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Comparative GC-MS analysis of ethanolic extract of *Alternanthera philoxeroides* collected from polluted and unpolluted site

[1] U Arul Pamila, [2] S Karpagam
[1] Ph.D. Research Scholar, Department of Botany, Queen Mary's College (A), Chennai, Tamil Nadu, India
[2] Associate Professor, Department of Botany, Queen Mary's College (A), Chennai, Tamil Nadu, India

Abstract
To analyze and characterize the phytoconstituents of ethanolic extract of the aerial parts of an edible plant *Alternanthera philoxeroides* (Mart.) Griseb collected from polluted and unpolluted sites using gas chromatography-mass spectroscopy (GC-MS). The shade dried aerial parts of plant powder *A. philoxeroides* were extracted with ethanol and crude ethanolic extracts was obtained. The concentrated extract was subjected to GC-MS analysis to detect the phytoconstituents. The GC-MS analysis was performed by using Perkin Elmer Clarus 500 model with the software Turbomass ver 5.2. GC-MS plays a key role in the analysis of unknown component of plant origin. The GC-MS analysis shows different peaks with low and high molecular weight, determining the presence of many bioactive compounds. The isolation and identification of phytoconstituents is based on the peak area, retention time, molecular weight and molecular formula. From GC-MS analysis, ethanol extracts of *A. philoxeroides* from polluted site showed higher phytoconstituents when compared to the ethanol extract of *A. philoxeroides* from unpolluted site. The ethanolic extract of aerial parts of *Alternanthera philoxeroides* proved to be a reservoir of bioactive compounds identified by GC-MS which could prove effective in the treatment of various diseases.

Keywords: GC-MS analysis, phytoconstituents, *Alternanthera philoxeroides* ethanol extract

Introduction
Traditional Ayurvedic, Homeopathy, Siddha and Unani medicines are widely used in developing countries like India, because they do not show any side effects compared to Allopathic medicines. Last few decades, people all over the world are aware of the uses of traditional herbal medicines. Nature has been as source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Various medicinal plants have been used as a source of medicine for years in daily life to treat diseases all over the world [1]. Herbal drugs obtained from plants are believed to be much safer; this has been proved in the treatment of various ailments. In the last few years GC-MS has become firmly established as a key technological platform for secondary metabolite profiling in plant species.

The family Amaranthaceae presents 180 genera and 2,500 species distributed mainly in cool temperate regions. This family represents the most species-rich lineage within the flowering plant order of Caryophyllales [2].

Amaranth is a very versatile crop that is grown in a wide range of agro-climatic conditions; it resists drought, heat, and pests, and adapts readily to new environments, including some that are inhospitable to conventional cereal crops [3-4]. It is one of the few multi-purpose crops, which can supply grains and tasty leafy vegetables of high nutritional quality as a food and animal feed, and additionally an ornamental plant, because of an attractive inflorescence colouration [5-6].

Alternanthera philoxeroides, commonly known as alligator weed is an emergent semi-aquatic herbaceous, perennial with prostrate, sprawling, creeping, floating hollow stems, often in a dense tangled mass, rooted in shallow water or growing from the shoreline, occasionally free-floating [7-8]. The hollow stems provide considerable buoyancy of the mat [9]. Roots form at stem nodes. Aqueous extracts of the plant demonstrated for inhibitory activity against human immunodeficiency virus [10]. The plant extracts has been found to possess inhibitory activity against dengue virus in-vitro [11]. The antitumor compounds alternanthin B and N-trans-feruloyl-3,5-dimethoxytyramine has been isolated from aerial parts of *A.philoxeroides* [12]. The preliminary phytochemical analysis showed the presence of alkaloids, carbohydrates, saponins, phenols, flavonoids, aminoacids, diterpenes, tannin, terpenoids, protein, steroid, oxalate, coumarin and quinone in the ethanol extract of *A. philoxeroides* from unpolluted site [13]. The aim of present work was to analyze phytochemical constituents present in aerial parts of the plant extract using ethanol by GC-MS in polluted and unpolluted site.

Materials and Methods
Collection and authentication of plant materials
A. philoxeroides specimens were collected from two different sites Pechiparai of Kanyakumari District and Cooum River, Maduravoyal Chennai, Tamil Nadu. The collected plants were identified and confirmed by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai.

