

***In vitro* phytochemical screening, evaluation, antioxidant potential and antibacterial activity of *Amorphophallus paeonifolius* (Dennst. Nicolson)**

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ABSTRACT

Phytochemicals show increasing demand in the market with their potent antioxidant, immune stimulant, growth promoting, anti-pathogenic, and anti-stress activity. Phytochemical constitution, antioxidant potential, antibacterial and antifungal activity of solvent extract (Aqueous, methanol, ethanol, ethyl acetate) of *Amorphophallus paeonifolius* tuber was studied. The phytochemical constituents and their composition in solvent extracts of the tuber from *Amorphophallus paeonifolius* were quantitatively estimated. DPPH, free radical scavenging activity, phosphor molybdenum assay, ABTS radical scavenging property, and ferric ion reducing antioxidant power assay were studied to measure the antioxidant properties of extracts of the tuber. Antibacterial activity was performed for four bacterial pathogens (both gram positive and gram negative) using DMSO as a solvent. In phytochemical estimation, phenolic and flavonoid contents were highest in methanolic extract (2.5±0.100 mg GA/100 mg extract and 10.7±0.6 mg rutin/100mg extract) respectively. In ethanolic extract tannin content (91.72±0.91 mg tannic acid/100mg extract). In antioxidant activity, ethanolic extract showed maximum activity for DPPH (53.01% at 40 µg/ml), hydroxyl radical (100%), and 56.03% of ABTS radical cation scavenging activity. Among four solvent extracts, aqueous extract exhibit maximum antibacterial activity for *Streptococcus aureus* – MTCC 737 (3.25 mm zone of inhibition), *Klebsiella pneumonia* - MTCC 109 (0.75 mm zone of inhibition), *Pseudomonas aeruginosa* - MTCC 424 (1.25 mm zone of inhibition) and 1.5 mm of zone of inhibition for *Bacillus subtilis* - MTCC 121. In phytochemical screening, presence of flavonoids, phenols, tannins, the tuber extracts may be used as anti-allergenic, anti-cancer, anti-pathogenic agents and used to treat cardiovascular disease.

KEY WORDS: *Amorphophallus paeonifolius*, phytochemicals, antioxidant, DPPH, ABTS, antibacterial.

1. INTRODUCTION

Amorphophallus paeonifolius commonly known as elephant foot yam is edible tuber crop, cultivated mainly in almost all tropical and subtropical regions. This plant grown in countries like Philippines, India, Srilanka, Thailand, Indonesia and Malaysia as a commercial plant. It is a perennial crop. The plant taxonomically classified under the family of Araceae. The plant is herbaceous and contain large corm grown underground. They are about 80-100 cm in height. The plant contains monoecious flowers, but both male and female flowers found in the same plant. The flowers give a putrid smell when it blooms. The yam rich in calcium oxalate crystals, which causes soreness in throat and tongue when consumed. Traditionally, the corm of this plant is used to treat jaundice and to treat piles. The leaf and stem juices are used to treat ulcers (Devi Prasad, 2013). They also found to possess analgesic and anti-protease activities.

Previously, Nine different species of *Amorphophallus*, such as, *A. corrugates*, *A. yunnanensis*, *A. albus*, *A. kachinensis*, *A. krausei*, *A. konjac*, *A. paeoniifolius* (Dennst.), *A. yuloensis*, and *A. nanus* have been reported and used as medicine, fodder, food, and some extent used for wine production (Liu, 2004). In ayurvedic medicine, this tubers are highly valued in vitiated conditions of Vata, Kapha in treatment of piles, haemophillic conditions, skin diseases, intestinal worms, restorative in dyspepsia, obesity. Moreover yam used as tonic, appetizer and in stomachic (Kirtikar and Basu, 1987; Sivaraj, 1994; Narayana, 2004).

Methanolic extract of the tubers showed anti-inflammatory, anti-histaminic (Shankhajit, 2010). Extract of the tuber showed immune modulatory activity (Tripathi, 2010). These biological properties are due to the phytochemicals present in the tuber. However, these free radicals, have negative effects over cell aging, lipid oxidization, carbohydrate damage, protein oxidation and DNA damage (Devasagayam, 2004). Antioxidants are chemical molecules that interact with free radicals, moreover neutralize their effect. Phytochemicals such as flavonoids, Tannins, Lycopene, Phenolic acids, Vitamin C serve as potential antioxidants (Hamid, 2010). Phytochemicals like flavonoids (Tim and Andrew, 2005), tannins (Giovana, 2013), ascorbic acid (Songlin, 2001), phenolic compounds (Vijaya Bharathi, 2012), alkaloids, and saponins etc., plant showed antimicrobial properties (Benziane, 2012). These phytochemicals, due to this property aids in prevention of infections and other harmful microbial diseases. The main objective of this study, to investigate the phytochemical constitution, phytochemical composition, antioxidant potential and antibacterial activity of various tuber extracts of *Amorphophallus paeonifolius*.

