GC-MS/MS ANALYSIS OF SOME *AMARANTHUS* SPECIES

SNEHAL WADKAR
Department of Botany,
Shivaji University
Kolhapur 416 004.

ADITYA MAGDUM
Department of Botany,
Shivaji University
Kolhapur 416 004.

D. K. GAIKWAD
Department of Botany,
Shivaji University
Kolhapur 416 004.

CHIRAG U. NARAYANKAR
Department of Botany,
Shivaji University
Kolhapur 416 004.

MAHESH MANE
Department of Botany,
Shivaji University
Kolhapur 416 004.

NIVAS DESAI
Shri Pancham Khemraj
Mahavidyalaya,
Sawantwadi.
nivasdesai88@gmail.com

Abstract

The present study was carried out identify its phytochemical by GC-MS/MS analysis. The present study deals with the Gas Chromatography Mass Spectroscopy analysis of Amaranthus cruentus, Amaranthus hypochondricus, Amaranthus cruentus, which have various medicinal properties. The GC-MS/MS study also carried out and it showed the presence of phytochemicals. Hence these plants extracts with the property of bioavailability and retention of certain compounds can be recommended for their use as a natural medicine for the treatment of infectious diseases.

Introduction

Amaranthus is a huge taxonomic gathering with a diverse variety of species that share characteristics like resistance to biotic and abiotic stresses, good yield, nutritional supplement, and economic qualities (Brenner *et al*., 200 and Rana *et al*., 2007). *Amaranthus* is a very narrow annual plant that had rapid growth, is drought resistant, and is adaptive to different surroundings. Its various species have unisexual flowers and seeds that are compact, black in colour with shiny lustre. Multiple raised in captivity aspects are grown up in Tamil Nadu, Andhra Pradesh, Karnataka, and Kerala in India (Khurana, 2014).

Amaranth is made up of 60-70 different species, 40 of which are native to the Continent of South America. Over 400 varieties of such species are found across the World in both temperate and tropical climates, and are classified as grain, vegetable, decoration, or weed types (Rana *et al*., 2007). Amaranth is also commonly known as Rajgira (king seed) and Ramdana (God's seed). It is typically a pseudo-cereal with amazing protein and nutritional content as compared to the actual cereal crops. Thus, recent decades have witnessed an increased use of amaranth flour blended with wheat or maize in households. Amaranth species are therefore gaining a lot of attention in developing countries to overcome protein malnutrition (Escudero, 2006). Various parts of plant are used for respiratory infections, vision defects, tuberculosis, fleshy tumors, liver problems and inflammations. In Ayurveda, leaf decoction used for chest afflictions and gastroenteritis. Seeds are also beneficial for sores. Seeds and leaves use as astringent for stopping diarrhea,

bloody excrement, hematuria and excessive menstruation (Sumner *et al*., 2003, Weckwerth, 2003 and Kopka *et al*., 2014).

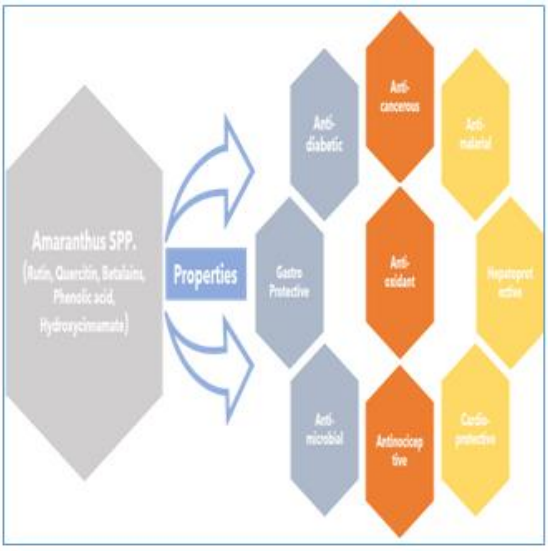

Figure 1. Pharmcological properties of *Amaranthus* species

GC-MS is the most popular technique for the identification and quantitation of secondary metabolites. The intention of the present study was to investigate the bio-chemical components by using GC-MS/MS analysis. This analysis provides a demonstrative spectral output for the entire compounds which are separated from the sample.

Material and Method Collection of the plant

In the current investigation different plant part of *Amaranthus cruentus* (Stem, leaves, root), *Amaranthus viridus* (Stem), *Amaranthus hypochondricus* (Leaves), *Amaranthus cruentus* (Leaves), *Amaranthus cruentus* (root). The plant was identified, Department of Botany, Shivaji University, Kolhapur.

