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Morphological, phytochemical, and anti-bacterial properties of wild and indigenous plant (*Amorphophallus commutatus)*

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This study was undertaken to investigate the phytochemical and anti-bacterial properties of *Amorphophallus commutatus* var. *wynadensis* tubers using solvents of increasing polarity (viz: petroleum ether, hexane, and water). The chemical constituents of various extracts were obtained by the phytochemical analysis. For the anti-bacterial studies, both gram positive bacteria (*Streptococcus pyogenes*, *Streptococcus faecalis* and *Bacillus cereus*) and gram negative bacteria (*Enterobacter aerogenes, Escherichia coli, Salmonella typhi,* and *Proteus mirabilis*) were used. Among the various extracts, the petroleum ether extract (PEE) showed maximum zone of Inhibition (ZOI) against *S. typhi and P. mirabilis* with ZOI as 35 and 34 mm, respectively. The morphological aspects of *A. commutatus* are also present.

Key words: *Amorphophallus commutatus* var. *wynadensis*, tuber, phytochemical analysis, anti-bacterial activity, petroleum ether extract, zone of inhibition.

INTRODUCTION

From time immemorial, traditional medicinal plants have been used as various types of active principles. WHO has estimated that 70% of the world population is dependent on traditional herbal medicines. Out of the total 225000 plants species, only less than 10% have been studied so far for their medicinal properties (Widjaja and Lester, 1978; Kelmanson et al., 2000). India has rich floral diversity of herbal plants and the traditional medical system which is several hundred years old. Medicinal plants attribute for physiologically active properties for the treatment of numerous ailments (Adebanjo et al., 1983; Natarajan et al., 2005). In tropical and subtropical Asia, the genus *Amorphophallus* has been used as a food source and as a traditional medicine for centuries. They are perennial plants with a highly dissected umbrella-shaped leaf blade and an underground stem in the form of a corm (Chua et al., 2010). Nine species of *Amorphophallus*, that is,*Amorphophallus corrugatus* N.E.Br.; *Amorphophallus yunnanensis* Engl.; *Amorphophallus albus* P. Y. Liu and J. F. Chen; *Amorphophallus kachinensis* Engl. and Gehrm.; *Amorphophallus krausei* Engl.; *Amorphophallus konjac* K. Koch ex N.E.Br.; *Amorphophallus paeoniifolius* (Dennst.) Nicolson; *Amorphophallus yuloensis* H. Li; and *Amorphophallus nanus* H. Li and C.L. long have been reported and used as medicine, fodder, food, and for wine production (Liu, 2004). In India, *Amorphophallus commutatus* Engl. belonging to the family Araceae is found to be distributed in the regions of Kerala, Karnataka, Maharashtra and Gujarat. It is commonly known as sherla, ujomut, vajramuth, and is one of the red listed medicinal plants of the South India. Its tuberous

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Figure 1. *A. commutatus* (Schott) Engl. var. *wayanadensis* (a) Tuber; (b) inflorescence; (c) Infructescence; (d) a portion of petiole showing mottling; (e) Lamina-side view.
Source: Sivad and Jaleel (2002).

corms are reported to be used for treatment of piles, cysts and tumors (Ravikumar and Ved, 2004). It is also used as cure for snake bite by tribal folks living in villages of Sitamata wildlife, sanctuary, Rajasthan, India (Jain et al., 2005; Kavitha et al., 2011). The morphology and taxonomy of *A. commutatus* has been studied by Sivadasan and Abdul Jaleel (2002), but its medicinal properties have not been studied till now. In this study, the petroleum ether, methanol, and water extracts of tuber corms of this plant species are investigated for its phytochemical analysis and their antimicrobial activity.