2. MATERIALS AND METHOD

Tuber collection: For solvent extract, disease free fresh tubers were obtained from local market of Coimbatore district, Tamil Nadu. Tubers initially washed twice with water, and slice into small pieces (1-2 cm. long) and shade dried. Shade dried slices were fine powdered and powdered samples kept in air tight container.

Test organisms: Bacterial pathogens, *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. All four bacterial pathogens were stored at 4°C and were sub-cultured for 24 h at 37°C prior to use.

Preparation of extracts: About 30 g of fine grinded sample was extracted with water, methanol, ethanol and ethyl acetate in a soxhlet for 20 cycles. Extracts were then condensed and concentrated using flash evaporator. Each sample was made to 1 mg/mL concentration using respective solvent (Ferreira, 2006).

Phytochemical analysis: The phytochemicals such as alkaloids, carbohydrates, glycosides, saponins, tannins, phytosterols, flavonoids, steroids, and terpenoids reported previously in the different extracts of *Amorphophallus paeonifolius* (Trease and Evans, 1978; Edeoga, 2005).

Phytochemical Evaluation:

Total phenolic contents: Total phenolics were determined according to Rajeshwar (2005). In these studies, phenolic content was estimated for all four solvent such as aqueous, methanol, ethanol and ethyl acetate extracts. 20 µL of each extracts were makeup with 1 mL with distilled water. 0.5 mL of freshly prepared Folin ciacalteau phenol reagent were added and finally, 2.5 mL of 20% sodium carbonate were added respectively to each extract. Entire reaction mixture gently agitated and left in dark for 40 minutes. The absorbance of each sample was measured at 725 nm. For estimation of total phenolic content, gallic acid was used as the standard. Phenolic content was expressed as mg of Gallic acid per 100 mg of the extract.

Tannin content: 100 µL of each sample were made up to 7 mL of distilled water. Followed by 8 mM potassium ferric cyanide and finally, 20 mM ferric chloride prepared in 0.1M hydrochloric acid were added. Entire reaction mixture was mixed and optical density was measured at 700 nm. Tannic acid used as standard for tannin estimation studies. Tannin content was expressed as mg of tannin per 100 mg of the extract.

Flavonoid content: Total flavonoid content was quantitatively estimated by the method of Sathishkumar (2013), using rutin used as standard. 0.1 mL of each samples were taken and make volume with 5 mL with distilled water. 0.3 mL of 5% NaNO₂ was added. 3 mL of 10% AlCl₃ was added after 5 minutes and were shaken well. 2 mL of 1M NaOH was added after 6 minutes of incubation and the absorbance was measured at 510 nm. The flavonoid content were expressed as mg rutin/ 100 mg of the extract.

Saponin content: Saponin content in the sample was estimated by Mbaebie (2012). 5 g of the fine powdered sample was added with 50 mL of 20% (v/v) of ethanol. The reaction mixture was kept in hot water bath at 55°C for 4 hours. The residue was collected and once again re-extracted with 50 mL of 20% (v/v) ethanol and kept in boiling water bath until the volume reduces to 20 mL. The solution so obtained was taken in separating funnel with 10 mL of diethyl ether. The contents were shaken vigorously. The aqueous layer was separated and about 10 mL of butan-1-ol was added to the filtrate and then washed with 10 mL of 5% aqueous sodium chloride. The whole mixture is kept in water bath for condensation and after it was dried in oven at 40°C.

$$\% \text{ saponin} = \frac{\text{Weight of final filtrate}}{\text{Weight of sample}} * 100$$

Antioxidant Assays:

DPPH free radical scavenging activity: Scavenging activity was estimated by the modified method of Navnath 2012. Methanolic solution of DPPH was made into the concentrations of 10, 20, 40, 60, 80, 100 µg/mL and make it volume of 1 mL. 3 mL of DPPH solution (100 mM) was added to all test tube containing extracts and finally incubated for 20 minutes in dark. Optical density was measured at 517 nm. Methanol and DPPH solutions were used as control and negative control respectively.