Preparation of extract

The plant was shadow dried and standard procedure using electrical blender was used for conversion of plant into powder. Ethanolic extract of selected plant powder was prepared by maceration process in which powder was soaked in analytical grade ethanolic and kept to macerate for 24 hours. After 24 hours macerated powder extract was filtered with the help of Whatman. filter No.1. Prepared extract was further used for GC-MS/MS analysis of different compounds from *Amaranthus* plant species.

GC-MS/MS analysis

The GC-MS/MS analysis of the Ethanolic extract was run on Shimadzu make QP- 2010 with non-polar 60 M RTX 5MS column. For these analysis helium gas was used as carrier gas and 400C temperature set at oven for initial 3min. and then final temperature 48^0C with rate at 10^0C (min.sup.-1). A2 (mu) L sample was injected with splitless mode. The mass spectra with electron impact ionization 70 eV energy were estimated across 35 to 650 amu scale.

Identification of phytochemical

The specimen running time was 45 min. and the extract's bioactive molecules have been established by analyzing chromatographic maximum persistence duration use the NIST Library. The amount inferences are rendered from those in the GC-MS/MS to the TIC areas by the corresponding maximum regions.

Result and discussion

The analysis of mass spectra of fruit of *Amaranthus* species it's extracted in alcohol is shown in Table 1, 2, 3, 4, 5 and 6. The unknown sample and library standard chromatogram are shown in figure 2, 3, 4, 5 and 6. The GC-MS/MS spectra of fruit, leaves, bark extract

shown various peaks the identification was made by percent pick area, retention time after comparing it with the library of National Institute of standard and technology (NIST).

Amaranthus cruentus

The various phytocomponents identified in the *Amaranthus cruentus* (Stem) shows total nine peak in stem ertract out of these n-Hexadecanoic acid have the human colon cancer cell line inhibition activity, Ethyl Oleate increase the medicinal drug solubility activity. The mass spectra Ethane, 1,1,1-triethoxy- (4.15 %), 2(2Butoxyethoxy)carbonyl)benzoic acid (2.66 %), Undec-10-ynoic acid, tetradecyl ester (2.93 %), trans-Geranylgeraniol (16.16 %), n- Hexadecanoic acid (19.67 %), Hexadecanoic acid, ethyl ester (15.48 %), trans,trans-9,12- Octadecadienoic acid, propyl e (16.98 %), Ethyl Oleate (15.66 %), Octadecanoic acid, 17-methyl-, methyl ester (6.31 %) (Table No. 1 and figure 2).

Table No. 1: Phytocomponents identified in the alcoholic extract of the stem of *Amaranth cruentus* by GC- MS/MS.

Peak	R. Time	Area %	Name	Biological properties	Reference
1	6.678	4.15	Ethane, 1,1,1-triethoxy-	-	-
2	16.44	2.66	2(2Butoxyethoxy) carbonyl)benzoic acid	-	-
3	19.07	2.93	Undec-10-ynoic ester	-	-
4	19.23	16.16	trans-Geranylgeraniol	-	-
5	22.74	19.67	n-Hexadecanoic acid	human	Ravi Zyakun *al*., (2012)
6	23.19	15.48	Hexadecanoic acid, ethyl ester	-	-
7	26.11	16.98	trans,trans-9,12-Octadecadienoic acid, propyl este		
8	26.20	15.66	Ethyl Oleate	Increasing drug solubility	Xing *et al*., 2016.
9	26.58	6.31	Octadecanoic methyl ester		

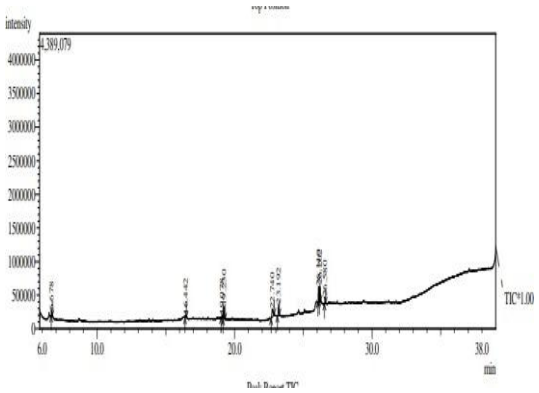

Figure No. 2: GC-MS/MS Chromatogam of *Amaranthus cruentus* stem.

The various phytocomponents identified in the *Amaranthus cruentus* (leaves) shows total eight peak in leaves extract out of these Phytol showing Anti-inflammatory, Anticarcinogenic and Antitumoral agent properties.

The mass spectra Naphthalene, decahydro-1,4a-dimethyl-7-(1-meth (1.68 %),Hexadecane (1.35 %), 2,6,10-Trimethyltridecane (2.16 %), Hexadecanoic acid, ethyl ester (19.82 %), Phytol (21.89 %)

Table No. 2: Phytocomponents identified in the alcoholic extract of the leaves of *Amaranth cruentus* by GC-MS.

