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Gas Chromatography-Mass Spectrometry (GC-MS) Determination of Bioactive Components from *Aervalanata.* (L.) Whole Plant

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ABSTRACT

Aervalanata L. belongs to the family of *Amaranthaceae*, found in the tropical regions. Traditionally, this plant is known for antimicrobial, anthelminthic, antiparasitic, antidiabetic, diuretic, nephroprotective, cytotoxic, and antihyperlipidemic activities. As yet no further characteristic study has been conducted from ethanolic extract of this species, therefore in this present study we seek to identify and evaluate the bioactive compounds from the ethanolic extracts of *Aervalanata* L whole plant by using the GCMS. Result of this research work reports twenty-eight compounds. The identified chemical compounds were correlated with the NIST Mass Spectrum Library.In conclusion, we seek to provide additional information on the clinical significance and pharmacological information associated with this plant.

Keywords: Aervalanata L; GC-MS; Traditional uses; Acetic acid; 3-O-Methyl-d-glucose; n-Hexadecanoic acid; Butanoic acid; Lactic acid

1. INTRODUCTION

Spectrometry and gas chromatography are to recognise diverse materials present in the unknown samples. It includes diagnosis of drugs, unidentified specimens, fire investigation and environmental analysis.[1] GC-MS-a combination of Gas Chromatography and Mass Spectrometry, is used to analyse complex organic and biochemical mixtures.[2] *Aervalanata* L one of the important medicinal plants belongs to the family of *Amaranthaceae*. This plant is 30-60cm tall with many branches and shrubs. Leaves are simple, alternate, with short petioles having dense hair. Flowers are small green clusters on spikes. The plant produces greenish rounded fruit having kidney shaped seed with shining black testa.[3]

Due to the presence of phytochemicals and minerals such as alkaloids, flavonoids, tannins, sodium, potassium, calcium, chloride, it plays a therapeutic role in pathological conditions. It exhibits diuretic activity, anti-inflammatory, hyperglycemic resistance, urolithic, anti-hyperlipidemic and many more. Hence, applying more scientific methods on this species may lead to the discovery of a new entity which will be helpful to the pharmaceutical industry for the production of novel therapeutic drugs from this species.[4] Ethnopharmacologically, the whole plant of *Aervalanata* L is used to cure diarrhea and malaria.[5,6] Administration of *Aervalanata* aqueous suspension to CaOx urolithic rats had reduced the oxalate synthesising enzymes, diminished the markers of crystal deposition in the kidney and hence can be used as curative agent for urolithiasis.[15]

In this current research work, an attempt has been made to compile and document information from the ethanol extract of the whole plant, using GC/MS to evaluate and identify bioactive compounds to demonstrate the biological properties required for the research purpose.

2. METHODOLOGY

2.1 Raw Materials

Dried plants of *Aervalanata* L (whole plant) were collected from the sources of small agriculture village, Vadaseri, Thanjavur District, Tamil Nadu, India. The plant materials were authenticated based on organoleptic, macroscopic examination and certified (Authenticated No. PARC/2019/4095) by Professor P. Jayaraman, Director, IHB, Plant Anatomy Research Centre, Tambaram, Chennai-45, India.

2.2 Formation of Ethanolic Extract

Extracts were prepared as described by the standard method.[7] Initially, the collected plant materials were allowed to dry for a few days in a sunshade so that the

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Table 1. Isolation of bioactive compounds from ethanolic extract of *Aervalanata* L. based on Retention time, Molecular formula, and Molecular weight by using (GC/MS).

S. No.	Isolates	IUPAC Name	Empirical formula	Retention time	MW g/mol
1	Acetic acid	Ethanoic acid	$C_2H_4O_2$	3.100	60
2	2-Propanone, 1-hydroxy	1-Hydroxypropan-2-one	$C_3H_6O_2$	3.367	74
3	Silanediol dimethyl	Dihydroxy(dimethyl)silane	$C_2H_8O_2Si$	3.647	92
4	Ethanone, 2-(formyloxy)-1-phenyl	Phenacyl formate	$C_9H_8O_3$	3.882	164
5	Propanoic acid, 2-hydroxy-, methyl ester, (±)	Methyl 2-hydroxypropanoate	$C_4H_8O_3$	3.984	104
6	Silane, diethoxydimethyl	Diethoxy(dimethyl)silane	$C_6H_{16}O_2Si$	4.150	148
7	Propanoic acid, 2-oxo-, methyl ester	Methyl 2-oxopropanoate	$C_4H_6O_3$	4.512	102
8	Glycolaldehyde dimethyl acetal	2,2-dimethoxyethanol	$C_4H_{10}O_3$	4.748	106
9	Furfural	Furan-2-carbaldehyde	$C_5H_4O_2$	5.129	96
10	Butanoic acid, 4-hydroxy	4-hydroxybutanoic acid	$C_4H_8O_3$	6.357	104
11	L-Lactic acid	2-hydroxypropanoic acid	$C_3H_6O_3$	6.784	90.08
12	Glycerine	Propane-1,2,3-triol	$C_3H_8O_3$	9.354	92.09
13	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	$C_6H_8O_4$	10.245	144.12
14	2-Methoxy-4-vinylphenol	4-ethenyl-2-methoxyphenol	$C_9H_{10}O_2$	12.560	150.7
15	Phenol, 2,6-dimethoxy	1,3-dimethoxy-2-hydroxybenzene	$C_8H_{10}O_3$	13.057	154.16
16	Phenol, 2,4-bis(1,1-dimethylethyl)	1-Hydroxy-2,4-di-tert-butylbenzene	$C_{14}H_{22}O$	15.061	206.32
17	2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)	2-Cyclohexen-1-one, 2-(2-methyl-2-propenyl)	$C_{10}H_{14}O$	16.352	150.22
18	Megastigmatrienone	Tabanone	$C_{13}H_{18}O$	16.492	190.28
19	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol	$C_{14}H_{22}O$	16.887	206.32
20	Tetradecanoic acid	Tetradecanoic acid	$C_{14}H_{28}O_2$	17.898	228.37
21	3-O-Methyl-d-glucose	(2R,3S,4R,5R)-2,4,5,6-tetrahydroxy-3-methoxyhexanal	$C_7H_{14}O_6$	18.267	194.18