$$\% \text{ inhibition} = \frac{\text{Control} - \text{test}}{\text{Control}} * 100$$

Hydroxyl radical scavenging activity: The ability of extracts to scavenge H₂O₂ was determined by the method of Ruch (1989). 0.1 mL of the sample was made up to 1.5 mL using phosphate buffer (pH 7). 0.5 mL volume of 10 mM 2-deoxy-D-Ribose, 0.25 mL of 20 mM sodium salt of EDTA, 0.25 mL of 20 mM ferrous chloride was added respectively. To this mixture 1.9 mL volume of distilled water and 0.5 mL volume of 10 mM hydrogen peroxide were added. Entire reaction mixture was incubated for 4 hours at 37°C in water bath. 2.5 mL of 2.8% of TCA was added to arrest the reaction. 2.5 mL of 1% TBA finally added and incubated at 100°C for 10 minutes in a water bath. After cooling the optical density was measured at 520 nm.

ABTS radical scavenging assay: The total antioxidant capacity of the sample was measured by the ABTS radical cation-decolorization assay (Pellegrini, 2003). For assay, 7 mM ABTS aqueous solution and volume of 2.4 mM of

ammonium persulphate were added in equal volume and incubated in dark for 14 hours at room temperature (28±2°C). The reaction mixture was diluted by addition ethanol until the absorbance reach 0.70. To 100 µL of each samples, 900 µL volume of the prepared ABTS reagent added to all the extracts and vortex it. Finally, entire reaction mixture incubated for 6 minutes at room temperature (28±2°C). Optical density measured at 734 nm.

$$\text{ABTS scavenging activity} = \frac{\text{Control} - \text{test}}{\text{Control}} * 100$$

Ferric reducing antioxidant assay: Ferric reducing power assay was performed by the method given in Barreira (2008). 0.1 mL volume of extracts was make up with 1 mL of 96% of ethanol. To this, 5 mL volume of distilled water, 1.5 mL of 1M hydrochloric acid, 1.5 mL of 1% potassium ferric cyanide, 0.5 mL of 1% sodium dodecyl sulphate and 0.5 mL of 0.2% ferric chloride were added in series. Finally, entire reaction mixture was incubated in a water bath at 50°C for 20 minutes. Test tubes were cooled rapidly and Optical density was measured at 750 nm. Ascorbic acid used as the standard. Antioxidant capacity was measured as mg of ascorbic acid per 100 mg of the extract.

Phospho molybdenum assay: To 100 µL of the extracts, 1 mL of the reagent- (28 mM sodium phosphate and 4 mM ammonium molybdate in 10 mL sulphuric acid) was added. Test tubes were covered with foil and were incubated at 90°C for 90 minutes. The samples were cooled and the optical density was read at 695 nm. Ascorbic acid was used as standard. Result reported as mg of ascorbic acid per 100 mg of the extract.

Antibacterial activity: Antibacterial activities of various solvent extracts were performed by the method followed by Guilherme (2007). *Streptococcus aureus* – MTCC 737, *Klebsiella pneumonia* - MTCC 109, *Pseudomonas aeruginosa* - MTCC 424 and *Bacillus subtilis* - MTCC 121. Mueller Hinton agar plates were prepared and allowed to solidify. The microbes were cultured in the nutrient broth for 24 h and were streaked over the plates. Sterile discs were poured with 20 µL of the various samples of concentration 100 µg/mL in DMSO. Discs were then impregnated over the plate, finally incubated at 37°C for 24 h. 20 µL of Streptomycin (100 mg/mL in DMSO) was poured in a disc to act as a positive control and 20 µL of DMSO was poured in another disc to act as a negative control.

3. RESULTS

Phytochemical extraction and analysis: Figure.1, shows different solvent extractive values of *Amorphophallus paeonifolius*. Similarly, In Phytochemical analysis of various solvent extracts of *Amorphophallus paeonifolius* were given in table.1.

