0.36, 0.47, 0.52, 0.73, 0.78, 0.84, 0.89 (Yellow)	0.36, 0.52, 0.73, 0.78, 0.84, 0.89 (Yellow)
After derivatization	0.05, 0.21, 0.36, 0.47, 0.52, 0.73, 0.78, 0.84, 0.89 (Grey)	0.36, 0.52, 0.73, 0.78, 0.84, 0.89 (Yellow)

Table 3. TLC analysis of *Amorphophallus commutatus*

	Rf values of Major spots	
	Methanol extract	Hexane extract
Visible light	0.02, 0.8 (Yellow)	Nil
UV 254nm	0.03, 0.08, 0.2 (Blue), 0.75 (Yellow)	0.2, 0.65, 0.75 (Blue), 0.72 (Yellow)
UV 365nm	0.02 (Pink), 0.05, 0.10 (Blue), 0.21, 0.26 (Greenish), 0.23, 0.36, 0.45 (Orange), 0.68 (Green), 0.76, 0.78, 0.84 (Red), 0.92 (Blue)	0.02 (Green), 0.05, 0.10 (Blue), 0.21, 0.26 (Greenish), 0.23, 036, 0.45 (Orange), 0.68 (Green), 0.76, 0.78, 0.84, 0.91 (Red)
Iodine	0.05, 0.21, 0.42, 0.52, 0.62, 0.78, 0.92 (Yellow)	0.05, 0.21, 0.42, 0.52, 0.62, 0.78, 0.92 (Yellow)
After derivatization	0.05, 0.16, 0.21, 0.40, 0.42, 0.52, 0.62, 0.78, 0.92 (Grey)	0.05, 0.16, 0.21, 0.40, 0.42, 0.52, 0.62, 0.78, 0.92 (Grey)

urinary tract infection, genital yeast infection, oral thrush, fungal skin infection and *A. niger*, a causative agent of aspergillosis and otomycosis. Methanol and Hexane extracts showed good inhibitory activity against all the bacterial and fungal pathogens used for the study but aqueous extracts of both the species did not show inhibitory activity against any pathogen. The comparative analysis of inhibitory effect of methanol, hexane and standard antibiotics against tested microorganisms by disc diffusion method is shown in Fig. 3.

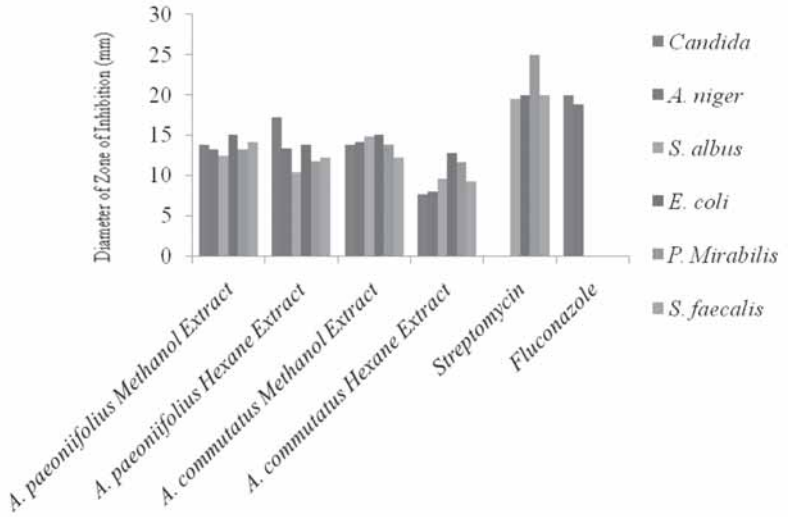

Fig.3. Comparative analysis of inhibitory effect of corm extracts and standard antibiotics against selected pathogens

TLC analysis

The results of Thin Layer Chromatography of Methanol and Hexane extracts given in Table 2 and 3 indicates presence of different phytochemicals in corms of both the species of *Amorphophallus* as many spots were observed. Antimicrobial activity of any medicinal plant is related to the presence of different secondary metabolites (Goldy and Kalra, 2008; Daisy et al., 2008). Majority of drugs used worldwide possess ethnomedicinal properties, associated with use of bioactive components of the plant (Daniel and Norman, 2001). Most of these drugs cannot be synthesized economically and hence are obtained directly from the plants (Hamburger Hostettman, 1991).

Conclusion

The presence of wide range of phytochemicals in corms of both the species of *Amorphophallus* confirms that these plants are potential source of medicinal dietary crops. The present study supports the ethnomedicinal use of *Amorphophallus* corms against different bacterial and fungal pathogens tested for antimicrobial activity. Further research can be done for isolation and purification of drugs for the treatment of various diseases.

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