22	Hexadecanoic acid, methyl ester	15-methylhexadecanoic acid	$C_{17}H_{34}O_2$	19.495	270.5
23	n-Hexadecanoic acid	Hexadecanoic acid	$C_{16}H_{32}O_2$	19.940	256.42
24	Hexadecanoic acid, ethyl ester	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	20.157	284.5
25	Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro-	Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro-	$C_{18}H_{18}N_4O$	20.367	306.4
26	trans-13-Octadecenoic acid	(E)-octadec-13-enoic acid	$C_{18}H_{34}O_2$	21.620	282.5
27	Octadecanoic acid	(E)-octadec-2-enoic acid	$C_{18}H_{36}O_2$	21.804	284.5
28	7-Oxabicyclo [4.1.0] heptane, 2,2,6-trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-	2,2,6-Trimethyl-1-[(1E)-3-methyl-1,3-butadienyl]-5-methylene-7-oxabicyclo [4.1.0] heptane	$C_{15}H_{22}O$	23.624	218.33

muddy portions were removed. Then the dried plants were roughly grinded by using a mixture. 50 g of powdered material was soaked with 300ml of ethanol for 72 hours with intermediate shaking separately in a beaker. The filter was filtered through paper and extracted with the Soxhlet apparatus. The extracts were concentrated to dryness by keeping them over a boiling water bath for 15 to 20 minutes at 80 ºC to 90 ºC. The last traces of solvent were removed by transferring them into a china dish and then allowedto heat through a sand bath at normal temperature carefully in order to prevent charring or denature of the compound due to overheating.[8,9] The yield of ethanolic extracts (1.5mg) was noted for future reference. The dried crude extract was stored in sterile amber-colored bottle or vials and stored in the refrigerator until used for this work.

2.3 GC-MS

The sample then injected into the column and the carrier gas helium is used to flow at a rate of 1ml/minute. The injector is operated to inject the sample into the column at an injection mode of 10 °C/minute. The oven temperature is programmed at 50-250 °C. The ionization voltage of 70 eV for ions and the mass range of 50-600 units of mass are used for chromatographic conditions. The National Institutional Standards and Technology (NIST) database of over 62,000 formats was used to describe the compounds isolated by GC-MS. Isolated components were then compared with the known mass spectrum of the NIST library.

3. RESULTS AND DISCUSSION

In this present study, we observed and reported about plenty of bioactive compounds by GC/MS analysis of ethanolic extracts from *Aervalanata*L. The identified bioactive compounds were compared & confirmed, with a mass spectral library of NIST. The names of isolated compounds along with the IUPAC name, composition, molecular formula, and molecular weight were shown in Tables 1. among which the most abundant were Acetic acid at Rt of 3.100, Ethanone, 2-(formyloxy)-1-phenyl at Rt of 3.882, Glycolaldehyde dimethyl acetal at Rt of 4.748, L-Lactic acid at Rt of 6.784, Glycerin at Rt of 9.354, 4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl at Rt of 10.245, 4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol at Rt of 17.898, 3-O-Methyl-d-glucose at Rt of 18.267, *n*-Hexadecanoic acid at Rt of 19.940, trans-13-Octadecenoic acid at Rt of 21.620 and2,2,6-Trimethyl-1-[(1E)-3-methyl-1,3-butadienyl]-5-methylene-7-oxabicyclo [4.1.0] heptaneat Rt of 23.624.Each compound has a unique property and its uses and nature was collected from PUBCHEM and PUBMED.[10]

