



GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS OF *AMARANTUS POLYGONOIDES* LINN. (AMARANTHACEAE)

M. Sharmila*, M. Rajeswari**, Indhiramuthu Jayashree***
& D. H. Geetha****

PG and Research Department of Botany, Vellalar College for Women, Erode,
Tamilnadu

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Abstract:

In the present study, the bioactive components of *Amarantus polygonoides* entire plant have been assessed using GC-MS analysis. Twenty nine compounds in ethanolic extract of the whole plant were identified. 9, 12, 15-Octadecatrienoic acid (Z, Z, Z) - (16.69%), Hexadecanoic acid (7.95%), Neophytadiene (5.62%) were the most prevailing compounds in ethanolic extract.

Key Words: GC-MS, *Amarantus*, Hexadecanoic Acid (7.95%) & Neophytadiene.

Introduction:

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organisation (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli *et al.*, 2009). Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutants, these are nonphytotoxic, more systematic and easily biodegradable (Vyas 1999; Kaushik *et al.*, 2002; Chamanlal, Verma 2006). Plants have great potential uses, especially as traditional medicine and pharmacopoeia drugs. Medicinal plants have been called part of nature's pharmacy. It is a sad fact that nowadays we are moving away from nature and due to our undisciplined life style new diseases are being identified. But the fact is that our rich nature contains remedy for all diseases. 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* (Brown, 1810). *Amarantus polygonoides* is an annual herb and also used to treat several disorders such as the leaves are used as a laxative and applied as an emollient poultice to abscesses, boils and burns. The juice of the root is used to treat fevers, urinary troubles, diarrhoea and dysentery. The seed is used as a poultice for broken ribs (Selvakumar *et al.*, 2014).

Materials and Methods:

Collection and Preparation of Plant Extract:

The whole plant parts of *Amarantus polygonoides* Linn. was collected from Erode district, Tamil Nadu, India and were authenticated and deposited at the PG and Research Department of Botany, Vellalar College for Women, Erode (Tamil Nadu), India. Fresh plants were collected and air-dried at room temperature and then homogenized to obtain coarse powder. The powder test plant was extracted (Mukherjee, 2002) with the solvent ethanol by hot extraction using soxhlet apparatus, collected and stored in a vial for further analysis.

Gas Chromatography-Mass Spectrometry Analysis:

Ethanolic extract of the whole plant of *Amarantus polygonoides* was analyzed for the presence of different volatile compounds by GC-MS technique. GC-MS analysis of the extract was performed at The South India Textile Research Association (SITRA), Coimbatore (Tamil Nadu), India. Using a GC-MS (Model; Thermo Trace GC Ultra Ver.5.0) equipped with a DB-35MS fused silica capillary column (30m length X outside diameter 0.25 mm X internal diameter 0.25 μ m) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with XCALIBUR software. For GC-MS detection, an electron ionization system with ionization energy of -70eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was 1 μ l; Injector temperature 250°C; Ion source temperature 200°C. The oven temperature was programmed from 70° to 200°C at the rate of 10°C/min, held isothermal for 1minutes and finally raised to 250°C at 10°C/min. Interface temperature was kept at 250°C. Total GC run time was 40.52 min. The relative percentage of each extract constituent was expressed as percentage with peak area normalization.

Identification of Components:

The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST (Mc Lafferly, 1989), WILEY (Stein, 1990) library sources were also used for matching the identified components from the plant material.

Results and Discussion:

Plant-derived substances have gained great interest due to their versatile applications. Use of herbal medicine for the treatment of diseases and infections is as old as mankind. The test plant *Amarantus*

polygonoides of the family Amaranthaceae was selected on the basis of ethanobotanical information collected from folk medicine and was subjected to GC-MS analysis. The presence of twenty nine bioactive compounds constituent that could contribute to the medicinal value of each plant. GC and MS total running time was 40.52. The GC-MS chromatogram of the test plant is showed in Figure 1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area are presented in Table 1. The structure and nature of the bioactive phytoconstituents are shown in Table 2.

Figure 1: GC-MS Chromatogram of the ethanolic extract of whole plant of *Amarantus polygonoides* Linn

