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Phytochemical Screening, GCMS Studies of *Amorphophallus* paeoniifolius (Dennst) Nicolson, and its Antimicrobial Activity

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ABSTRACT

Plants have been a vital source of medicine since ancient times. Various plant parts are highly medicinal due to the presence of bioactive compounds. The Elephant foot yam, Amorphophallus paeoniifolius. (Dennst.) Nicolson. It is a highly medicinal plant in which the tuber-mediated plant uses the leaf Part to evaluate the Phytochemicals (Flavonoids, Tannins, Phenols, Quinons, Protein, and Carbohydrates, etc.) present with the non-polar solvent Petroleum ether and high-polar solvent Methanol, respectively. The various chemical components were estimated using GC-MS, the antibacterial activity was used to evaluate disease drug resistance against pathogens or contaminants, and the antioxidant activity was used to conclude free radical scavenging activity.

Keywords: Antimicrobial, Antioxidant activity, Elephant foot yam, GC-MS, Phytochemicals Analysis.

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INTRODUCTION

Amorphophallus paeoniifolius (Elephant foot yam), Family Araceae, is a perennial herb known as Suran or Jimikand (Hindi) (Figure 1). Genus Amorphophallus has more than 200 species worldwide (1) and 18 in India. The plant was identified using evidence shreds (2). The plant is cultivated for its edible tubers in many regions, for its potential therapeutic use (3). It is a crop of Southeast Asian origin in tropical and subtropical Asia. The genus Amorphophallus has been used as a food source and a traditional medicine for centuries (4). It grows in the wild in the Philippines, Malaysia, Indonesia, and other Southeast Asian countries. Herbs are the source of magnificent inhibitors that can act on various diseases (5). Most species are endemic (6). Northern mountain provinces of Vietnam contain glucomannan, a plant component with many health benefits, such as treatment for constipation, improvement of type 2 diabetes, and lowering of blood cholesterol and sugar (7). Despite great advances in modern scientific medicine, traditional medicine is still the primary form of treating diseases in the majority of people in developing countries, including India. Even among those to whom Western medicine is available, the number of people using one form or another of complementary or alternative medicine is rapidly increasing worldwide. Increasing knowledge of metabolic processes and the effect of plants on human physiology has enlarged the range of applications of medicinal plants (8). A. paeoniifolius tuber had analgesic, gastro-protective, anti-helminthic, and antibacterial activities (9,10). Especially in the concentration of antioxidant compounds such as flavonoids, triterpenoids, and polyphenols. These Bioactive compounds are important in preventing cancer, neurodegenerative diseases, cardiovascular diseases, and diabetes (11).

MATERIAL AND METHODS

Collection and Preparation of Plant Material:

The Collection of the Leaf part of *Amorphophallus paeoniifolius* is based on the Identification of the place of the specimen in Belagavi District, Jambooti village, Karnataka, India (Fig. 1). The collected leaf part was washed thoroughly with sterile distilled water, and then all Leaves were shade-dried and powdered using a Blender machine and used for further analysis.

Preparation of Plant Leaf Extract:

The fresh leaf was collected and washed with distilled water to remove the unwanted wastes on the surface of the leaves. The Leaves were placed in a shaded place for the proper drying process (moisture removal). The dried leaf material is blended with the help of a Blender machine. The Leaf powder weighed about 25 grams and was packed with the help of filter paper. Soxhlet extractor is used for further extraction process with temperatures about 50°C for Petroleum ether and 60°C for Methanol, having 250mL of solvent respectively. The Collected Crude extract is used for further analysis.

Phytochemical Analysis:

Test for flavonoids: To check the presence of flavonoids, 2 ml of each extract was taken and mixed with a few drops of 20% NaOH. This resulted in the formation of an intense yellow color. A few drops of 70% dilute HCl were added, resulting in the disappearance of the yellow color. The Formation and disappearance of yellow color indicate the presence of flavonoids in the extract.

Test for Saponins: To test for the presence of saponins, 2 mL of each extract was added to 6 mL of distilled water and shaken vigorously. The formation of bubbles or persistent foam indicates the presence of saponins.

Test for Tannins: 10% of alcoholic ferric chloride was added to 2 ml of the solvent extract. The formation of brownish blue or black color indicates the presence of tannins.

Test for Phenols: To 2 mL of each extract, 2 mL of 5% aqueous ferric chloride was added. The formation of a blue color indicates the presence of Phenols in the extract.

