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Chemistry

ISOLATION OF ACTIVE PHYTOCHEMICALS FOR ANTI-MALARIAL ACTIVITY FROM TUBER EXTRACT OF AMORPHOPHALLUS CAMPANULATUS PLANT

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ABSTRACT Amorphophallus campanulatus, commonly known as Suran, is cultivated in India and other Asian countries. It is extensively used as a vegetable due to its high nutritive values and medicinal properties. The objective of this research work is to investigate the potential medicinal applications of the corm of the proposed plant anti-malarial activities. The Corm of Amorphophallus campanulatus was collected from village Karwale near Saphale, Palghar District and Taluka, Maharashtra State, India. The Corm crop produced on this land is organic and grown without the use of any chemical fertilizers. A total of 5 kg of corm was procured, washed with water, cut into small pieces, sun-dried, and converted into a fine powder. The sample was then subjected to sequential solvent extraction using a Soxhlet apparatus, starting with petroleum ether (A), followed by chloroform (B), ethanol (C), and water (D) in increasing order of polarity. The extracts obtained were further used for preliminary phytochemical analysis through qualitative methods. The ethanolic (C) extract showed the most promising results among the four extracts, and therefore the ethanolic extract was analyzed for its antibacterial and antifungal properties by using the Agar cup method and minimum inhibitory concentration. The antibacterial properties were compared with the standard drugs Gentamycin Ampicillin Chloramphenicol Ciprofloxacin and Norfloxacin. While the antifungal properties are compared with the standard drug Nystatin and Griseofulvin. Further it was chosen for minimal inhibition concentration (MIC) testing for the anti-malarial assay, following the protocol developed by Rieckmann and colleagues. Two standard drugs, Quinone and Chloro-quinone, were taken as references for anti-malarial activity. The plant extract was also investigated for its metal contents by Flame atomic adsorption spectroscopy for Zn Ag and Co. The sample was investigated for its organic chemical compounds by GC Mass Spectroscopy.

KEYWORDS : Amorphophallus campanulatus, Ethanol extract, Anti-malarial, GC mass spectroscopy.

INTRODUCTION

Elephant foot yam, scientifically known as Amorphophallus campanulatus and commonly referred to as Zaminkand, belongs to a large genus of around 200 tropical and subtropical tuberous herbaceous plants in the Arum family (Araceae). It is native to Africa, Australia, and Southeast Asia and is also known as Suran, Oal, or Jimikand in India. This herbaceous perennial C3 crop is cultivated in various states of India, including Andhra Pradesh, West Bengal, Gujarat, Kerala, Tamil Nadu, Maharashtra, Uttar Pradesh, Bihar, and Jharkhand. In India, it is often grown as an intercrop alongside ginger under coconut or banana trees, with a production yield of 50-80 tons per hectare However, in Indonesia, its production is relatively low, and the crop is underutilized (Ravi, 2009)

Elephant foot yam serves as a source of both protein and starch. The nutrient composition of Amorphophallus campanulatus includes approximately 2.14-7.56% crude protein, 1.04% crude fat, 9.43% crude fiber, 50 mg/100 g of calcium, 34 mg/100 g of phosphorus, and 0.78-6.24% oxalic acid The tuber of this plant holds high medicinal value and is consumed as food by many people. It is an important ingredient in many Ayurvedic preparations (Singh et al., n.d.)

The corms of Amorphophallus campanulatus contain various phytoconstituents such as quercetin, rutin, sitosterol, etc. (Dey et al., n.d.; Sharstry et al., n.d.). A water-soluble polysaccharide containing galactose, glucose, 4-O-acyl-methyl galacturonate, and arabinose has been isolated from the aqueous extract of the tuber. Glucomannan, characterized by spectroscopy, has also been isolated from the tuber. ((Ramachandra Setty, n.d.) ((Ramalingam et al., n.d.) High-performance thin-layer chromatography analysis has revealed the presence of quercetin and gallic acid. The corms contain Betulinic acid, β-sitosterol, stigmasterol, triacontane, lupeol, and β-sitosterol palmitate, along with glucose, galactose, sharp crystals of calcium oxalate, rhamnose, and xylose (Srivastava et al., 2014). The corms have been reported to possess antibacterial, antifungal, and cytotoxic activities due to the presence of a diterpenoid called salviasperanol and amblyone a triterpenoid (Srivastava et al., 2014) (Madhurima et al., n.d.; Singh et al., n.d.)

With the reference of above-mentioned activities and the presence of medicinal values our project to test the antimalarial activity for the best extractive solvent system with MIC (minimal inhibition concentration) for the same biological activities is under study in the current research.

METHODOLOGY

Collection of Plant Material

The tubers of Amorphophallus campanulatus were collected from a specific house in village Karwale near Saphale, Palghar District and Taluka, Maharashtra State, India in April 2018. The crop procured from this land is grown without the use of chemical fertilizers. The tuber, along with the flower, was identified by the Blleter Herbarium, specimen no. R-1274 of R. R. Fernandez, Department of St. Xavier's College. The tuber was washed thoroughly, chopped and dried under sunlight. The dried pieces were ground to a fine powder using a mill.