MATERIALS AND METHODS

Plant materials

The plant species were collected from the forest hills (Western Ghats) of Wayanad district, Kerala (11°36′18″N latitude and 76°04′59″E longitude). The plant species were identified and authenticated by Dr. V. Abdul Jaleel, Department of P.G Studies and Research in Botany, Sir Syed College, Thaliparamba, Kerala and was deposited (Herbarium specimen no. RIA 62) in Calicut University Herbarium (CALI), Malappuram, Kerala. For preparation of extracts, disease free fresh tuber corms were used.

Bacterial strains

Seven species of human pathogenic bacteria were obtained from the National Collection of Industrial Microorganisms (NCIM), Pune, India, and Microbial Type Culture Collection (MTCC), Chandigarh, India. The gram positive bacterial species used for testing were *Streptococcus pyogenes* (NCIM 2606), *Streptococcus faecalis* (NCIM 2405), and *Bacillus cereus* (NCIM 2458); and the gram negative tested for the activity were *Enterobacter aerogenes* (NCIM 5139), *Escherichia coli* (NCIM 2810), *Salmonella typhi* (MTCC 733), and *Proteus mirabilis* (NCIM 2388**)**. These strains were preserved at 4°C in the nutrient broth as stock cultures and were sub-cultured for 24 h at 37°C prior to use.

Preparation of extracts

The tuber corms were properly washed followed by surface sterilization using 1% of sodium hypochlorite (Maina et al., 2010). The tubers were chopped into pieces, sun dried, and powered using an electric blender. Extraction was done by Soxhlet apparatus (Gennaro et al., 2008) using solvents viz: petroleum ether, methanol, and water in the increasing order of their polarity. The obtained solvent extracts were concentrated using vacuum distillation process (Ferreira et al., 2006). The extracts were stored in refrigerator at 4°C (Ayvaz et al., 2008; Sultana et al., 2009).

Phytochemical analysis

Phytochemical tests were carried out on the petroleum ether, methanol and aqueous extract of plant materials using standard procedures (Trease and Evans, 1978; Edeoga et al., 2005). The analysis was done to test the presence of phytochemicals like alkaloids, amino acids, carbohydrates, phenols, terpenoids, glycosides, fixed oils and fats, proteins, flavonoids, anthoquinones, saponins, coumarin, Anthocyanin, Flavanones, tannins, quinones, and steroids.

Anti-bacterial assay

The anti-bacterial assay was performed on both gram positive and gram negative bacterial species by well diffusion method (Onkar and Dhingra, 1995). The petriplates were poured with approximately 25 ml autoclaved nutrient agar media (HIMEDIA) each. Using a micropipette, standardized inoculum (0.1 ml) of 0.5 McFarland turbidity standards, equivalent to 5×10^8 cfu/ml (McFarland, 1907; Lopez-Brea et al., 2008) was aseptically spread on the surface of nutrient agar plate. After drying, four wells were punched on each plate using a sterile cork borer of 8 mm diameter (Bradshaw, 1992). 0.1 ml of each extract (concentration of 100 mg/ml each) was pipetted into respective wells (Ayfer and Turgay, 2003; Ramesh et al., 2010). 10% Dimethyl sulfoxide HIMEDIA was used as vehicle control. Erythromycin (HIMEDIA) (0.5 mg/ml), a broad spectrum antibiotic was used as positive control. The Petri plates were incubated overnight at 37°C and the anti-bacterial activity was measured after 18 h of incubation. The diameter of ZOI was also measured. All the plant extracts were tested and calculated in triplicates with the mean values (Gupta et al., 1962).