Test for Proteins: The presence of proteins was determined by adding 1 ml of 40% NaOH and a few drops of 1% copper sulfate to 2 ml of the extract. The Formation of a violet color indicates the presence of peptide linkage molecules in the extracts.

Test for Cardiac Glycosides: 1 ml of each extract was added to 0.5 ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution. The Formation of a brown ring at the interface indicates the presence of cardiac glycosides in the sample.

Test for Terpenoids: 1 ml of each solvent extract was taken with 0.5 ml of chloroform, followed by a few drops of concentrated sulphuric acid. The formation of a reddish-brown precipitate indicates the presence of terpenoids in the extract.

Test for Carbohydrates: 1 mL of Extract was added with a few drops of Molisch's reagent and then with 1 mL of concentrated sulphuric acid on the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. The formation of a red or dull violet color indicates the presence of carbohydrates in the extract. **Test for Quinones:** To check the presence of quinones, 1 ml of extract was added to concentrated Hydrochloric Acid. The Formation of yellow precipitate or Coloration indicates the presence of Quinones in the Extract. Otherwise, it suggests the absence of Quinones in the given extract.

Gas Chromatographic Mass Spectrophotometric (GC-MS) Method:

The analysis was conducted on a Shimadzu-GCMS-QP2010S fitted with a 30 m \times 0.25 μm Rtx-5 ms capillary column. The oven temperature was programmed from 60 to 180°C at 3° C/min and isothermally held for 15 min. HP-5 MS (5% phenylmethyl siloxane). Helium was used as carrier gas flow at 1.61 mL/min, and 1 μl of the sample was injected for analysis. The ion source was maintained at 180 – 250 °C and 70 eV electron energy. The methanol was added to the extracts before injecting 1 μl into the column. The extracted name and molecular weight of unknown compounds were found by comparing their mass spectrum with the reference spectrum available in the Wiley GC/MS Library, Mass Finder Library, and Adams Library (13,14).

Ferric Reducing Antioxidant Power (FRAP) Assay:

The ferric-reducing capacity of extracts was investigated by using the potassium ferricyanide method. Briefly, 0.2mL of each of the extracts at different concentrations, 2.5mL of phosphate buffer (0.2 M, pH 6.6), and 2mL of potassium ferricyanide $K_3\text{Fe}$ (CN) $_6$ (1%) were mixed and incubated at 50°C for 300 min, to reduce ferricyanide into ferrocyanide. The reaction was stopped by adding 2.5 mL of 10% (w/v) trichloroacetic acid. Finally, 0.5 mL of FeCl $_3$ (0.1%) and the absorbance was measured at 700 nm. The sample concentration absorbance (IC $_{50}$) was calculated by plotting the absorbance against the corresponding sample concentration. (12)

Preparation of Antibacterial Activity: (Kirby-Bauer disc diffusion method):

The disk diffusion method, or Kirby-Bauer disc diffusion method, is among the most flexible susceptibility testing methods for antibacterial agents that can be tested. The technique consists of placing paper disks saturated with antimicrobial agents on a lawn of bacteria seeded on the surface of Nutrient Agar (NA) medium incubating the plate overnight, and adding the three different concentrations of solvent extracts and control as likewise $0.5~\mu$, $1.0~\mu$, and $1.5~\mu$ respectively and measuring the presence or absence of a zone of inhibition concentration around the disks.

RESULT AND DISCUSSION:

Phytochemical analysis:

The Qualitative phytochemical analysis of *A. paeoniifolius* Leaf extract showed that it was rich in phytoconstituents. It shows the presence of Flavonoids, Tannins, Cardiac Glycosides, Terpenoids, and Carbohydrates in the Methanol extract, and Flavonoids, Tannins, Cardiac Glycosides, and Carbohydrates present in Petroleum ether. (Table 1). the tuber part reported that the petroleum ether extract contains Alkaloids, Steroids, Flavonoids, and Carbohydrates respectively then the aqueous extract showed the presence of Flavonoids, Tannins, Protein, and Carbohydrates (15,) the qualitative and quantitative phytochemical analysis on *A. peaoniifolius* shows the presence of phenol, alkaloid, and glycosides and also saponins present in hexane extract. Also rich in Alkaloid content $(7.60 \pm 0.52 \text{ mg/g})$ in ethanol extract, total flavonoid rich in ethanol extract $(18.03 \pm 0.81 \text{ mg/g})$ dry weight). A. peoniifolius shows the presence of alkaloids, glycosides, saponin glycosides, cardiac glycosides, tannin and phenolics, flavonoids, proteins, amino acids, steroids and triterpenoids, and carbohydrates (17).

