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RESEARCH ARTICLE

GC-MS analysis of bio-active compounds in the ethanolic extract of *Amorphophallus sylvaticus*

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ABSTRACT: *Amorphophallus sylvaticus* (Roxb) (Araceae) seed acquired with different solvent extraction. The investigation aimed to carry out the GC-MS analysis of methanolic extracts of the seed *Amorphophallus sylvaticus* (Roxb) (Araceae). The preliminary analysis revealed the seeds contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been shown to be due to Eugenol, the major component of the essential oil which is also present in the fixed oil. The present study deals with the analysis of phytochemicals by qualitative and quantitative procedures using the ethanolic extracts of seeds of *Amorphophallus sylvaticus* by using soxhlet apparatus. Phytochemical constituents like alkaloids, flavanoids, saponins, Eugenol, glycosides in the ethanolic extract of *Amorphophallus sylvaticus* were also identified by GC-MS technique.
Keywords: *Amorphophallus sylvaticus*(Roxb), Eugenol, β-Sitosterol, phenol, GC-MS.

INTRODUCTION

Plant medicines are the most widely used medicines in the world today. A full eighty-five percent (85%) of the world's population employs herbs as their primary medicines (Hook., 1956). Plants are the original source materials for as many as 40% of the pharmaceuticals. Natural plant-based remedies are used for both acute and chronic health problems. As late as the early 1950's, many of the larger pharmaceutical companies still offered a broad variety of plant-based drugs in tablet, liquid and ointment forms (Bahman *et al*., 2003). Plant medicines are far and away safer, gentler and better for human health than synthetic drugs. This is so because human beings have co-evolved with plants over the past few million years. The results of synthetic drug explosion have been unfortunate (Prajapathi *et al*., 2003). Synthetic drugs often act in the body as irritants and toxins, upsetting the balance of whole systems, producing side effects that can be lethal. The World Health Organization (WHO) has shown great interest in documenting the use of medicinal plants by tribal in different parts of the world. India has a matchless position in the world, where a number of predictable ethnic systems of medicine viz., Siddha, Ayurveda, Unani, Yoga, Homeopathy, and Naturopathy are being used for the health care of people (Hook., 1956). Ayurvedic herbal arrangements based on the medicinal plants are used in present health care as nutritive supplements to avoid common bacterial diseases (Hook., 1956; Bahman *et al*., 2003; Prajapathi *et al*., 2003; Boham., 1994).

Consequently, many medicinal plants have been used by traditional medicine practitioners for the treatment of various diseases. Amongst the different evidence revealing that medicinal and culinary herbs have some common species, a diet rich in vegetables, fruits and Phytochemical which reduce the risk of cardiac and cancer diseases (Javanmerdi *et al*., 2003). Most of these plants were under consumed. Phytochemicals are biologically active chemical compounds found naturally in plants that protect against different ailments. They are non-nutritive compounds that contribute to flavor and colour (John., 1996; Craig., 1999). Considering the importance of plants as a source of medicine, even today we have selected *Amorphophallus sylvaticus* (Roxb.), family-Araceae, which is in use for centuries in the treatment of dental ailments. However, it is less explored plant for its varying activities. Hence an effort has been made here to investigate the potential uses of this plant.

1. MATERIALS AND METHODS

1.0. PREPARATION OF EXTRACTS

The specimen of *Amorphophallus sylvaticus* (Roxb.) were collected from Poombaarai forest, Dindigul. The *Amorphophallus sylvaticus* seeds were processed and crushed into powder and the extract was collected by using different solvents.

The seed powder of *Amorphophallus sylvaticus* (Roxb.) were subjected to extraction of solvents (Ethanol). The prepared extracts were then subjected to preliminary phytochemical analysis. The plant seeds were dried in shade, separated and made to dry powder. A weighed quantity (10 gm each) of the powder was subjected to continuous cold extraction in separate apparatus with solvents such as ethanol, methanol, water and hexane respectively for 24 hours in room temperature. The extracts were evaporated under reduced heat using water bath until all the solvent have been removed to obtain concentrated extract (Karthikeyan, R., 2016).

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1.1. PRELIMINARY PHYTOCHEMICAL ANALYSIS

Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration reaction to identify the major natural chemical groups such as alkaloids, saponins, tannins, glycosides, flavanoids, steroids, phenols, proteins, carbohydrates, fats, and amino acids vitamins and minerals. General reactions in this analysis revealed the presence (or) absence of these compounds in the crude extract tested (Herborne JB, 1973).