Preparation of Plant Extracts
Fresh plants were washed thoroughly three to four times with

running tap water then finally with sterile water followed by shade drying at room temperature for 20-30 days and powdered by using an electric blender and stored in airtight container. The sample of 10g was taken and soaked for 24h in 30 ml of ethanol. The extract was filtered using Whatman filter paper No. 1, evaporated to dryness and re-dissolved in DMSO (Dimethyl Sulphoxide). The extracts were preserved in airtight container and kept at 4-5^{0}C for further use.

Gas Chromatography-Mass spectrometry (GC-MS) Analysis

The GC-MS was performed by using PerkinElmer Clarus 500 Model and the software used is Turbomass ver 5.2. The fused silica column was packed with Elite -5MS(5% Phenyl 95% dimethylpolysiloxane, 30 m x 250 µm) The oven temperature was set up from 50 ºC with an increase of 8 ºC/min to 220 ºC for 5 min and 7 ºC /min to 280 ºC for 15 mins. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min. An aliquot of 2 µl of sample was injected into the column with the injector temperature at 280 ºC and the Split ratio of 10:1. The ionizing energy of 70 eV was used and the electron ionization is involved. The mass range is 40-600 amu. The inlet line temperature was 200 ºC and source temperature was 150 ºC Total GC running time was 50 minutes. The compounds were identified referring to NIST 2005 library.

Identification of compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technique (NIST Version-Year 2005) having more than 62,000 patterns. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST data library (version 2005). The name, molecular weight, molecular formula and structure of the components of the test material were determined.

Results and Discussion

GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, ethers etc [14]. Plants are a tremendous source for the discovery of new products of drug development. Today several distinct chemicals derived from plants are important drugs that are currently used in more countries in the world [15]. The phytoconstituents present in the ethanol extract of aerial parts of *Alternanthera philoxeroides* in unpolluted site and polluted site were identified by GC-MS analyzed (Figures 1 and 2). It clearly showed twelve peaks indicating the presence of twelve phytochemical compounds. The active principals with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extracts of *A. philoxeroides* in unpolluted and polluted site are presented in Tables 1 and 2.

Twelve components were detected in ethanol extracts of aerial parts of *Alternanthera philoxeroides* in unpolluted site. The results revealed that Ar-tumerone (8.14%), Phenol, 5-(1,5-dimethyl-4-hexeny (% 2.77), Bicyclo[3.1.1]heptane, 2,6,6-tri...(% 2.04), 2-Pentadecanone, 6,10,14-trimethyl-(% 1.23), n-Hexadecanoic acid (% 29.23), 3-Acetoxypentadecane (% 2.10), 9,12-Octadecadienoic acid (Z,Z)- (% 12.50), 9,12-Octadecadienoic acid (Z,Z)-(% 13.61), Octadecanoic acid (% 3.45), Tris(tert-butyldimethylsilyloxy)...(% 7.27), Temazepam (% 13.20), 5-Methyl-2-phenylindolizine (% 4.47) in (table 1). Seventeen components were detected in ethanol extracts of *A. philoxeroides* in polluted site.