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

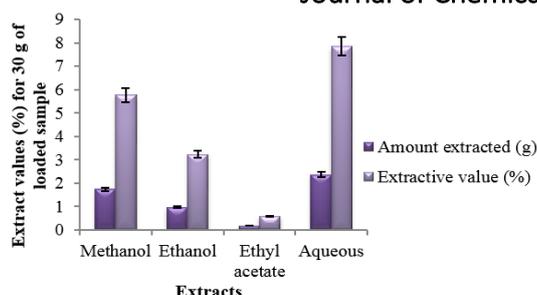


Figure.1. Extractive value of solvent extracts from *Amorphophallus paeoniifolius*

Phytochemical evaluation: In phytochemical evaluation, total phenolic, tannin, saponin and flavonoid content was estimated. For quantification of total phenolic content, concentration of phenolic content expressed as Gallic Acid (GA)/100 mg extract. Total tannin content was estimated for all solvent extracts of *Amorphophallus paeoniifolius* and expressed as tannic acid equivalent (mg tannic acid (TA) / 100 mg extract). In saponin estimation, concentration found to be 1.78 $\mu\text{g}/\text{mg}$ of the powdered sample. Saponins help in the reduction of risk of heart disease. Similarly, Total flavonoid content of all four extracts were found using rutin standard. The total phenolic content, tannin, saponin and flavonoid content in all four extracts were given in table.2.

Table.2. Total phenolic, tannin, flavonoid and saponin content of various extracts of *Amorphophallus paeoniifolius*

Extracts	Total phenolic-GAE (mg GA/100mg extract)	Tannin-TAE (mg tannic acid/100mg extract)	Total flavonoid Rutin (mg rutin/100mg extract)	Saponin content
Aqueous	1.53 \pm 0.78	17.12 \pm 1.73	5.2 \pm 0.152	1.78 $\mu\text{g}/\text{mg}$ of sample (dry weight)
Methanol	2.5 \pm 0.100	45.86 \pm 1.58	10.7 \pm 0.6	
Ethanol	2.25 \pm 0.278	91.72 \pm 0.91	2.467 \pm 0.45	
Ethyl acetate	1.12 \pm 0.368	21.47 \pm 1.21	6.00 \pm 0.61	

Antioxidant assay:

Free radical scavenging activity of DPPH: The DPPH scavenging activity all four extract was given in the figure.2. DPPH scavenges free radicals and gives intense colour. All the four extracts showed noticeable free radical scavenging activity. Ethanol extract showed maximum activity. This indicates the presence of hydrogen donating antioxidant group in the extracts.

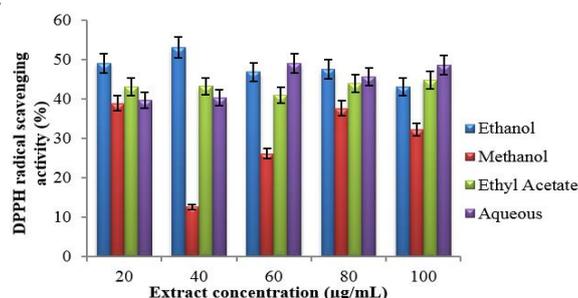


Figure.2. DPPH free radical scavenging activity of various extracts of *Amorphophallus paeoniifolius*

Phospho molybdenum assay: Figure.3, denotes the antioxidant assay for different extracts using phosphor molybdenum. Reduction of phosphor molybdenum (VI) to phosphor molybdenum (V) by the sample was recorded as antioxidant property of the extract. In this study, methanolic extract showed significant activity. Among all other extracts, methanolic extract showed significant AEAC equivalent of 335.53 \pm 1.18 and ethanolic, aqueous, ethyl acetate extracts showed 207.37 \pm 0.98, 94.26 \pm 0.92, and 125.95 \pm 0.56 of AEAC equivalent.

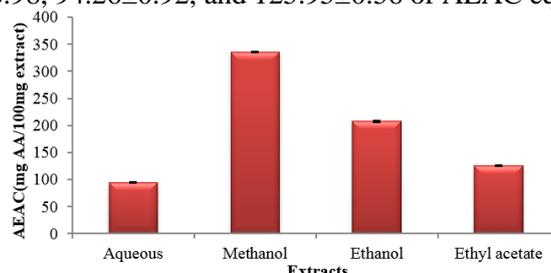


Figure.3. Phospho molybdenum assay of various extracts of *Amorphophallus paeoniifolius*

Hydroxyl radical scavenging activity: Figure.4, denotes the ethanolic, methanolic, ethyl acetate and aqueous extracts hydroxyl radical scavenging activity. Of all the extracts ethanol showed highest activity 99%. Methanolic extract with 92% of activity and ethyl acetate with 82% of free radical scavenging activity. Aqueous extract showed only about 3% of activity.