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis:

The GC-MS analysis of A. Peoniifolius leaf methanol and petroleum ether extract showed Formamide, N, Ndimethyl, Hexadecanoic acid, methyl ester, n-hexadecanoic acid, 9(2,3 Diphenylcyclopropyl) methyl phenyl sulfoxide, trans, Triphenylphosphine oxide, Heptacosyl heptafluorobutyrate, 1-Hexacosanol, Campesterol, Stigmasterol, gamma.-Sitosterol presence in methanol extract. 2-Pyrrolidinone, 1-methyl, n-Hexadecanoic acid, Phytol, Heptatriacontane, Tetrapentacontane, Campesterol, Stigmasterol, gamma.-Sitosterol, present in petroleum ether extract respectively RT in min and area of percentage shows various chemical component listed in (Table 2 and 3), even the structure isolated through the GC MS library to make the entire deliberation in a comprehensive format (Fig 2 & 3). The Amorphophallus campanulatus shows the presence of compounds in ethanolic and aqueous extracts are 1-Nonadecene, 1-Octadecene, Tetradecane, 1-Undecene, 1- Hexadecene, Hexadecane, etc; saturated and unsaturated fatty acids and their esters like Tetradecanoic acid, Hexadecanoic acid and its methyl and ethyl esters, Pentadecanoic acid, Heptadecanoic acid, Octadecanoic acid, Dodecanoic acid, Linoleic acid and its ester, Oleic acid, Ethyl oleate, etc, and plant sterols like Stigmasterol, β-Sitosterol, Campesterol, Fucosterol, etc. Apart from these, the extracts are also rich sources of tocopherol, the common and most popular antioxidant vitamin. It is also worth mentioning that the basic difference between the ethanolic and aqueous extracts of *Amorphophallus campanulatus* lies in their polyphenols, flavonoids, and triterpene content. The ethanolic extract contains a greater variety of phenolic compounds like 1, 3, 5, 5-benzenetriol, dodecanol, Phenol, 2,4-bis(1,1-dimethyleth..., the flavonoid fraction 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-, and the triterpene Squalene. These pharmacologically active compounds are present in higher amounts in the ethanolic extract than in aqueous extract (18).

Antioxidant activity by FRAP assay:

FRAP Assay of A. paeoniifolius Leaf powder was taken. The five concentrations were taken to evaluate the ferric reducing antioxidant power assay in Methanol extract, showing 3.57 ± 0.12, 13.83 ± 0.30, 26.87 ± 0.18, 35.06 \pm 0.17, 42.23 \pm 0.14, respectively, and the increasing rate of points results in the IC₅₀ value. $280.30 \mu g$ ml. Petroleum ether extract showing 13.45 ± 0.20 , 24.62 ± 0.25 , 33.66 ± 0.15 , 41.69 ± 0.15 , and 51.01 ± 0.13 respectively, and the increasing rate of points results in the IC₅₀ value 242.75 µg ml, graph (1), DPPH invitro antioxidant activity shows the concentration of Alcohol extract of A. paeoniifolius (402.01 4.32%) had a greater scavenging effect than water (310.42 \pm 5.72%), ethyl acetate (253.83 \pm 7.2%), chloroform $(225.05 \pm 5.67\%)$, hexane $(153.19 \pm 2.33\%)$, and ascorbic acid $(295.35 \pm 0.36\%)$. (16). The five concentrations were taken to evaluate the ferric reducing antioxidant power assay in Methanol extract showing 3.57 ± 0.12 , 13.83 ± 0.30 , 26.87 ± 0.18 , 35.06 ± 0.17 , 42.23 ± 0.14 respectively, and the increasing rate of points results in the IC₅₀ value. 280.30 μg ml. Petroleum ether extract showing 13.45 ± 0.20, 24.62 \pm 0.25, 33.66 \pm 0.15, 41.69 \pm 0.15, and 51.01 \pm 0.13 respectively, and the increasing rate of points results in the IC50 value 242.75 μg ml (Table 4). The extract at 50 $\mu g/mL$ concentration had higher inhibitory activity of 68.6%. The IC₅₀ value was observed to be 30 μg/mL. The results reveal that extracts contain powerful inhibitor compounds, which may act as primary antioxidants that react with free radicals. (20). The leaf of A. peoniifolius has some potential value in the field of pharmacology.