Peak	R.Time	Area%	Name	Biological properties	Reference
1	13.11	1.68	Naphthalene, decahydro-1,4a-dimethyl-7-(1-meth	-	-
2	13.77	1.35	Hexadecane	-	-
3	14.60	2.16	2,6,10-Trimethyltridecane	-	-
4	23.17	19.82	Hexadecanoic acid, ethyl ester	-	-
5	25.56	21.89	Phytol	Anti-inflammatory, Anticarcinogenic and Antitumoral agent.	Olofsson *et al.*, 2014, Islam *et al.*, 2018.
6	26.10	21.07	trans,trans-	-	-

			9,12-Octadecadienoic acid, propyl ester		
7	26.19	23.44	Ethyl Oleate	-	-
8	26.57	8.59	Octadecanoic acid, ethyl ester	-	-

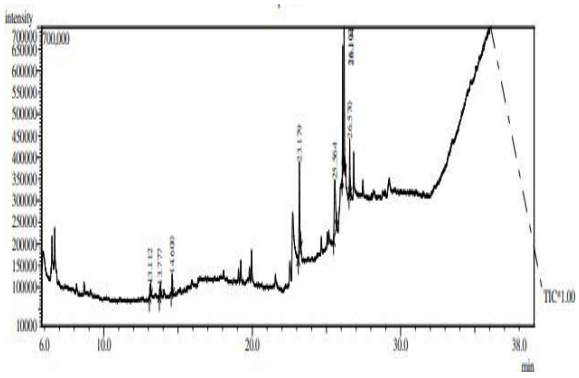

Figure No. 2: GC-MS/MS chromatogram of *Amaranthus cruentus* leaves.

The various phytocomponents identified in the *Amaranthus cruentus* (root) shows total six peak in root extract out of these Squalene showing Drug and vaccine delivery, Inhibit the development of various tumors, Rich Antioxidant properties and Linoleic acid ethyl ester showing antioxidant activity.

The mass spectra Butane, 1,1-diethoxy-3-methyl- (5.71 %), Ethane, 1,1,1-triethoxy- (6.54 %), Squalene (2.56 %), Ethyl 13-methyl-tetradecanoate (31.28 %), Linoleic acid ethyl ester (23.50 %), Ethyl Oleate (30.41 %).

Table No. 3: Phytocomponents identified in the alcoholic extract of the root of *Amaranthus cruentus* by GC-MS/MS.

Peak	R. Time	Area %	Name	Biological properties	Reference
1	5.71	5.71	Butane, diethoxy-3-methyl-		
2	6.672	6.54	Ethane, 1,1,1-triethoxy-		
3	19.230	2.56	Squalene	Drug properties	Fox, 2009. Red

					d y
4	23.189	31.28	Ethyl methyl-tetradecanoate	-	-
5	26.112	23.50	Linoleic acid ethyl ester	Antioxidant Activities	Kim 2020
6	26.200	30.41	Ethyl Oleate	-	-

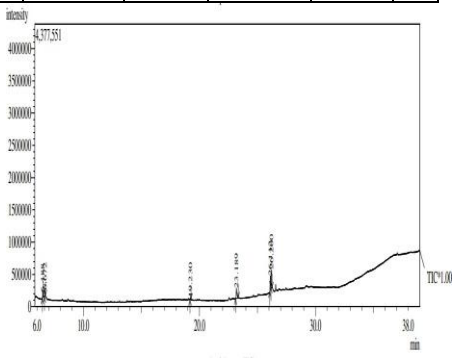

Figure No. 3: Chromatogram GC-MS/MS of *Amaranthus cruentus* root.

Amaranthus viridus

The various phytocomponents identified in the *Amaranthus viridus* shows total thirteen peak in stem extract out of these 2,4-Decadienal, (E,E)- showing Nematicidal Activity, 2,4-Dodecadienal inhibition of smooth muscle cells, n-Hexadecanoic acid having human colon cancer cell line.

Mass spectra 2,4-Decadienal, (E,E)- (25.94 %), 2-Undecenal (6.12 %), 2,4-Dodecadienal (9.95 %), Diethyl Phthalate (2.11 %), 1-Dodecanol, 3,7,11-trimethyl- (1.66 %), 3,5-di-tert-Butyl- 4-hydroxybenzaldehyde (3.53 %), Undec-10-ynoic acid, tridec-2-yn-1-yl ester (0.62 %), trans- Geranylgeraniol (11.29 %), n-Hexadecanoic acid (24.21 %), Hexadecanoic acid, ethyl ester (1. 41 %), 1-Decanol, 2-octyl- (1.38 %), 13-Hexyloxacyclotridec-10-en-2-one (2. 26 %), 9-Octadecenoic acid, (E)- (9. 52 %).