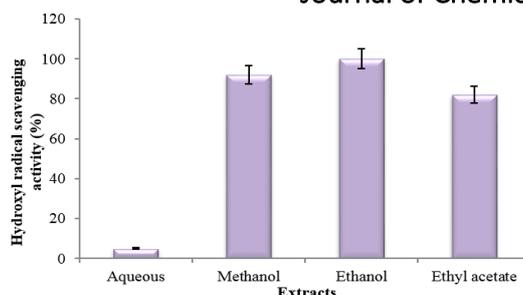


Figure.4. Hydroxyl radical scavenging activity of various extracts of *Amorphophallus paeoniifolius*
ABTS radical cation scavenging activity: The efficiency of various extracts to scavenge ABTs free radical is given in the figure.5. This method of ABTS is used to quantify the antioxidant activity of phenolic compounds.

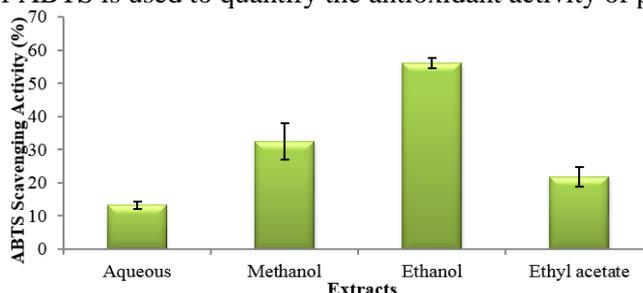


Figure.5. ABTS scavenging activity (%) of various extracts of *Amorphophallus paeoniifolius*
Modified FRAP: Ferric reducing/antioxidant property was estimated, expressed as ascorbic acid equivalent per mg of extract obtained in various solvents in figure.6. Potassium ferricyanide (Fe^{3+}) is reduced by the antioxidant compounds to potassium ferrocyanide (Fe^{2+}) which forms complex with ferric chloride. Ethyl acetate extract have highest AEAC value of 26.24 ± 2.66 . Aqueous extract showed 19.43 ± 1.80 AEAC/100 mg extract, methanolic extract, ethanolic extract showed 23.60 ± 3.26 , 24.68 ± 1.91 AEAC equivalent/100 mg extract respectively.

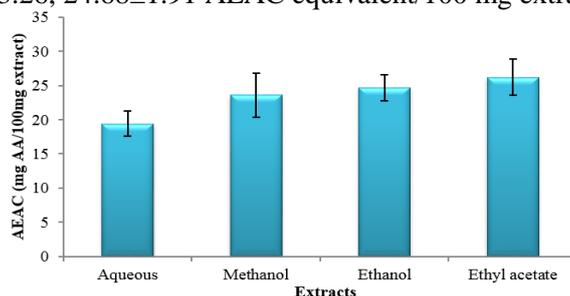


Figure.6. FRAP of various extracts of *Amorphophallus paeoniifolius*

Antibacterial assay: Antibacterial activity of the shade dried tuber (100 μ g) was performed against 4 different human bacterial pathogen and results were given in figure.7. Studies suggest presence of phytochemicals such as 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.

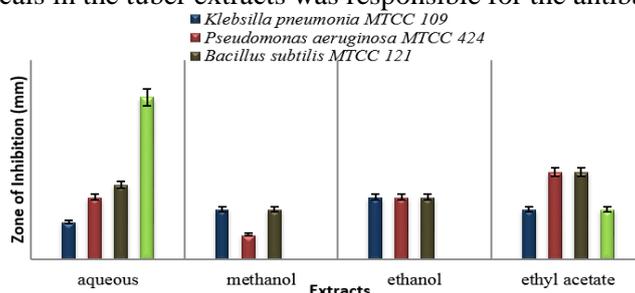


Figure.7. Antibacterial activity of various extracts of *Amorphophallus paeoniifolius*

DISCUSSION

For phytochemical analysis, Alkaloids and glycosides were absent in all four extracts. Saponins were present in methanolic and aqueous extracts. Polyesters, phenols, flavonoids, tannins, terpenoids, steroids, gum and mucilage and carbohydrates presence in all extracts. Tannins show green black colour confirms the presence of Catecholic tannins. In all other phytochemical evaluation, Methanolic extract with significant phenolic content of 2.5 ± 0.100 mg of GA/100 mg extract. Ethanolic extract with 2.25 ± 0.278 mg GA/100 mg extract. Aqueous extract with 1.53 ± 0.78 mg GA/100 mg extract. Ethyl acetate showed 1.12 ± 0.368 mg GA/100 mg extract.