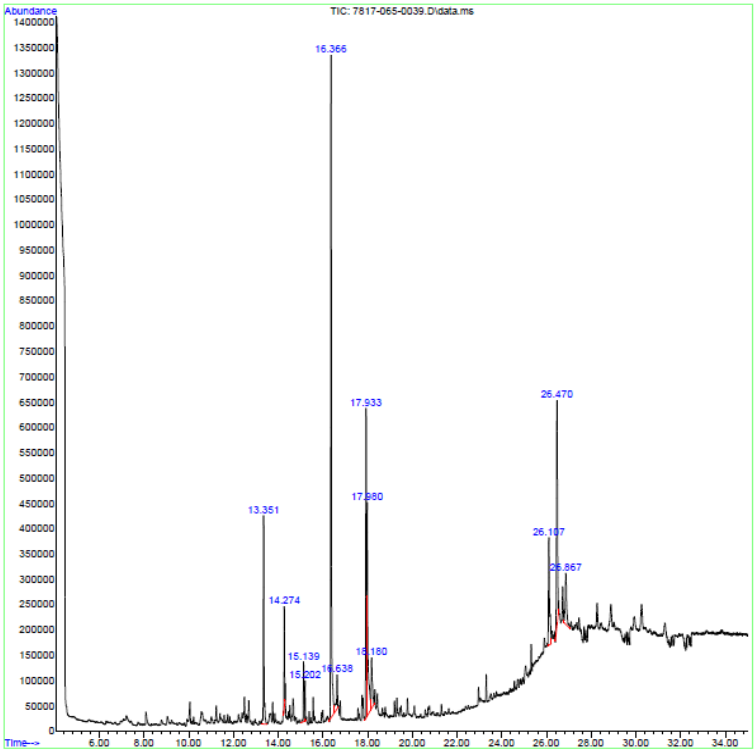

Fig 1: GC-MS analysis of phytochemicals identified from ethanolic extracts of *Alternanthera philoxeroides* in unpolluted site

Table 1: Phytochemical compounds identified in ethanol extracts of *Alternanthera philoxeroides* in unpolluted site

PK#	PEAK NAME	Molecular formula	Molecular weight	Retention time	% peak area
1.	Ar-tumerone	$C_{15}H_{20}O$	216.324 g/mol	13.351	8.14
2.	Phenol, 5-(1,5-dimethyl-4-hexeny	$C_{15}H_{22}O$	218 g/mol	14.272	2.77
3.	Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	138.249 g/mol	15.141	2.04
4.	2-pentadecanone, 6,10, 14-trimethyl	$C_{18}H_{36}O$	268.485 g/mol	15.200	1.23
5.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424 g/mol	16.367	29.23
6.	3-Acetoxypentadecane	$C_{17}H_{34}O_2$	270.451 g/mol	16.641	2.10
7.	9,12-octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.445 g/mol	17.934	12.50
8.	9,12-octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	280.445 g/mol	17.979	13.61
9.	Octadecanoic acid	$C_{18}H_{36}O_2$	284.477 g/mol	18.179	3.45
10	Tris (tert-butyldimethylsilyloxy)arsane	$C_8H_2O_2Si$	871.302 g/mol	26.105	7.27
11.	Temazepam	$C_{16}H_{13}ClN_2O_2$	300.742 g/mol	26.469	13.20
12.	5-Methyl-2phenylindolizine	$C_{15}H_{13}N$	207.276 g/mol	26.871	4.47

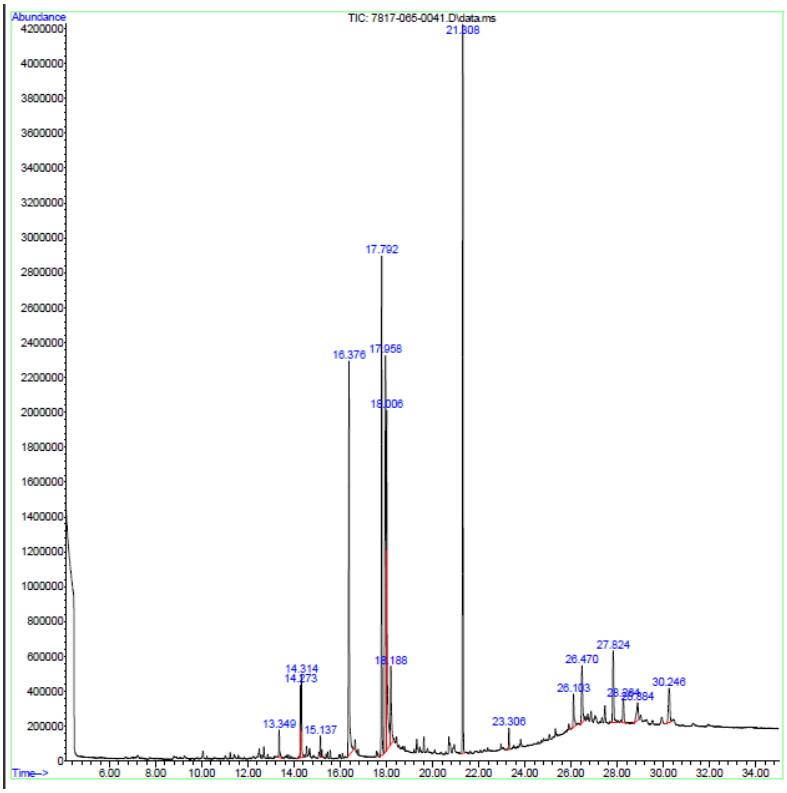

Fig 2: GC-MS analysis of phytochemicals identified from ethanolic extracts of *Alternanthera philoxeroides* in polluted site