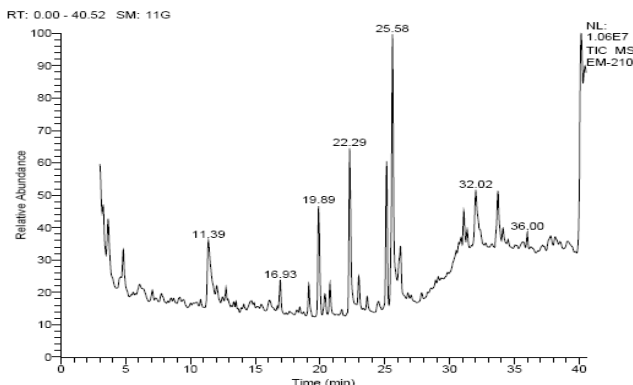
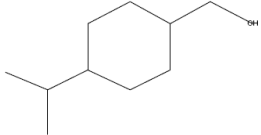
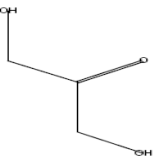
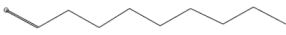
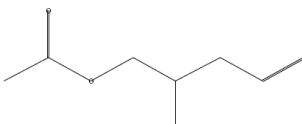
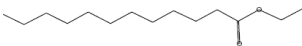
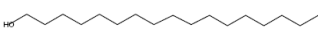
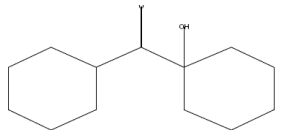
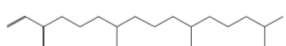
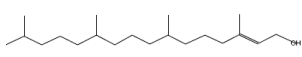


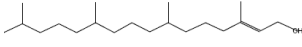
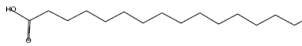
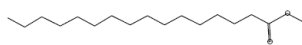
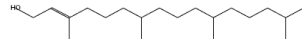
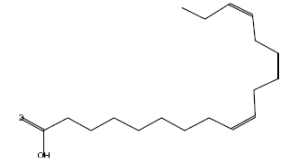
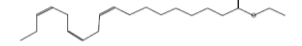
Table 1: Bioactive compounds identified in the ethanolic extract of an entire plant part of *Amarantus polygonoides* Linn. by GC-MS

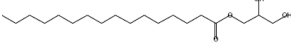
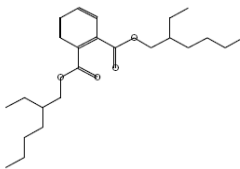
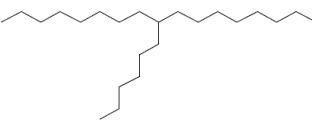
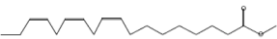
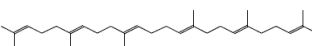
S.No	R.T	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %
1	3.26	p-Cymen-7-ol	C ₁₀ H ₁₄ O	150	1.02
2	3.64	Dihydroxyacetone	C ₃ H ₆ O ₃	190	2.38
3	4.83	4-Chlorophenyl tert-pentyl sulphate	C ₁₁ H ₁₅ ClO ₄ S	278	4.29
4	6.06	4-(4-Nitro-1H-Pyrazol-1-yl) Butanoic Acid	C ₇ H ₉ N ₃ O ₄	199	1.21
5	6.42	Nonanal	C ₉ H ₁₈ O	142	0.93
6	11.39	4-(Acetoxy)-3-Methyl-1-butanone	C ₇ H ₁₂ O ₃	144	4.37
7	12.03	2,2,6-Trimethyl-1-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptan-3-ol	C ₁₄ H ₂₂ O ₂	222	0.72
8	14.74	Dodecanic acid, ethyl ester	C ₁₄ H ₂₈ O ₂	228	0.93
9	16.14	1-Heptadecanol	C ₁₇ H ₃₆ O ₂	256	0.94
10	16.93	Methanone, (1-Hydroxycyclohexyl)Phenyl-	C ₁₃ H ₁₆ O ₂	204	1.58
11	19.13	4,7-Methano-1H-indene, 3a,4,5,6,7,7a-hexahydro-5-(2-propenyloxy)-	C ₁₃ H ₁₈ O	190	1.63
12	19.89	Neophytadiene	C ₂₀ H ₃₈	278	5.62
13	20.29	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.37
14	20.77	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.58
15	22.29	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	7.95
16	23.00	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	1.47
17	23.63	4-(Dimethylamino)-2-(methylthio)-3-thioxocyclobutenylium-1-thiolate	C ₇ H ₉ NS ₃	203	0.72
18	24.51	Methyl 2-Methyl-1-deuterio-2-propenyl Ether	C ₅ H ₉ DO	86	0.86
19	25.13	2-Hexadecen-1-ol- 3,7,11,15-tetramethyl-, [R*,R*-(E)]-	C ₂₀ H ₄₀ O	296	7.06
20	25.58	9,12,15-Octadecatrienoic acid(Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	278	16.69
21	26.20	Ethyl 9,12,15-Octadecatrienoate	C ₂₀ H ₃₄ O ₂	306	6.59
22	31.09	Glycerol 1-palmitate	C ₁₉ H ₃₈ O ₄	330	5.84
23	31.35	1,2-Benzene dicarboxylic acid, bis(2-ethylhexyl)ester	C ₂₄ H ₃₈ O ₄	390	0.92
24	32.02	Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324	3.64
25	33.72	9,12,15-Octadecatrienoic, Methyl ester (Z,Z,Z)-	C ₁₉ H ₃₂ O ₂	292	4.04
26	36.00	2,6,10,14,18,2-Teracosa hexaene, 2,6,10,15,19,23-Hexamethyl-	C ₃₀ H ₅₀	410	0.73
27	37.81	N-Benzyl-N-(2-bromobenzyl)-N-(2-bromo-3-(2-bromophenoxy)propyl)amine	C ₂₃ H ₂₂ Br ₃ N O	565	1.24