1.2. GC –MS ANALYSIS

1.2.0. PREPARATION OF EXTRACT

Amorphophallus sylvaticus (Roxb.) were shade dried. 20 g of the powdered tubers were soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatmann filter paper No.41 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phyto components of the plant material used. 2 µl of these solutions was employed for GC/MS analysis GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 El was employed (split ratio of10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da (Pattabiraman, K *et al*., 2017).

1.3. IDENTIFICATION OF COMPONENTS

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The spectrum of unknown components was compared with the version, 2014, software, Turbo mass 5.2. This is done in order to determine whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extraction these compounds.

2. RESULTS AND DISCUSSION

Seeds of *Amorphophallus sylvaticus* (Roxb.) were analyzed by proximate amount (Table 1) in the crude extract. Fat was high when comparing to other protein and fibers. Qualitative test proved that saturated and unsaturated fatty acids are very much abundant and Flavonoids in Secondary stage (Table 1). Secondary metabolites of *Amorphophallus sylvaticus* (Roxb.) also were analyzed using GC-MS technique. The GC-MS method conform that *Amorphophallus sylvaticus* (Roxb.) contain all the classes of secondary metabolites especially the eugenol and Flavonoids was given by Mass- Spectroscopy coupled with GC. The GC-MS analysis of the *Amorphophallus sylvaticus* (Roxb.) volatile oil showed 28 compounds such as (in %) Phenol, 4-(2-propenyl)-0.51, Eugenol-53.96, alfa-Copaene-1.11, Caryophyllene-8.44, Humulene-1.39, Chavibetol-4.22, Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-6.81, trans-calamenene-1.20,α-Cubebene-0.44,Santalol, cis,α-1.69,cis-Z-α- Bisabolene epoxide-0.87,Lanceol, cis-0.42, trans-Z-α-Bisabolene epoxide-0.64,Isoaromadendrene epoxide-0.72,2,4,6-Trimethoxyacetophenone-1.30,Farnesol, acetate-0.65, Calarene epoxide-0.21,n-Hexadecanoic acid-0.88,9,12-Octadecadienoic acid (Z,Z)-0.95,9,12,15-Octadecatrienoic acid, (Z,Z,Z)-0.51,α-Cadinol-0.62, Phenol, 2-methoxy-4-(2-propenyl)-, acetate-0.27, Eicosane, 7-hexyl-0.45, Heptacosane-0.86, β-Sitosterol-0.96(Table 2 & 3).

3.CONCLUSION

These results suggest *Amorphophallus sylvaticus* (Roxb.) is a source of alkaloids, flavonoids, saponins, Glycosides etc. In ethanolic extracts of *Amorphophallus sylvaticus* (Roxb.) GC-MS screening of eugenol and Flavonoids of *Amorphophallus sylvaticus* (Roxb.) was recorded and which confirm the presence of all classes of eugenol, phenol, β-Sitosterol and Flavonoids. *Amorphophallus sylvaticus* (Roxb.) holds nutraceutical potential against various physiological threats owing to its rich phytochemistry especially due to presence of eugenol, phenol, phenyl propanoid(Chavibetol) and β-Sitosterol. Finally fixed and essential oil supplement in food products especially bakery items is feasible and can be employed to achieve the allied health charging.

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Table.1: Proximate composition of *Amorphophallus sylvaticus* (Roxb.) in seeds

S.NO	Proximate composition	Results(g/100g)
1.	Moisture	6.72
2.	Crude protein	23.18
3.	Crude Fat	33.11
4.	Crude Fiber	7.21
5.	Ash	3.67
6.	Carbohydrate	26.11

Table.2: Preliminary Phytochemical Screening of *Amorphophallus sylvaticus* (Roxb.)