The result of this present studies reveals that Acetic acid, 4-hydroxy butanoic acid, Lactic acid, Glycerine, 3-O-Methyl-d-glucose, Hexadecanoic acid, Propanoic acid, Octadecanoic acid, 2-Methoxy-4-vinylphenol, Phenol, 2,6-dimethoxy, Furfural, etc., obtained through GCMS of *Aervalanata* L have more medicinal properties and are also used as raw materials for many chemicals as well as pharmaceutical industry. Acetic acid is used in the production of vinyl acetate monomer, acetic anhydride (analgesics), etc., and is also used as a solvent to purify organic compounds and recrystallisation. Used as an antibiotic to treat bacterial or fungal infections and aids in dissolving the kidney stone formation. 4-hydroxy Butanoic acid is a compound related to the production of pharmaceutical products or drugs. Lactic acid, due to the presence of its disinfectant and keratolytic properties, it is used as an antiseptic in ointments to remove warts, calluses and other wounds.

Glycerine is used as a moisturizer to treat dry, rough, scaly, itchy skin and also applied to prevent the skin irritations. 3-O-Methyl-d-glucose is frequently used to study blood-brain barrier transport and distribution sites of hexose in the brain. An important requirement for this application is that it should not be chemically modified in tissues. *n*-Hexadecanoic acid present in ethanolic extract of *Aervalanata* L is the raw material for the production of soap,

lubricating oils, waterproofing materials, food additives etc. Hexadecanoic acid ethyl ester is used as a hair and skin conditioning agent. Hexadecanoic acid methyl ester plays the role as a metabolite needed for growth, reproduction, and maintaining health. Propanoic acid, 2-hydroxy, methyl ester, is used in the manufacture of varnishes which is applied on surfaces of wooden furniture or art objects to provide a glossy and also as a protective agent. Octadecanoic acid and trans-13-Octadecenoic acid are a component of animal fats and vegetable oils. It is used as an emulsifying or dissolving agent in aerosol products. 2-Methoxy-4-vinylphenolpresent in ethanolic extract of *Aervalanata* L is an aromatic substance used as a flavoring agent. Phenol, 2,6-dimethoxy is a compound including spices, juice, colors, flavors, etc, that are added to food for human consumption. Furfural is an imperative renewable, chemical ingredient that is essential for the chemical industry.

Early reports says that Isorhamnetin-3-O-β-D-glucoside and narcissin are thought to be separated from ground and air-dried materials.[11] GC-MS analysis of leaves, stems, roots, flowers, and seeds from *Aervalanata* L exhibits pyridine, hydroquinone monobenzyl ether, *n*-Docosane, dotriacontane, Ricinoleic acid, 2-isopropyl-2,5-dihydrofuran.[12] The plant *Aervalanata* L comprised rich in phenolic compounds, alkaloids and steroids, along with that kaempferol, tiliroside, *β*-sitosterol, aervoside, syringic acid, and canthin-6-one has also been isolated.[13] Earlier report says that four different flavanols such as quercetin, kaempferol, 4'-methoxy kaempferol, 4',7dimethoxy kaempferol, were identified from this *Aervalanata* L.

In addition, phenolic acids such as vanillic acid, syringic acid, p-hydroxy benzoic acid, p-coumaric acid, trans-ferulic acid, melilotic acid and betacyanin were identified.[14] The aqueous extract of *Aervalanata* L contains flavonoids such as kaempferol and related compounds, triterpenes such as betulin and tannins, which may act as curative agents for Urolithiasis.[15]

In previous phytochemical screening *Aervalanata* L is said to contain a wide variety of phytochemicals, including alkaloid, steroid, flavonoid, tannin, amino acid, protein, carbohydrate, cardiac glycoside, saponin, and terpenoid.[16] The *Aervalanata* L plant is endowed with a variety of flavonoid, alkaloid, steroid, polysaccharide, tannin, phenolic compound and saponin.[17,18,19] Previous reports through Fourier Transform Infrared Spectroscopy analysis from the roots, stems, leaves, and flowers of *Aervalanata* L consists of various functional groups including amide, alcohol, aldehydes, nitro compounds, ethers, amines, phenols, etc. This indicates that *Aervalanata* L comprises diversity of the chemical constituents in it.[20] The result of this research work reveals that plant *Aervalanata* L comprise plenty of medicine as well as pharmaceutical compounds. It may possess various biological properties such as antioxidant, anti-diabetic, hepatoprotective, zanti-cancer properties as well as urolithic, anti-inflammatory, and diuretic activity. Hence, *Aervalanata* L may be an excellent remedy for the treatment of various diseases.

4. CONCLUSION

In conclusion, after systematic analysis of this research work, plenty of bioactive compounds that have been identified and documented. It will provide additional information about the efficacy of diverse biological properties such as urolithic, anti-inflammatory, diuretic activity, anti-inflammatory, anti-hyper glycaemic, and anti-hyper lipidemic activities related to this plant. This documentation and valuable information will definitely support the efficacy of *Aervalanata* L.

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