Antibacterial Activity:

The Antibacterial activity in various concentrations of $50\,\mu$ l, $100\,\mu$ l, and $150\,\mu$ l and Amoxicillin as a control $100\,\mu$ g/mL respectively, which the inhibition zone created after the incubation period of bacterial plates has recorded with respective Zone of Inhibition sensitivity of Methanol comes with 09 mm, 12 mm, and 15 mm respectively and Petroleum ether extract 07mm, 10mm, and 14mm (Table 5) hence The overall inhibition zone sensitivity reports resistance of the Leaf part of *A. paeoniifolius* against bacterial inoculum *S. aureus.* antibacterial activity resulting in various concentrations of $50\,\mu$ l, $100\,\mu$ l, and $150\,\mu$ l and Amoxicillin as a control $100\,\mu$ g/mL, with the inhibition zone created after the incubation period of bacterial

plates has recorded with respective Zone of Inhibition sensitivity of Methanol comes with 09 mm, 12 mm, and 15 mm respectively and Petroleum ether extract 07 mm, 10 mm, and 14 mm (Figure 4), Methanol and Hexane extracts showed good inhibitory activity against all the bacterial pathogens used for the study but aqueous extracts of both the species did not show inhibitory activity against any pathogen (19).

Table 1: Qualitative analysis of two different solvent extracts.

Serial Number	Tests	Plant leaf extract		
Serial Number		Methanol extract	Pet Ether extract	
1.	Flavonoids	+	+	
2.	Saponins	-	-	
3.	Tannins	+	+	
4.	Phenols	-	-	
5.	Proteins	-	-	
6.	Cardiac Glycosides	+	+	
7.	Terpenoids	+	-	
8.	Carbohydrates	+	+	
9.	Quinones	-	-	

Note: + = Present, - = Absent

Table 2: GC MS Analysis of A. peoniifolius Methanol Leaf Extract.

Table 2. GC 115 Analysis of A. peonlybrius Methanol Leaf Extract.						
Solvent	Sl No	RT (Min)	Name of the Compounds	Molecular Formula	Molecular Weight (g/mol)	Area (%)
	1	3.468	Formamide, N, N-dimethyl	C3H8N2O	88	15.38
	2	19.678	Hexadecanoic acid, methyl ester	C17H34O2	270	3.86
٠.	3	20.153	n-Hexadecanoic acid	C16H32O2	256	4.00
extract	4 27.563		9(2,3Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-	C22H20OS	332	14.40
	5	28.702 Triphenylphosphine oxide		C18H15OP	278	1.94
Methanol	6	34.201	Heptacosyl heptafluorobutyrate	C31H55F02	592	1.70
let	7	36.862	1-Hexacosanol	C26H54O	382	3.20
2	8	38.309	Campesterol	C28H48O	400	3.20
	9	38.661	Stigmasterol	C29H48O	412	4.74
	10	39.517	gammaSitosterol	C29H50O	414	8.56

Note: RT = Retention Time

Table 3: GC MS Analysis of A. peoniifolius Pet Ether Leaf Extract.

Solvent	Sl No.	RT (Min)	Name of the Compounds	Molecular Formula	Molecular Weight (g/mol)	Area (%)
	1	7.723	2-Pyrrolidinone, 1-methyl	C5H9NO	99	1.31
ıct	2	20.189	n-Hexadecanoic acid	С16Н32О2	256	4.52
xtra	2 20.189 n-Hexadecanoic acid 3 22.453 Phytol 4 34.203 Heptatriacontane		Phytol	C20H40O	296	1.95
_			С37Н76	520	4.94	
Ether	5	36.824	Tetrapentacontane	C54H110	758	7.87
Pet]			Campesterol	C28H48O	400	12.07
<u> </u>	7	38.660	Stigmasterol	C29H48O	412	15.53
8 39.517 gammaSitosterol		C29H50O	414	23.96		

Note: RT = Retention Time

Table 4: Antioxidant Activity (FRAP) Relative % of Reducing Power Assay.