Extraction of Phytochemical

Three batches of the powdered sample, weighing 100 grams each, were subjected to solvent extraction. The powdered sample, packed in muslin cloth, was placed in a Soxhlet apparatus for successive extraction using 250 ml of solvent. The solvents were used in increasing order of polarity, starting with petroleum ether(A), followed by chloroform(B), ethanol(C), and finally distilled water(D). This procedure was repeated twice.

Preliminary Phytochemical Screening

This study involved the testing of different extracts(A-D) of Amorphophallus campanulatus to detect the presence of phytoconstituents using qualitative chemical tests. The qualitative tests for various phytoconstituents were conducted on all four extracts of Amorphophallus campanulatus.

Sr. No.	Phytochemical	Tests performed
1	Alkaloids	Mayer's test, Dragendorff's test, Wagner's test, Hager's test, tannic acid test
2	Tannins & Phenolic Compound	Gelatin test, Ferric chloride test, Vanillin Hydrochloride test, Alkaline reagent test, Mitchell's test
3	Flavonoids	Shinoda test, Magnesium Hydrochloride reduction test, Zinc Hydrochloride reduction, Alkaline reagent test
4	Proteins & Amino Acids	Millon's test and Ninhydrin test
5	Sterols & Triterpenoids	Liebermann- Buchard test and Salkowski test

Antimicrobial Analysis (Agar cup diffusion method)
The test organisms were grown in Mueller Hinton broth (in case of fungal strain Sabouraud Dextrose broth) providing incubation period of 48hr. The optical density of the culture was adjusted using 0.5 McFarland standards (10^6 cfu/ml). The cell suspension was mixed to homogeneity to give a final density of 1 x 10^4 cfu/ml and then taken ahead for checking the antimicrobial activity using agar cup diffusion method. Each plate contained four samples. 0.1mL volume of each sample was loaded into the wells and the plate was incubated at room temperature for 30°C for 48hr.

MIC (ANTI-MALARIAL ACTIVITY)
The concentrated ethanol extract was studied for the Minimum Inhibitory Concentration (MIC) of antimalarial activity.

All the synthesized compounds were screened for anti-malarial activity at the Microcare Laboratory & TRC in Surat, Gujarat. The antimalarial assay was conducted in 96-well microtiter plates, following the micro assay protocol of Rieckmann and co-workers with minor modifications. The cultures of P. falciparum strain were maintained in RPMI 1640 medium supplemented with 25mm HEPES, 1% D-glucose, 0.23% sodium bicarbonate, and 10% heat-inactivated human serum. The asynchronous parasites of P. falciparum were synchronized after treatment with 5% D-sorbitol to obtain only the ring stage parasitized cells. (Rieckmann et al., 1968)

For the assay, an initial ring stage parasitemia of 0.8 to 1.5% at 3% hematocrit in a total volume of 200µl of RPMI-1640 medium was determined using Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitemia (rings), and it was uniformly maintained with 50% RBCs (O+). A stock solution of 5mg/ml was prepared for each test sample in DMSO, and subsequent dilutions were prepared using the culture medium. The diluted samples (20µl each) were added to the test wells to obtain final concentrations (at fivefold dilutions) ranging between 0.4µg/ml to 100µg/ml in duplicate wells containing the parasitized cell preparation. The culture plates were incubated at 37°C in a candle jar.

After 36 to 40 hours of incubation, thin blood smears were prepared from each well and stained with JSB stain. The slides were microscopically observed to record the maturation of ring stage parasites into trophozoites and schizonts in the presence of different concentrations of the test agents. The concentration of the test sample that completely inhibited maturation into schizonts was recorded as the minimum inhibitory concentration (MIC). Chloroquine was used as the reference drug.

The mean number of rings, trophozoites, and schizonts recorded per 100 parasites from duplicate wells after 38 hours of incubation, along with the percent maturation inhibition with respect to the control group.

Analysis Of Gas Chromatography - Mass Spectroscopy Spectrum
For further analysis of phytochemicals and to find out the probable compound which are responsible for medicinal and biological activity we did Gas chromatography with mass spectroscopy, and we found 12 active compounds

RESULTS AND TABLE
Phytochemical Screening
The Ethanol and Water extracts tested positive for the presence of alkaloids and flavonoids, suggesting the potential presence of bioactive compounds that could have medicinal benefits.

Tannins were detected only in aqueous extract.

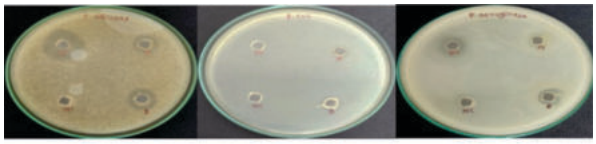
Terpenoids and steroids, which can have various medicinal properties, were present in both the chloroform and ethanol extracts. This indicates that these extracts could contain compounds that may contribute to potential therapeutic effects.