28	39.09	Diethyl(N-4-nitrophenylcarbamiyl) phosphonate	$C_{11}H_{15}N_2O_6$ P	302	0.90
29	40.13	Cholest-7-en-3-one, 4,4-dimethyl-, (5a)-	$C_{29}H_{48}O$	412	11.76

Table 2: The structure and nature of the bioactive phytoconstituents of ethanolic extract of entire plant parts of *Amarantus polygonoides* Linn

S.No	RT	Name of the Compound	Structure	Nature/Synonym	Bioactive/ Uses
1	3.26	p-Cymen-7-ol		Ether, Ethyl a-methyl benzyl, Benzene,(1-ethoxy ethyl)	Anti-cancer, Cytotoxicity
2	3.64	Dihydroxy acetone		Glycerone, simple carbohydrate (a triose)	Skin colouring agent
3	6.42	Nonanal		Aldehyde	Anti-diarrhoeal
4	11.39	4-(Acetoxy)-3-Methyl-1-butanone		Levulinic acid, ethyl ester, ethyl levulate, ethyl levulinate	Cardiotonic activity, mild anti-allergic activity
5	14.74	Dodecanic acid, ethyl ester		Ethyl laurate, Ethyl dodecylate, Lauric acid, Ethyl ester	Anti-cancer, Genotoxicity
6	16.14	1-Heptadecanol		Heptadecyl alcohol, Heptadecan-1-ol, 1-heptadecyl alcohol	Anti-cancer, Anti-microbial activity
7	16.93	Methanone, (1-Hydroxy cyclohexyl) Phenyl-		1-Hydroxy cyclohexyl phenyl ketone, Irgacure184, 1-Hydroxy cyclohexyl-1-Phenyl methanone	Anti-cancer
8	19.89	Neophytadiene		Fungistatic	Anti-microbial
9	20.29	3,7,11,15-Tetramethyl-2-hexadecen-		Terpene alcohol	Cancer preventive, Anti-inflammatory, Fragrance compound, Anti-microbial

		1-ol			
10	20.77	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Terpene alcohol	Cancer preventive, Anti-inflammatory, Fragrance compound, Anti-microbial
11	22.29	Hexadecanoic acid		Palmitic acid, Fatty acid, White crystal	Antioxidant, Pesticide, Flavor, 5-Alpha-Reductase-inhibitor, Anti-fibrinolytic, Hemolytic, Lubricant, Nematicide, Antiallopathic, Hypocholesterolemic, Anti-inflammatory, Anti-bacterial
12	23.00	Hexadecanoic acid, ethyl ester		Palmitic acid, ester compound	Antioxidant, Hypocholesterolemic, Nematicide, Antiandrogenic, Pesticide, Anti androgenic, Flavor, Hemolytic, 5-Alpha-Reductase- inhibitor, Lubricant
13	25.13	2-Hexadecen-1-ol-3,7,11,15-tetramethyl-[R-[R*,R*-(E)]]-		Transphytol, Phytol, 3,7,11,15-Tetramethyl-2-hexadecene-1-ol, Diterpene	Anti-cancer, Antioxidant, Anti-inflammatory, Diuretic, Cytotoxicity, Anti-microbial, Cancer preventive
14	25.58	9,12,15-Octadecatrienoic acid(Z,Z,Z)-		Linolenic acid	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Nematicide, Antihistaminic, Antieczemic, Antiacne, 5-Alpha-Reductase- inhibitor, Antiandrogenic, Anticoronary
15	26.20	Ethyl 9,12,15-Octadecatrienoate		Linolenic acid, Ethyl ester, Ethyl octadec-9,12,15-trienoate	Cell viability