Table 2: Phytochemical compounds identified in ethanol extracts of *Alternanthera philoxeroides* in polluted site

PK#	PEAK NAME	Molecular formula	Molecular weight	Retention time	% peak area
1.	Ar-tumerone	$C_{15}H_{20}O$	216 g/mol	13.351	0.91
2.	Phenol, 5-(1,5-dimethyl-4-hexeny	$C_{15}H_{22}O$	218.33 g/mol	14.272	1.78
3.	Ethyl p-methoxycinnamate	$C_{12}H_{14}O_3$	206.241 g/mol	14.316	2.28
4.	Bicyclo[3.1.1]heptane, 2,6,6-tri...	$C_{10}H_{18}$	138.25 g/mol	15.141	0.51
5.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42 g/mol	16.374	14.79
6	Phenol, 4-(3,7-dimethyl-3-etheny...	$C_{18}H_{24}O$	256 g/mol	17.793	13.50
7.	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45 g/mol	17.956	15.43
8.	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264 g/mol	18.008	14.19
9.	Octadecanoic acid	$C_{18}H_{36}O_2$	284 g/mol	18.187	3.51
10.	Phthalic acid, di(2-propylpentyl...	$C_{24}H_{38}O_4$	390 g/mol	21.307	19.49
11	Squalene	$C_{30}H_{50}$	410.72 g/mol	23.305	0.56
12.	Tris(tert-butyldimethylsilyloxy)...	$C_{48}H_{66}O_9Si_3$	871.302 g/mol	26.105	1.41
13.	Ethanone, 2-(2-benzothiazolylthi...	$C_{14}H_{13}N_3OS_2$	303 g/mol	26.469	3.00
14.	Urs-12-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	468.72 g/mol	27.821	3.62
15.	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46 g/mol	28.267	1.33
16.	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.462 g/mol	28.884	1.33
17	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.462 g/mol	30.243	2.36

Table 3: Biological activity of phytoconstituents identified in the ethanol extracts of *A. philoxeroides* unpolluted site

PK#	Name of the compounds	Biological Activity
1.	Ar-tumerone	Antiviral.
2.	Phenol, 5-(1,5-dimethyl-4-hexeny	Antioxidant, Antibacterial/Antiseptic and disinfectant aromatic alcohol., Topical Anesthetic.
3.	Bicyclo [3.1.1] heptane, 2,6,6-trimethyl-	No Activity reported.
4.	2-pentadecanone, 6,10, 14-trimethyl	Cancer-preventive.
5.	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavour, hemolytic, 5-alpha reductase inhibitor.
6.	3-Acetoxypentadecane	Antinephrotoxic and antioxidant activities
7.	9,12-octadecadienoic acid (Z, Z)-	Anti-inflammatory Hypocholesterolemic Cancer preventive Hepatoprotective Nematicide Insectifuge, Antihistaminic Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic Antiarthritic Anticoronary.
8.	9,12-octadecadienoic acid (Z, Z)-	Anti-inflammatory Hypocholesterolemic Cancer preventive Hepatoprotective Nematicide Insectifuge, Antihistaminic Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic Antiarthritic Anticoronary.
9.	Octadecanoic acid	Cancer preventive Insectifuge/Cosmetic, Flavor, Hypocholesterolemic, Lubricant, Perfumery, Propecic, Suppository
10	Tris (tert-butyldimethylsilyloxy) arsane	No Activity reported.
11.	Temazepam	Therapy of insomnia, anti-anxiety agent, anticonvulsant activity, motor coordination, and memory loss.
12.	5-Methyl-2phenylindolizine	No Activity reported.

Table 4: Biological Activity of phytoconstituents identified in the ethanol extracts of *A.philoxeroides* polluted site