RESULTS

Morphological properties

A. commutatus (Figure 1: a to e) is a tuberous herb. The

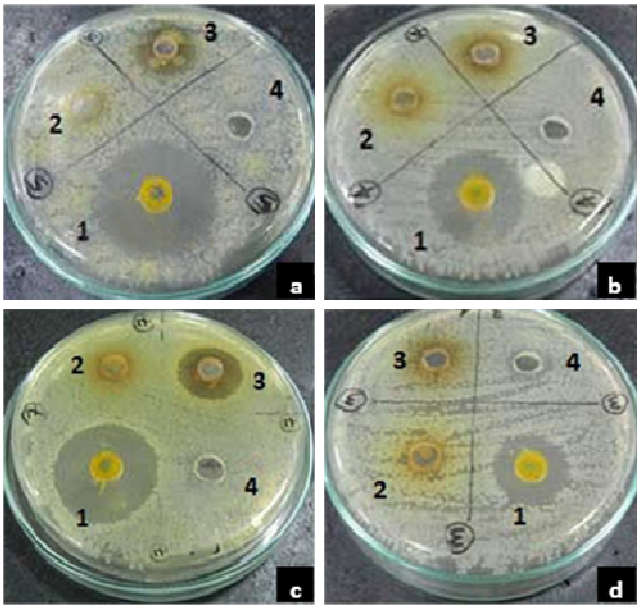

Figure 2. Anti-bacterial activity of tuber extracts of *A. commutatus* var. *wynadensis* with four different solvents. The solvents are petroleum ether (1); Methanol (2); Water (3); dimethyl sulfoxide (4); against a: *S. typhi*; b: *P. mirabilis*; c: *E. coli; and* d: *E. aerogenes*.

tuber (Figure 1a) is subglobose, circa (ca.) 14 cm diameter, and ca. 5.5 cm thick, skin pale brown in color with numerous roots. The petiole (Figure 1d) is cylindrical, smooth, light greenish with whitish mottles. Lamina (Figure 1e) is ca. 80 cm broad, with ovate-oblong leaflets and an acuminate apex. The leaflets are ca. 15 cm long and ca. 4.5 cm broad, with its upper surface dark green, and lower surface light green in color. Its margin is entire and the marginal surface is undulate. The peduncle is ca. 85 cm long and ca. 1.5 cm in diameter at base, and tapers towards the top. It is light green with greenish black and purplish green spots. The spathe is ovate-oblong and is as long as the spadix, ca. 28 cm long, and 11 cm broad, with a convolute basal portion. Its limb is expanded, erect with acute apex, purplish at the top, and has greenish purplish at the base with white mottles.

The spadix is sessile, ca. 29 cm long. The female zone is ca. 2.4 cm long and ca. 1.4 cm in diameter while, the male zone is ca. 3.8 cm long and 3.8 cm in diameter. The appendix of terminal spadix is an elongate conoid with a rounded apex ca. 22 cm long, and 1.5 cm diameter, tapering towards the tip, smooth and reddish brown in color. The female flowers have a subglobose ovary, ca. 1.7 mm long and 0.2 mm diameter, green in color, and unilocular with a single basal anatropous ovule. The style is very short or absent with light purplish color. The stigma is discoid with orange color. Male flowers are sessile, loosely arranged, pale yellowish, with inconspicuously 2-lobed purplish connectives ca. 2 mm long and 2.25 mm broad. Fruits are broadly ovate red-colored berries, ca. 1.2 cm long and 0.8 cm diameter. The seeds are ca. 0.8 cm long and 0.5 cm diameter.

Phytochemical analysis

The preliminary test carried out on the petroleum ether, methanol and aqueous extract (Table 1) showed the presence of wide variety of phytochemicals. The presence of terpenoids and glycosides were confirmed in all the three extracts. PEE also showed the presence of fixed fats and oils, and coumarins. Most of the phytochemicals were found in methanol and aqueous extracts. Thick red precipitate formed in the Fehlings' test confirmed the strong presence of carbohydrates in methanol and aqueous extract of tuber corms.

Anti-bacterial assay

The results of anti-bacterial assay (Table 2) revealed that PEE was most effective against *S. typhi* (Figure 2a) and *P. mirabilis* (Figure 2b) with a large inhibitory zone. It had also shown significant activity against *E. coli* (Figure 2c) and *E. aerogenes* (Figure 2d). Methanol extract had a good potency against *S. pyogenes* and *S. faecalis* with ZOI 19.06 and 18.86 mm, respectively. Aqueous extract had shown appreciable activity against gram negative bacteria viz: *E. coli, S. typhi,* and *P. mirabilis* except *E. aerogenes*. While it showed no considerable activity against gram positive bacteria. The ZOI of erythromycin (positive control) was also presented in Table 2. There was no ZOI with the vehicle control.