S.NO	Phytochemicals	Observation
1.	Carbohydrates	+
2.	Proteins	+
3.	Fats	+
4.	Saturated & Unsaturated fatty acids	+
5.	Free Fatty acids	+
6.	Alkaloids	+
7.	Flavonoids	+
8.	Saponins	+
9.	Carvacrol	+
10.	4-Terpinol	+
11.	Tanins	+
12.	Glycosides	+
13.	Pholbatanins	-

Table:3. Compounds identified in the *Amorphophallus sylvaticus* (Roxb.) sample

S.No	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
1.	6.32	Phenol, 4-(2-propenyl)-	$C_9H_{10}O$	134	0.51
2.	8.07	Eugenol	$C_{10}H_{12}O_2$	164	53.96
3.	8.43	alfa.-Copaene	$C_{15}H_{24}$	204	1.11
4.	9.10	Caryophyllene	$C_{15}H_{24}$	204	8.44
5.	9.56	Humulene	$C_{15}H_{24}$	204	1.39
6.	9.77	γ-Muurolene	$C_{15}H_{24}$	204	0.35
7.	10.28	Chavibetol	$C_{10}H_{12}O_2$	164	14.22
8.	10.35	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	$C_{15}H_{24}$	204	6.81
9.	10.40	trans-calamenene	$C_{15}H_{22}$	202	1.20
10.	10.55	α-Cubebene	$C_{15}H_{24}$	204	0.44
11.	11.23	Santalol, cis,α-	$C_{15}H_{24}O$	220	1.69

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12.	11.56	cis-Z-α-Bisabolene epoxide	$C_{15}H_{24}O$	220	0.88
13.	11.74	Cubenol	$C_{15}H_{26}O$	222	0.29
14.	11.82	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	220	0.23
15.	11.87	Lanceol, cis	$C_{15}H_{24}O$	220	0.42
16.	12.07	trans-Z-α-Bisabolene epoxide	$C_{15}H_{24}O$	220	0.64
17.	12.24	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220	0.72
18.	12.35	2,4,6-Trimethoxyacetophenone	$C_{11}H_{14}O_4$	210	1.30
19.	14.03	Farnesol, acetate	$C_{17}H_{28}O_2$	264	0.65
20.	14.89	Calarene epoxide	$C_{15}H_{24}O$	220	0.21
21.	15.69	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.88
22.	18.10	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.95
23.	18.17	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	0.51
24.	25.47	α-Cadinol	$C_{15}H_{26}O$	222	0.62
25.	27.94	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206	0.27
26.	29.15	Eicosane, 7-hexyl-	$C_{26}H_{54}$	366	0.45
27.	32.64	Heptacosane	$C_{27}H_{56}$	380	0.86
28.	37.57	β-Sitosterol	$C_{29}H_{50}O$	414	0.96

GC- MS Chromatogram

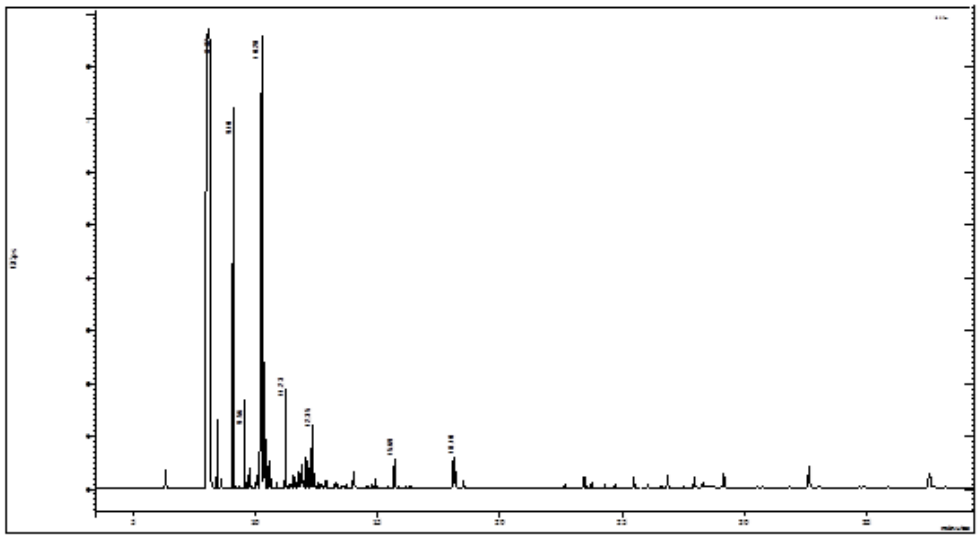

Figure 1: Secondary Metabolite screening of *Amorphophallus sylvaticus* (Roxb.) by GC-MS technique

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CONFLICTS OF INTEREST

"The authors declare no conflict of interest".