In case of tannin estimation, Ethanolic extract with maximum tannin content of 91.72 ± 0.91 Tannic acid equivalent/100 mg extract. Methanolic, ethyl acetate and aqueous extracts showed 45.86 ± 1.58 mg TA/100 mg extract, 21.47 ± 1.21 mg TA/100 mg extract, and 17.12 ± 1.73 mg TA/100 mg extract respectively. Similarly, in flavonoid estimation, maximum flavonoid content in methanolic extract of 10.7 ± 0.6 mg rutin/100 mg extract. Ethyl acetate, Aqueous and ethanolic extracts showed 6.00 ± 0.61 mg rutin/100 mg extract, 5.2 ± 0.152 mg rutin/100 mg extract and 2.467 ± 0.45 mg of Rutin/100 mg extract respectively.

Total phenolic content of methanol extract and hydroalcoholic extract were 6.16 ± 1.8 mg and 5.14 ± 2.1 mg equivalent to Catechol / gm were previously studied (Nataraj, 2008). Similarly, phenolic content in petroleum ether extract showed 44.58 mg GAE/g (Angayarkanni, 2010). Hydroxyl radical scavenging activity, Reactive oxygen species (RoS) react with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell (Battu, 2010). In previous study reported that, IC₅₀ values were reported as 19.56 and 18.40 µg/ml respectively for methanol extract of *A. paeoniifolius* var. *campanulatus* corm and ascorbic acid (Arockia and Mohan, 2013).

DPPH (Diphenyl picryl hydrazyl) Radical scavenging activity, previous studies showed that, *A. paeoniifolius* ethanolic extract at the concentration of 50 µg/mL had higher inhibitory activity of 68.6%. (Angayarkanni, 2010). Similarly, methanol and hydro alcoholic extract at concentrations 500 µg exhibited 42.83% and 72.13% inhibition (Nataraj, 2008). Ethanolic extracts of tuber showed 54.78 % inhibition at Concentration (60 µg/ml) (Jagatheesh, 2010). In ABTS radical scavenging activity, Ethanol extract with maximum free radical scavenging activity of $56.03 \pm 1.56\%$. methanolic extract showed $32.57 \pm 5.46\%$. Aqueous extract showed $13.29 \pm 1.08\%$ and Ethyl acetate with $21.89 \pm 2.9\%$ of scavenging activity. Similarly previous report, ethanolic extract of tuber showed good scavenging effects. The IC₅₀ value of the extract was found to be 35 µg/mL (Angayarkanni, 2010). Similarly, Jagatheesh (2010), reported 73.58 % of inhibition at Concentration (100 µg/ml).

In general, antimicrobial activity is due to presence of plant metabolites like terpenoids, glycosides as previously reported (Daisy, 2008; Goldy and Kalra, 2011). Similarly, previous study on the anti-bacterial activity of Petroleum ether extract of tuber is attributed due to presence of amblyone and triterpenoid (Khan, 2008). Various reports showed that, Aqueous and other various solvent extracts of tubers were evaluated for its antibacterial properties (Khan, 2008; Krishnaa, 2011). Previous study showed that, methanolic extract showed appreciable antibacterial activity against *S. pyogenes* and *S. faecalis* with 19.06 and 18.86 mm of zone of inhibition, respectively. Similar studies, aqueous extract showed appreciable activity against *E. coli*, *P. mirabilis* and *S. typhi*, except *E. aerogenes* (Arjun, 2013).

From this study, aqueous extracts showed maximum inhibition for *Staphylococcus aureus* - MTCC 737. Methanolic and ethanolic extracts not showed any inhibition for *Staphylococcus aureus* - MTCC 737. Ethyl acetate extract with zone of inhibition of 1 mm. *Klebsilla pneumonia* - MTCC 109 was inhibited by a zone of 1 mm in all the extracts. Ethyl acetate extract showed maximum inhibition for *Pseudomonas aeruginosa* - MTCC 424. Aqueous and ethanolic extracts showed the zone of inhibition of 1.25 mm and methanolic extract reported 0.75 mm of inhibition for *Pseudomonas aeruginosa* - MTCC 424. *Bacillus subtilis* - MTCC 121 showed 1.0 mm, 1.25 mm, 1.50 mm, 1.75 mm as zone of inhibition for methanol, ethanol, aqueous and ethyl acetate respectively. Thus all the extracts have a considerable effect over *Bacillus subtilis* - MTCC 121. From this study, aqueous extract showed maximum antibacterial activity when compared with all the solvent extracts.