DISCUSSION

Emergence of resistant strains of bacteria and potent side effects of modern chemical based drugs demands the need for new antibiotics (Eloff, 1998). Among the globally used drugs, 80% of them possess ethnomedical properties, indistinguishable or associated to the present use of the active constituents of the plant (Daniel and Norman, 2001). It is also estimated that 60% of anti-infectious drugs commercialized or under clinical trials are from natural sources (Yue-Zhong, 1998). Majority of these cannot be synthesized economically and are acquired directly from plants (Hamburger and Hostettmann, 1991; Rates, 2001).

The strong presence of carbohydrates, absence of proteins, and amino acids revealed in the phytochemical analysis of *A. commutatus* var. *wynadensis* (Table 1) showed similarity with the previous reports on *Amorphophallus campanulatus*, another species of the same genus (Firdouse and Alam, 2011). The anti-microbial activity of plant extracts (solvent based) is proportionate with the type and quantity of active chemical constituents in it (Fokunang et al., 2000).

Table 1. Preliminary phytochemical analysis of tuber extracts of *A. commutatus* var. *wynadensis.*

Chemical constituent	Test	Tuber extract		
		Petroleum ether	Methanol	Water
Alkaloids	Wagner's test	-	+	-
Amino acids	Ninhydrin test	-	-	-
Carbohydrates	Fehlings test	-	+	+
Phenols	Ferric chloride test	-	+	+
Terpenoids	Salkowski test	+	+	+
Glycosides	Keller kiliani test	+	+	+
Fixed oils and fats	Spot test	+	+	-
Proteins	Biuret test	-	-	-
Flavanoids	Shinoda test	-	-	-
Anthoquinones	Borntrager's test	-	-	-
Saponins	Foam formation test	-	-	-
Coumarin	Alkaline test	+	-	-
Anthocyanin	Alkaline test	-	-	-
Flavanones	Sodium hydroxide test	-	+	+
Quinones	Sulfuric acid test	-	+	+
Tannins	Ferric chloride test	-	+	+
Steroids	Libermann burchard test	-	+	-

+ indicates presence; - indicates absence.

Table 2. Anti-bacterial assay of tuber extracts of *A. commutatus* var. *wynadensis*.

Micro-organism	Zone of inhibition (in mm) ± SD			
	Petroleum ether	Methanol	Water	Standard drug (erythromycin)
S. pyogenes	14.83 ± 0.76	19.06 ± 0.30	0 ± 0.00	20.3 ± 0.7
S. faecalis	10.96 ± 0.5	18.86 ± 0.15	0 ± 0.00	23 ± 0.2
E. aerogenes	21.9 ± 0.17	11.23 ± 0.68	0 ± 0.00	22.03 ± 0.25
E. coli	27.13 ± 0.32	10.73 ± 0.25	11.8 ± 0.2	24.93 ± 0.11
S. typhi	34.7 ± 0.7	11.96 ± 0.05	22.13 ± 0.15	25 ± 0.9
B. cereus	0 ± 0.00	18.56 ± 0.30	0 ± 0.00	22.9 ± 0.15
P. mirabilis	34.06 ± 0.20	11.56 ± 0.20	23.96 ± 0.45	20.2 ± 0.72