Concentration	Relative percentage of reducing power		
(µg/ml)	Methanol	Petroleum Ether	
50	3.57 ± 0.12	13.45 ± 0.20	
100	13.83 ± 0.30	24.62 ± 0.25	
150	26.87 ± 0.18	33.66 ± 0.15	
200	35.06 ± 0.17	41.69 ± 0.15	
250	42.23 ± 0.14	51.01 ± 0.13	
IC ₅₀ Value (in μg/ml)	280.30 μg/ml	242.75μg/ml	

Table 5: *A. paeoniifolius* Methanol and Pet Ether extracts Inhibition zone sensitivity against *S. aureus.*

Serial	Concentration (in	Measurement (in mm)			
Number	μg/ml)	Methanol	Petroleum Ether		
1.	50	9	7		
2.	100	12	10		
3.	150	15	14		
Control	Amoxicillin (500mg)		20		

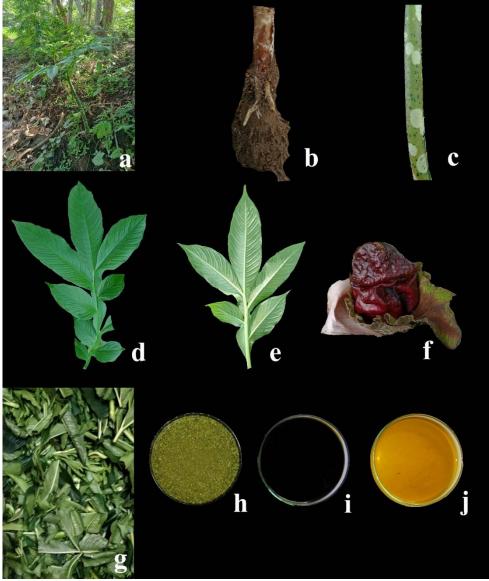
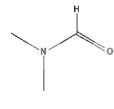


Figure 1: *Amorphophallus paeoniifolius* (Dennst.) Nicolson. a. Habit, b. Tuber c. Stem d. Leaf dorsal, e. Leaf ventral, f. Flower, g. Chopped Leaf, h. Fine Powder of Leaf, i. Methanol Extract, j. Petroleum Ether Extract.



Formamide, N, N-dimethyl

Hexadecanoic acid, methyl ester

Heptacosyl heptafluorobutyrate

n-Hexadecanoic acid

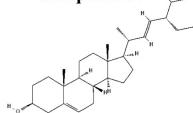
Triphenylphosphine oxide

н 0

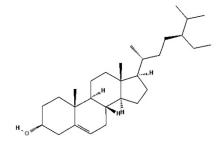
1-Hexacosanol

9(2,3Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-

Campesterol

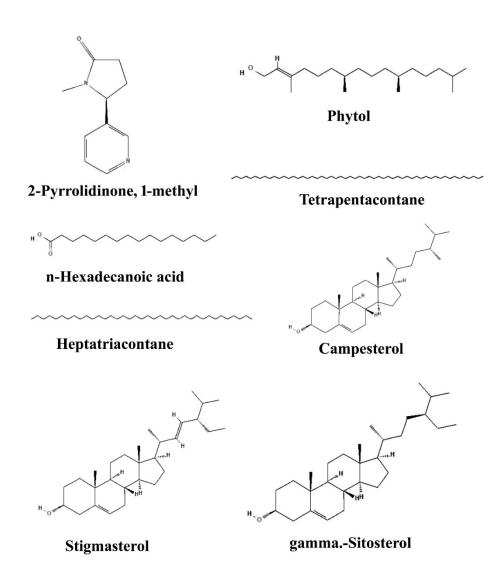


Stigmasterol

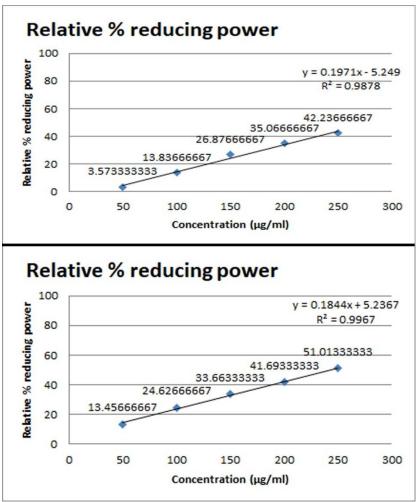


gamma.-Sitosterol

Figure 2: Amorphophallus paeoniifolius GCMS Compound structures of Leaf part methanol extract.



 $\label{lem:compound} \textbf{Figure 3:} \ \textit{Amorphophallus paeoniifolius} \ \textbf{GCMS} \ \textbf{Compound structures of Leaf part petroleum ether} \\ \textbf{extract.}$



Graph 1: Amorphophallus paeoniifolius. Antioxidant assay by FRAP method, the upper graph shows methanol extract & Lower graph shows petroleum ether relative percentage of reducing power.

Figure 4: *Amorphophallus paeoniifolius* Antibacterial activity shows inhibition zones a. Methanol Extract b. Petroleum Ether.



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