4. CONCLUSION

Traditional medicine, the compounds from plants are attracting much of attention. Scientists aim at exploiting these plants for application in pharmaceuticals and cosmetic based industries. From the experimental results, tuber extracts of *Amorphophallus paeoniifolius* indicated the presence of phyto-chemicals. Hence the crude extracts of *Amorphophallus paeoniifolius* tuber has high antimicrobial potential which are nearly on par with growth inhibition of standard antibiotic, which indicated appreciable antimicrobial potency of extracts. Similarly extracts shows good antioxidant potential even at very low concentrations. Further investigations that would identify the bioactive compound from the *Amorphophallus paeoniifolius* for animal studies helps in drug development.

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REFERENCES

- Angayarkanni J, Ramkumar KM, Priyadharshini U, Poornima R, Antioxidant potential of *Amorphophallus paeoniifolius* in relation to their phenolic content, Pharmaceutical Biology, 48 (6), 2010, 659–665.
- Arjun RK, Asis S, Abdul VJ, Sreena R, Karthikeyan S, Gothandam KM, Morphological, phytochemical, and antibacterial properties of wild and indigenous plant (*Amorphophallus commutatus*), Journal of Medicinal Plants Research, 7 (13), 2013, 744-748.

Arockia JA, Mohan VR, *In vitro* antioxidant activity of *Amorphophallus paeoniifolius* (Dennst.) Nicolson var. *campanulatus* (Blume ex. decne) Sivadasan, International journal of Pharmaceutical research and Bio-science, 2 (5), 2013, 211-222.

Barreira JCM, Isabel CFR, Ferreira M, Beatriz PP, Oliveira, Jose AP, Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars, Food Chem. Toxicol, 46, 2008, 230-235.

Battu CR, Ethadi SR, Priya, GV, Priya KS, Chandrika K, Rao AV, Reddy SO, Evaluation of antioxidant and anti-inflammatory activity of *Euphorbia heyneana* Spreng, Asian Pac. J. Trop. Biomed, 2010, 191-194.

Benziane MM, Kambuche BN, Bellahouel S, Merah B, Fortas Z, Soulimani R, Saidi S, Derdour A, Antimicrobial activity of the alkaloids and saponin extracts of *Anabasis articulata*, E3, Journal of Biotechnology and Pharmaceutical Research, 3 (3), 2012, 54-57.

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

Devasagayam TPA, Tilak JC, Bloor KK, Ketaki SS, Saroj SG, Lele RD, Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects, Journal of the association of Physicians of India, 52, 2004, 794- 804.

Devi Prasad AG, Shyma TB, Raghavendra MP, Plants used by the tribes for the treatment of digestive system disorders in Wayanad district, Kerala, Journal of Applied Pharmaceutical Science, 3 (08), 2013, 171-175.

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

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

Giovana MLF, Ana LF, Valéria SC, Correa, Bianca WB, Silvana G, Saulo F A, Suzelei de CF, Ana MSP, Antimicrobial Activity and Rates of Tannins in *Stryphnodendron adstringens* Mart. Accessions Collected in the Brazilian Cerrado, American Journal of Plant Sciences, 4, 2013, 2193-2198.

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

Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A, Antioxidants: Its medicinal and pharmacological applications, African Journal of Pure and Applied Chemistry, 4 (8), 2010, 142-151.

Jagatheesh K, Arumugam V, Elangovan N, Pavankumar P, Evaluation of the Anti-Tumor and Antioxidant Activity of *Amorphophallus Paeoniifolius* on DMBA Induced Mammary Carcinoma, International Journal of Chemical and Pharmaceutical Sciences, 1 (2), 2010, 40-50.

Khan A, Rahman M, Islam MS, Antibacterial, antifungal and cytotoxic activities of amblyone isolated from *Amorphophallus campanulatus*, Indian J Pharmacol, 40, 2008, 41-44.

Kirtikar KR, Basu BD, Indian Medicinal Plants, 4, 2nd ed, In: Kirtikar KR, Basu BD (eds), Dehra Dun, India, International book distributors, 1987, 2061- 2062

Krishnaa RK, Karthikeyani S, Kumari SK, Antibacterial activity of *Amorphophallus commutatus*, an endemic plant of Western Ghats, South India, Journal of Pharmacy Research, 4 (3), 2011, 710-711.

Lanchoti FG, Fachin A, Correa V, Bertoni B, Giuliatti S, Amui S, Castro FS, Soares PA, Antimicrobial Activity and Rates of Tannins in *Stryphnodendron adstringens* Mart. Accessions Collected in the Brazilian Cerrado, American Journal of Plant Sciences, 4 (11), 2013, 2193-2198.