The anti-bacterial test results (Table 2) suggested that the extracts showed a better activity against the gram negative bacteria. Among the three extracts, PEE presented maximum anti-bacterial activity against the human pathogens. Especially, significant activity was shown against the typhoid causing pathogen, *S. typhi* with a ZOI of 34.7 mm. Generally, the antimicrobial activity is attributed to the secondary metabolites like terpenoids, glycosides as proposed by previous reports (Daisy et al., 2008; Goldy and Kalra, 2011). The PEE showed potent activity with a ZOI of 34.06 mm against *P. mirabilis.* This facultative anaerobe is capable of expressing multiple antibiotic resistances, characterized as plasmid-borne drug resistance (Khan and Musharraf, 2004). In this concern, these extracts would be of substantial importance in exterminating these pathogenic attacks. Moreover, the activities of PEE against these bacterial pathogens were efficacious than erythromycin (0.5 mg/ml), the standard commercial anti-bacterial drug. The methanol extracts depicted a moderate activity against all the test pathogens. Except for *S. typhi* and *P. mirabilis*, the aqueous extract showed the least activity against other test pathogens.

A previous study on the anti-bacterial activity of PEE of *A. campanulatus* is attributed to amblyone, a triterpenoid (Khan et al., 2008). Similarly, the anti-bacterial activity of PEE of *A. commutatus* might be due to the presence of terpenoids, reported in the phytochemical analysis. This research might aid in fabricating wide spectrum plant based antibiotic effective against antibiotic resistance infection caused by pathogenic bacteria.

Conclusion

These phytochemical and anti-bacterial analyses are the

first reported for *A. commutatus* var. *wynadensis*. Potent anti-bacterial activities proved by the experimentation defend the claims of the traditional use of *A. commutatus* in ayurvedic medicine preparation, its use as a remedy against various bacterial infections, and its ethno-botanical uses. Nevertheless, the exact molecular mechanisms of the Indian systems of medicines like Ayurveda are not being revealed and it is not accepted by the International Society of Science. Further studies are required to isolate and characterize the structural elucidation of the bioactive compounds from *A. commutatus* for future commercialization.

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REFERENCES

Adebanjo AO, Adewama CO, Essein EE (1983). Anti- infective agents of higher plants. Proceedings of an international symposium of Medicinal plants, Fifth edition, University of Ife, Nigeria. pp. 152-158.

Ayfer AD, Turgay ZE (2003). Antimicrobial activities of various medicinal and commercial plant extracts. Turk. J. Biol. 27:157-162.

Ayvaz A, Osman S, Salih K, Ismet O (2008). Insecticidal activity of the essential oils from different plants against three stored product insect. J. Insect Sci. 10:1-13.

Bradshaw LJ (1992). Laboratory Microbiology, 4th ed. Saunders College Publishing. Fort Worth, USA. P 435.

Chua M, Timothy CB, Trevor JH, Kelvin C (2010). Traditional uses and potential health benefits of *Amorphophallus konjac* K. Koch ex N.E.Br. J. Ethnopharmacol. 128:268-278.

Daisy P, Salu M, Suveena S, Nirmala AR (2008). A Novel Terpenoid from *Elephantopusscaber*–Anti-bacterial Activity on *Staphylococcus aureus*: A Substantiate Computational Approach. Int. J. Biomed. Sci. 4(3):196-203.

Daniel SF, Norman RF (2001). The Value of Plants Used in Traditional Medicine for Drug Discovery. Environ. Health Perspect. 109(1):69-75.

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 4(7): 685-688.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64:711-713.

Ferreira A, Proenc C, Serralheiro MLM, Ara´ujo MEM (2006). The in vitro screening for acetylcholine esterase inhibition and antioxidant activity of medicinal plants from Portugal. J. Ethnopharmacol. 108:31-37.

Firdouse S, Alam P (2011). Phytochemical investigation of extract of *Amorphophallus campanulatus* tubers. Int. J. Phytomed. 3(1):32-35.

Fokunang CN, Ikotun T, Dixon AGO, Akem CN, Tembe EA, Nukenine EN (2000). Efficacy of antimicrobial plant crude extracts on the growth of *Colletotrichum gloeosporioides* f. sp. *manihotis*. Pak. J. Biol. Sci. 3(6):928-932.