No	Name of the compound	Biological Activities
1.	Ar-tumerone	Antiviral.
2.	Phenol, 5-(1,5-dimethyl-4-hexeny	Antiseptic, Topical Anesthetic.
3.	Ethyl p-methoxycinnamate	Antifungal activity.
4.	Bicyclo[3.1.1]heptane, 2,6,6-tri...	No Activity reported.
5.	n-Hexadecanoic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide,Lubricant, Antiandrogenic, Flavor, Hemolytic, 5-Alpha reductase inhibitor.
6	Phenol, 4-(3,7-dimethyl-3-etheny...	No Activity reported
7.	9,12-Octadecadienoic acid (Z,Z)-	Anti-inflammatory Hypocholesterolemic Cancer preventive Hepatoprotective Nematicide Insectifuge, Antihistaminic Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic Antiarthritic Anticoronary Insectifuge.
8.	9,17-Octadecadienal, (Z)-	Antimicrobial property.
9.	Octadecanoic acid	No Activity reported.
10.	Phthalic acid, di(2-propylpentyl...	Antimicrobial [16].
11	Squalene	Antibacterial,Antioxidant,Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor,Pesticide Diuretic.
12.	Tris(tert-butyldimethylsilyloxy)...	No Activity reported.
13.	Ethanone, 2-(2-benzothiazolylthi...	No Activity reported.
14.	Urs-12-en-3-ol, acetate, (3.beta.)-	No Activity reported.
15	Cyclotrisiloxane, hexamethyl-	Antimicrobial potential, antimicrobial, antioxidant.
16.	Cyclotrisiloxane, hexamethyl-	Antimicrobial potential, antimicrobial, antioxidant:
17.	Cyclotrisiloxane, hexamethyl-	Antimicrobial potential, antimicrobial, antioxidant.

Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database]

The results revealed that Ar-tumerone (% 0.91), Phenol, 5-(1,5-dimethyl-4-hexeny (% 1.78), Ethyl p-methoxycinnamate (%2.28), Bicyclo[3.1.1]heptane, 2,6,6-tri...(%0.51), n-Hexadecanoic acid (14.79), Phenol, 4-(3,7-dimethyl-3-etheny...(%13.50), 9,12-Octadecadienoic acid (Z,Z)-(%15.43), 9,17-Octadecadienal, (Z)- (%14.19), Octadecanoic acid (% 3.51), Phthalic acid, di(2-propylpentyl (% 19.49), Squalene (%0.56), tert-butyldimethylsilyloxy).(%1.41), Ethanone, 2-(2-benzothiazolylthi (%3.00), Urs-12-en-3-ol, acetate, (3.beta-) (%3.62), Cyclotrisiloxane, hexamethyl-(%1.33), Cyclotrisiloxane, hexamethyl-(%1.33), and Cyclotrisiloxane, hexamethyl (%2.36) in (table 2). In the present study, the GC-MS analysis of the ethanolic extracts of *A. philoxeroides* aerial parts in unpolluted and polluted site showed the presence of many bioactive compounds. Huang reported that ethyl propionate was identified as the most abundant component of the root aqueous extracts of *A. philoxeroides* [17]. Seven phytoconstituents are commonly identified in both of the samples; they are n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z), Ar-tumerone, Tris-(tert-butyl dimethyl), Octadecanoic acid, Phenol, 5-(1,5-dimethyl-4-hexeny, and Bicyclo [3.1.1] heptane. The 12 and 20 phytoconstituents were detected in ethanol extract of aerial parts of *Alternanthera philoxeroides* and *Alternanthera bettzickiana* [18]. In polluted site, *A. philoxeroides* contains more number of phytoconstituents when compared to unpolluted site. Constituent squalene is also having anti-cancer, anti-oxidant, anti-tumor, chemo-preventive, pesticidal and sun-screen properties [19]. Phenolic compounds have also been known as antioxidant agents, which act as free radical

terminators and have shown medicinal activity as well as exhibiting physiological functions. Octadecanoic acid is used against Hypocholesterolemic, antiarthritic, nematicide, 5-alpha reductase inhibitor, antiacne, and hepatoprotective activity [20]. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios [21]. The n-Hexadecanoic acid has antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha reductase inhibitor activities [22-24].

Conclusion

Twelve phytoconstituents have been identified from ethanol extracts of *A. philoxeroides* from unpolluted site and seventeen phytoconstituents have been identified from ethanol extracts of *A. philoxeroides* from polluted site by GC-MS analysis. In the present study, in polluted site *A. philoxeroides* ethanol extracts showed higher phytoconstituents when compared to ethanol extracts of *A. philoxeroides* from unpolluted site. The stress of environmental pollution induces the plants to produce more of secondary metabolites as a defense mechanism. The present study reveals the presence of components in *Alternanthera philoxeroides* suggests that the contribution of these compounds on the pharmacological activity should be evaluated.

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