Table No. 4: Phytocomponents identified in the alcoholic extract of the stem of *Amaranthus viridus*

by GC-MS/MS.

Peak	R.Time	Area%	Name	Biological activity	Reference
1	12.64	25.94	2,4-Decadienal, (E,E)-	Nematicidal Activity	Caboni 2012
2	13.30	6.12	2-Undecenal		
3	14.19	9.95	2,4-Dodecadienal	Cytotoxic smooth	Cabre *et al*., 2003
4	16.41	2.11	Diethyl Phthalate		
5	18.05	1.66	1-Dodecanol, 3,7,11-trimethyl-		

6	18.77	3.53	3,5-di-tert-Butyl-4-hydroxybenzaldehyde		
7	19.06	0.62	Undec-10-ynoic acid, tridec-2-yn-1-yl ester		
8	19.21	11.29	trans-Geranylgeraniol		
9	22.75	24.21	n-Hexadecanoic acid	human	Ravi Krishnan, (2011)
10	23.16	1.41	Hexadecanoic ethyl ester		
11	23.27	1.38	1-Decanol, 2-octyl		
			-		
12	24.58	2.26	13-Hexyloxacycltridec-10-en-2-one		
13	25.97	9.52	9-Octadecenoic acid, (E)-		

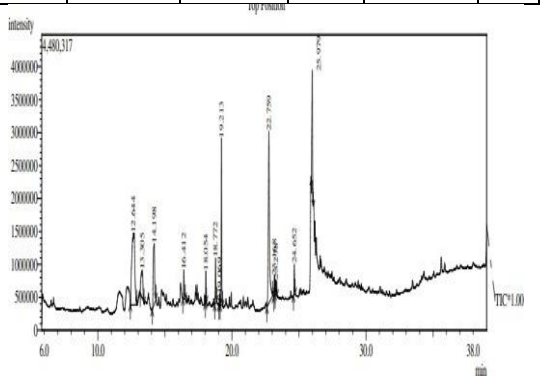

Figure No. 5: Chromatogram GC-MS/MS of *Amaranthus viridus* stem.

Amaranthus hypochondricus

The various phytocomponents identified in the *Amaranthus hypochondricus* shows total four peak in the leaves extract. Squalene having used in drug and vaccine delivery, Inhibit the development of various tumors, Rich Antioxidant properties.

Mass spectra Squalene (7.81 %),3,7,11,15-Tetramethyl-2-hexadecen-

1-ol (5.68 %), Cholesta-4,6-dien-3-ol, (3.beta.)- (28.58 %), Stigmast-5-en-3-ol, oleate (32.91 %).

Table No. 5: Phytocomponents identified in the alcoholic extract of the leaves of *Amaranthus hypochondricus* by GC-MS/MS.

Peak	R.Time	Area%	Name	Biological properties	Reference
1	19.22	7.81	Squalene	Drug and vaccine delivery, Inhibit the development of various tumors, Rich Antioxidant properties	Fox, 2009 Reddy and
2	19.82	5.68	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		
3	31.64	28.54	Cholesta-4,6-dien-3-ol, (3.beta.)-		
4	32.91	57.97	Stigmast-5-en-3-ol, oleate		

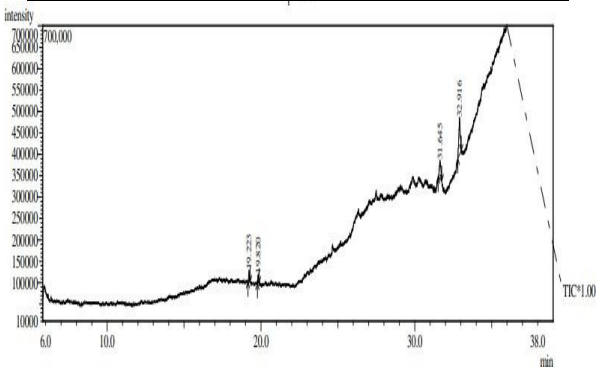

Figure No. 6: Chromatogram of GC-MS/MS analysis of *Amaranthus hypochondricus* leaves.

Conclusion

In GC-MS/MS results indicated various phytochemical constituents have been identified from ethanolic extract. Several compound showing antioxidant activity, antibacterial activity anticancerous activity, nematicidal Activity while some compound used in drugs delivery. The presence of these phytochemicals in *Amaranthus cruentus* (Stem, leaves, root), *Amaranthus viridus* (Stem), *Amaranthus*

hypochondricus (Leaves) is a significant finding in this present study.

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