Gennaro L, Sukhdev SH, Suman Preet S, Khanuja, Dev Dutt R (2008). Extraction Technologies for Medicinal and Aromatic Plants. United Nations Industrial Development Organization and the International Centre for Science and High Technology, Trieste, Italy. P 23.

Goldy S, Kalra SS (2011). Antimicrobial activity pattern of certain terpenoids. Int. J. Pharm. Biol. Sci. 2(1):87-91.

Gupta RBL, Singh S, Dayal Y (1962). Effect of Punarnava on visual activity and refractive errors. Ind. J. Med. Res. 50:428-434.

Hamburger M, Hostettmann K (1991). Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry 30(12):3864-3874.

Jain A, Katewa SS, Galav PK, Sharma P (2005). Medicinal plant diversity of Sitamata wild life sanctuary, Rajasthan, India. J. Ethnopharmacol. 102:143-157.

Kavitha KR, Karthikeyani S, Krishna KS (2011). Anti-bacterial activity of *Amorphophallus commutatus,* an endemic plant of Western Ghats, South India. J. Pharm. Res. 4(3):710-711.

Kelmanson JE, Jager AK, Van SJ (2000). Zulu medicinal plants with anti-bacterial activity. J. Ethnopharmacol. 69:241-246.

Khan AU, Musharaf A (2004). Plasmid-mediated multiple antibiotic resistances in *Proteus mirabilis* isolated from patients with urinary tract infection. Int Med. J. Exp. Clin. Res. 10(11):598-602.

Liu PY (2004). Konjac. China Agriculture Press, Beijing (in Chinese).

Lopez-Brea M, Alarcon T, Domingo D, Diaz-Reganon J (2008). Inhibitory effect of Gram-negative and Gram-positive microorganisms against *Helicobacter pylori* clinical isolates. J. Antimicrob. Chemother. 61:139-142.

McFarland J (1907). The nephelometer: an instrument for estimating the number of bacteria used for calculating the opsonic index for vaccines. J. Am. Med. Assoc. 49:1176.

Natarajan D, John BS, Srinivasan K, Nagamurugan N, Mohanasundhari C, Perumal G (2005). Anti-bacterial activity of *Euphorbia fusiforms*: A rare medicinal herb. J. Ethnopharmacol. 102:123-126.

Onkar D, Dhingra JB (1995). Basic plant pathology methods, 2nd edition. Lewis Publishers, Boca Raton. pp. 287-305.

Ramesh AS, Christopher JG, Setty CR, Thankamani V (2010). Comparative study on the yield ratio and bioactive compounds of *Terminalia arjuna* bark and corewood. J. Pharm. Res. 3(6):1420-1422.

Rates SMK (2001). Plants as source of drugs. Toxicon 39:603613.

Ravikumar K, Ved DK (2004). Illustrated Field Guide- 100 Red listed medicinal plants of conservation concern in South India. FRLHT, Bangalore. pp. 1- 467.

Maina, SM, Emongor Q, Sharma, KK, Gichuki ST, Gathaara M, de Villiers SM (2010). Surface sterilant effect on the regeneration efficiency from cotyledon explants of groundnut (*Arachis hypogea* L.) varieties adapted to Eastern and Southern Africa. Afr. J. Biotechnol. 9(20):2866-2871.

Sivadasan M, Abdul Jaleel V (2002). Two new varieties of *Amorphophallus commutatus* (Schott) Engl. (Araceae) from India. Rheedea 12(2):159-168.

Sultana B, Farooq A and Ashraf M (2009). Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. Molecules 14:2167-2180.

Trease GE, Evans WC (1978). A Text book of Pharmacognosy, 11th edition. Bailliere Tindall London. P 530.

Widjaja EA, Lester RN (1978). Morphological, Chemical & Anatomical analysis of *Amorphophallus campanulatus* tuber. Reinwadtia 10(3):271-280.

Yue-Zhong S (1998). Recent natural products based drug development: a pharmaceutical industry perspective. J. Nat. Prod. 61:1053-1071.