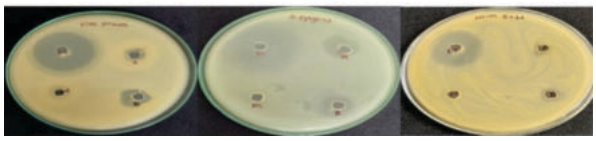
Proteins were detected in the ethanol and water extracts. While proteins may not always directly relate to therapeutic properties, their presence might indicate a more comprehensive profile of bioactive compounds in these extracts.

Considering the presence of alkaloids, flavonoids, terpenoids, steroids, and proteins in the ethanol and water extracts, these extracts appear to be more suitable for further investigation to explore the potential therapeutic or medicinal properties of the plant.

Antimicrobial Analysis
From the agar Cup method of antimicrobial analysis significant zones of inhibitions were observed for the organisms as mentioned in the table below and shown in the image.

Sr.No	Organisms	Zone of Inhibition(mm)		
		Blank	Positive Control	Negative Control
1	Candida albicans	16	23	-
2	Escherichia coli	12	24	-
3	Pseudomonas aeruginosa	12	14	-
4	Klebsiella pneumonia	13	25	-
5	Streptococcus pyogenes	14	28	-
6	Staphylococcus aureus	12	23	-





The ethanolic extract of Amorphophallus campanulatus showed inhibitory effect against Candida albicans, E. coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus pyogenes and Staphylococcus aureus. Since the sample showed antimicrobial activity against test organisms effective work should be done to utilize the benefits of this antimicrobial property.

ACE_GC-MS Peak Profile

Sr.no.	RT [mins]	Compound Name	Molecular weight	Area	% Area
1	6.63	n,n-Dimethyl-o-(1-methyl-butyl)-butyl-hydroxylamine	131	1745472	5.992
2	7.06	Iso-Menthol	156	2067489	7.097
3	11.47	2,2,4,4,6,6-pentamethyl-heptane	170	721501	2.477
4	14.43	3,5-Dimethyl-4-heptanone	142	534908	1.836
5	16.62	Undecan-4-ol	172	429563	1.475
6	17.1	1-Fluorododecane	188	390925	1.342
7	17.85	Isobutyl-Tiglate	156	258327	0.887
8	19.16	n-Hexadecanoic acid	256	7274080	24.971
9	20.19	Butyl pentadecanoate	298	280875	0.964
10	21.03	Methyl 10,11-octadecadienoate	294	12970599	44.547
11	21.35	4-Ethyl-octanoic acid	172	2167356	7.440
12	30.84	Oleic acid	282	283303	0.973
				29130418	

MIC- Anti-Malarial Activity
The minimum inhibitory concentration of the antimalarial activity of the plant extract was obtained and compared with the minimum inhibitory concentration of the standard drugs Chloroquine and Quinine. The MIC of the ethanolic extract is 0.95µg/ml while Chloroquine is 0.020µg/ml and Quinine is 0.268µg/ml

Analysis of Gas Chromatography- Mass Spectroscopy Spectrum
From GC-Mass Spectroscopy Peak profile we were able to observe 12 compounds in the Ethanol Extract. The details regarding the retention time (RT), chemical formula, and compound names of the detected peaks are given in the table below.

The identified compounds, including n, n-Dimethyl-o-(1-methylbutyl)-butyl) hydroxylamine, iso-Menthol, 2,2,4,4,6,6-pentamethylheptane ,3,5-Dimethyl-4-heptanone, Undecan-4-ol,1-Fluorododecane, isobutyl-Tiglate, n-Hexadecenoic acid, Butyl pentadecanoate, Methyl 10,11-octadecadienoate, 4-Ethyl-octanoic acid and Oleic acid.

All these bioactive compounds are responsible for the antibacterial and antimalarial property and other therapeutic uses of the plant.

CONCLUSION AND DISCUSSION
Amorphophallus Campanulatus has a nutritious value without any heavy metal content, only having calcium and potassium in high content which makes it more useful for human as a vegetable and shows so many different biological activities.

From above mentioned results of phytochemical tests Ethanolic extract shows presence of alkaloids, flavonoids and proteins and when antibacterial test was performed Ethanolic extract shows better antibacterial properties therefor ethanolic extract was taken for further testing of biological activities

Ethanolic extract of Amorphophallus Campanulatus were taken for

MIC test of Anti-tuberculosis, antimalarial, antifungal and antibacterial activities and results were compared with standard drugs. Results were shows that Ethanolic extract of plant have these activities in remarkable amount when results were compared with standard drugs for respective activities its shows that there is an activity but not as accurate as standard drugs. Standard drugs were more concentrated and more isolated there for its show's same activity with less quantity of drugs were as this plant Ethanolic extracts shows same activity with more quantity of extract.

But since it's a concentrated Ethanolic extract only results can be magnified and MIC can be improving with further isolation of active phytochemical and, it's a natural edible vegetable plant extract with no harmful metals so it can be taken in more quantity compared to standard drugs because it will have no harmful side effects.

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