16	31.09	Glycerol 1-palmitate		Hexadecanoic acid, Palmitin, Glycerol 1-monopalmitate, 1-Monopalmitin glycerol Monopalmitate	Cytotoxicity, Anti-viral
17	31.35	1,2-Benzene dicarboxylic acid, bis(2-ethylhexyl) ester		Plasticizer compound	Anti-fouling, Anti-microbial
18	32.00	Heptadecane, 9-hexyl-		9-n-Hexylheptadecane, Heptadecane	Carminative
19	33.72	9,12,15-Octadecatrienoic, Methyl ester (Z,Z,Z)-		Linolenic acid, Fatty acid ester	Anti-inflammatory, Hypochloesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Nematicide, Antihistaminic, Antieczemic, Antiacne, 5-Alpha-Reductase- inhibitor, Antiandrogenic, Anticoronary, Anti-arthritis
20	36.00	2,6,10,14,18, 2-Teracosahex aene, 2,6,10,15,19, 23-Hexamethyl-		Squalene, Organic compound, Triterpene	Anti-bacterial, Antioxidant, Anti-Tumor, Anti-inflammatory, Hypochloesterolemic, Immunostimulant

Twenty nine compounds were noticed in ethanolic extract of the whole plant part of *Amarantus polygonoides* Table 1 and Figure.1. The most prevailing major compounds were 9,12,15-Octadecatrienoic acid (Z,Z,Z)- (16.69%), Cholest-7-en-3-one,4,4-dimethyl-, (5a)-(11.76%), Hexadecanoic acid (7.95%), 2-Hexadecen-1-ol-3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(7.06%), Ethyl 9,12,15-Octadecatrienoate (6.59%), Glycerol 1-palmitate (5.84%), Neophytadiene (5.62%), 4-(Acetoxy)-3-Methyl-1-butanone (4.37%), 4-Chlorophenyl tert- pentyl sulphate (4.29%), 9,12,15-Octadecatrienoic, methyl ester (Z,Z,Z)- (4.04%), Heptadecane, 9-hexyl- (3.64%), Dihydroxyacetone (2.38%), 4,7-Methano-1H-indene,3a,4,5,6,7,7a-hexahydro-5-(2-propenyloxy)-(1.63%), Methanone, (1-Hydroxycyclohexyl) Phenyl-(1.58%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.58%), Hexadecanoic acid, ethyl ester (1.47%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol(1.37%), N-Benzyl-N-(2-bromobenzyl)-N-(2-bromo-3-(2-bromo phenoxy)propyl)amine (1.24%), 4-(4-Nitro-1H-Pyrazol-1-yl) butanoic Acid (1.21%) and p-Cymen-7-ol (1.02%) were also obtained. Some biological activities are shown in Table 2.

Among the identified compound, 9,12,15-Octadecatrienoic acid (Z,Z,Z)- and 9,12,15-Octadecatrienoic, methyl ester (Z,Z,Z)- which is a linoleic acid compound and reported to have an anti-inflammatory, anti-arthritis, hypocholesterolemic, hepatoprotective, anti-cancer, anti-histaminic, anti-acne, nematicide, insectifuge and anti-eczemic properties. Similarly, the presence of 9-octadecenoic acid was observed in the ethanolic root of

Plumbago zeylanica by Ajayi *et al.*, (2011). Hexadecanoic acid methyl ester is also known as palmitic acid ester. The fatty acid being effective in the treatment of antioxidant, pesticide anti-androgenic, nematocide, flavouring agent, hypocholesterolemic and lubricant. Similarly, Vinay Kumar *et al.*, (2011) and Gopalakrishnan and Vadivel (2011) reported the presence of hexadecanoic acid methyl ester in the methanol extract of *Spirulina platensis* and the ethanolic extract of *Mussaenda frondosa*, respectively. In contrast to the above study, the present investigation reported Hexadecanoic acid ethyl ester in the methanolic extract of entire plant of *Kyllinga triceps* (Siddabathuni *et al.*, 2014).

Hexadecanoic acid has the property of antioxidant and antimicrobial activities (Bodoprost and Rosemeyer 2007) and larvicidal effect (Falodun *et al.*, 2009). In this present study related to Amzad Hossain *et al.*, (2013) reported the presence of Hexadecanoic acid in the methanolic extract of leaf of *Thymus vulgaris*. The compounds like Neophytadiene and Heptadecene are reported in the present study. Similarly, presence of these compounds was noted in fresh leaves of ethyl acetate fraction of *Annona squamosa* (Vijayalakshmi *et al.*, 2011) and chloroform extract of aerial parts of *Fagonia lonjispina* (Hamidi *et al.*, 2012). Neophytadiene, a terpenoid compound has antipyretic, anti-inflammatory, anti-microbial and antioxidant activity (Venkata Raman *et al.*, 2012). 3,7,11,15-Tetramethyl-2-hexadecen-1-ol is an alcoholic compound and possessed cancer preventive, anti-inflammatory, fragrance compound and anti-microbial activity.

Conclusion:

The present study helps to predict the formula and structure of biomolecules which can be used as drugs. It enhances the traditional usage of *Amarantus polygonoides* which possesses some known and unknown bioactive compounds. However, isolation of individual phytochemical constituents and subjecting it to pharmacological activity will definitely give fruitful results.

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