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

Mbaebie BO, Edeoga HO, Afolayan AJ, Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq, Asian Pacific Journal of Tropical Biomedicine, 2012, 118-124.

Nadkarni KM, Nadkarni AK, Indian materia medica-1, Popular Prakashan, Mumbai, 1992, 1-508.

Narayana DP, Purohit SS, Arun KS, Tarun K, A Hand Book of Medicinal Plants – A complete source book, Agrobios (India), Jodhpur, 41, 2004.

Nataraj HN, Murthy RLN, Ramachandra SS, *In vitro* antioxidant and free radical scavenging potential of *Amorphophallus paeoniifolius*, Oriental Journal of Chemistry, 24 (3), 2008, 895-902.

Navnath MP, Xivanand NV, Dattatray KG, Tanaji GJ, Nutraceutical properties of the marine macroalga *Gayralia oxysperma*, *Botanica Marina*, 55 (6), 2012, 581–589.

Oliveira GFD, Furtado NAJC, Silva Filho AAD, Martins CHG, Bastos JK, Cunha WR, Antimicrobial activity of *Syzygium cumini* (Myrtaceae) leaves extract, *Brazilian Journal of Microbiology*, 38 (2), 2007, 381–384.

Pellegrini N, Del RD, Colombi B, Bianchi M, Brighenti F, Application of the 2, 2'-azobis (3-ethylene benzothiazoline-6- sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages, *J Agric Food Chem*, 51, 2003, 260–264.

Rajeshwar Y, Gupta M, Mazumder UK, Antitumor activity and *in vivo* antioxidant status of *Mucuna pruriens* (Fabaceae) seeds against Ehrlich ascites sarcoma in Swiss albino mice, *Iran J Pharmacol Therap*, 4, 2005, 46–53.

Ruch RJ, Cheng SJ, Klaunig JE, Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea, *Carcinogenesis*, 10, 1989, 1003–1008.

Saira B, Muruga L, Screening of Bioactive Compounds and antimicrobial effect of *Orithazh thamarai* chooranam, *International Journal of Pharm. Tech Research*, 4 (2), 2012, 572-575.

Sathishkumar T, Baskar R, Aravind M, Tilak S, Deepthi S, Bharathikumar VM, Simultaneous extraction optimization and analysis of flavonoids from the flowers of *Tabernaemontana heyneana* by high performance liquid chromatography coupled to diode array detector and electron spray ionization/mass spectrometry, *ISRN Biotechnology*, 2013, 1–10.

Sen A and Batra A, Determination of antimicrobial potentialities of different solvent extracts of the medicinal plant, *Phyllanthus amarus* Schum and Thonn. *Int J Green Pharm.*, 6, 2012, 50-56.

Shankhajit D, Yadu ND, Ajoy KG, Anti-Inflammatory Activity of methanolic Extract of *Amorphophallus paeoniifolius* and its possible mechanism, *International Journal of Pharma and Bio Sciences*, 1 (3), 2010, 1-10.

Sivaraj VV, Indira Balachandran, *Ayurvedic Drugs and Their plant sources*, Oxford and IBH publishing Co. Pvt. Ltd., New Delhi, Bombay and Calcutta, 1994, 457.

Songlin Li, Kenneth B, Taylor SJ, Kelly, Mark J, Jedrzejewski, Vitamin C inhibits the enzymatic activity of *Streptococcus pneumoniae* hyaluronate lyase, *The Journal of biological chemistry*, 276 (18), 2001, 15125-15130.

Tim Cushnie TP, Andrew JL, Antimicrobial activity of flavonoids, *International Journal of Antimicrobial Agents*, 26, 2005, 343–356.

Trease GE, Evans WC, *A textbook of Pharmacognosy*, 11th edition, Bailliere Tindall, London, 1978, 530.

Tripathi AS, Chitra V, Sheikh NW, Mohale DS, Dewani AP, Immuno modulatory Activity of the Methanol Extract of *Amorphophallus campanulatus* (Araceae) Tuber, *Tropical Journal of Pharmaceutical Research*, 9 (5), 2010, 451-454.

Vijaya BS, Nithya TG, Sivakumar SB, Muruga L, Screening of Bioactive Compounds and Antimicrobial effect of *Orithazh Thamarai Chooranam*, *International Journal of PharmTech Research*, 4 (2), 572-575.