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Research Article

PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS OBTAINED FROM "*AMARANTHUS HYBRIDUS*"

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ABSTRACT

Phytochemicals are non-nutritive, chemical compounds that occur naturally in plants and have diverse protective properties. There is currently an upsurge of interest in phytochemicals as a new source of natural pharmaceutical agents to be used in foods and pharmaceutical preparations to replace synthetic medicines, which are being restricted due to their potential health risks and toxicity. Thus, the objective of the present investigation was to actuate the biochemical components of *Amaranthus hybridus* using Perkin Elmer Gas Chromatography–Mass Spectrometry techniques. *Amaranthus hybridus* belonging to family Amaranthaceae is an annual Indian herb, is a tall herb with clusters of dark pink flowers. Methanolic extracts of *Amaranthus hybridus* was used for the qualitative phytochemical screening. Standard phytochemical screening tests were conducted for selected plant species and found that extract contains a variety of phytochemicals. Perkin Elmer Gas Chromatography–Mass Spectrometry technique was used to chemically identify important bioactive compounds isolated from methanolic extract of *Amaranthus hybridus.* Present investigation revealed that GC-MS analysis of methanolic extract of *Amaranthus hybridus* confess the existence 28 compounds at 36 different peaks. Many compounds were presented such as Squalene, n-Hexadecanoic acid, 9,12-octadecadienoic acid (Z, Z)-, methyl ester, Chondrillasterol, 6-Octadecenoic acid, methyl ester, (Z)-, 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-,Pentacosane, Tetracontane, Pentatriacontane and Benzenepropanoic acid. The present study provided evidence to approve the existence of various medicinally important bioactive compounds or phytochemicals that has got biological importance and it rationalizes their use in the traditional drugs for the treatment of different ailments.

Keywords: Family Amaranthaceae, *Amaranthus hybridus*, Rajgira, bioactive compounds, GCMS, squalene, herbal medicine.

INTRODUCTION

India is a land of rich biodiversity and is properly called the botanical garden of the earth[1]. *Amaranthus hybridus* is an annual herbaceous plant. The entire plant may be reddish at maturity. It is commonly called *Rajgira or Ramdana*. It belongs to the family Amaranthaceae and also known as Amaranth. They are strongly resisted to drought, warmth and pests and also expert to adept new environment[2]. *A. hybridus* grows on wrecked waste ground, in short grassland and in shady spaces in forests.

It has been described as extremely nourishing herbage and a valuable forage crop[3]. Cooked amaranth leaves have rich amount of vitamin A, vitamin C, Calcium, Manganese and folate. Leaves considered as admirable source of protein.

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.

Pharmacists are interested in the secondary metabolites because of their therapeutic performance and low toxicity[4]. For the analysis of certain class of metabolites there are many techniques like Gas chromatography and Mass spectroscopy (GC-MS), Liquid chromatography (LC), Nuclear magnetic resonance spectroscopy (NMR), UV and light visible spectroscopy[5-8].

GC-MS is the most popular technique for the identification and quantitation of secondary metabolites[9-11]. The intention of the present study was to investigate the bio-chemical components by using GC-MS analysis. This analysis provides a demonstrative spectral output for the entire compounds which are separated from the sample.

MATERIALS AND METHODS

Collection of the plant

In the current investigation whole plant of *Amaranthus hybridus* was collected from Ajmer, Rajasthan. The plant was identified and authenticated at the Herbarium, Department of Botany, University of Rajasthan, Jaipur.

Preparation of extract

The plant was shadow dried and standard procedure using electrical blender was used for conversion of plant into powder. Methanolic extract of selected plant powder was prepared by maceration process in which powder was soaked in analytical grade methanol 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 phytochemical screening and GC-MS analysis of bioactive compounds from *Amaranthus hybridus* plant.

Phytochemical screening

Phytochemical screening of methanolic extracts of *Amaranthus hybridus* was done according to the established protocols. Analysis of carbohydrate, protein, flavonoids, tannins, triterpenes, phenols, phytosterols, alkaloids and saponins was carried out according to the methods described by Peach and Tracey[12.]

GCMS analysis

GCMS is the best technique for the effective chemical analysis. About 1 µl of methanolic extract was injected into the GC-MS device using a micro syringe. As the phytochemical compounds were separated, they produce a definite spectral peak and recorded on a paper as a graph that is called chromatogram. Peaks are studied from the base to the tip in the Total ion chromatogram (TIC). The time between elution and injection is termed as "retention time" or "RT". Retention time is used to differentiation between the compounds. For the current investigation GCMS analysis was carried out at Research laboratory of The Jawaharlal Nehru University (JNU), Delhi.

Identification of compounds by GCMS

The Mass-Spectrum of unknown compounds was compared with the spectrum of the known compounds which was stored in the JNU library. The name of compound, molecular weight, molecular structure and molecular formula of the compound which were revealed in the test materials were identified. Peaks which are shown in GCMS of methanol extract of *Amaranthus hybridus* revealed the existence of the bioactive compounds.

RESULT

The phytochemical components have been investigated qualitatively in our laboratory through the common procedures by using various chemicals. Carbohydrates, Proteins, flavonoids, alkaloids, tannins, terpenoids and phenols were presented in the methanolic extract of this plant (Table 1).

GC-MS is the best procedures to identification for the compounds of long-branched chain hydrocarbons, volatile matters, acids, alcohols and esters etc. In the present study GC-MS analysis was done in methanolic extract of plant material. Total ion chromatogram (TIC) of the methanolic extract from *Amaranthus hybridus* showing the GC-MS analysis of the compounds which are identified in the given Figure 1. The peaks existed in the chromatogram were recognized and were compared with the data of spectrum of the identified compounds, reserved in the GC-MS library. For the approval of phytochemical, constituent's peak area, RT and molecular formula were used. Peak area is presented in percentage. The detailed tabularizations of GC-MS analysis of the extract are given in Table 2.

Many compounds like Squalene, n-Hexadecanoic acid, 9, 12-octadecadienoic acid (Z, Z)-, methyl ester, Chondrillasterol, 6-Octadecenoic acid, methyl ester, (Z)-,1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-, Pentacosane, Tetracontane, Pentatriacontane, Benzenepropanoic acid and 2,2-Dimethyl-3-[(3E,7E,11E,15E)-3,7,12,16,20-Pentame were revealed in GC-MS analysis of methanolic extract of *Amaranthus hybridus.* Some of the phytochemicals which are having more peak area % and importance are listed in Table 2.

Table 1: Phytochemical constituents in the methanol extract of *Amaranthus hybridus*

Phytochemical constituents	Methanolic extract
Carbohydrates	+
Proteins	+
Flavonoids	+
Tannins	+
Triterpenes	+
Phenols	+
Phytosterols	+
Alkaloids	+
Saponins	-

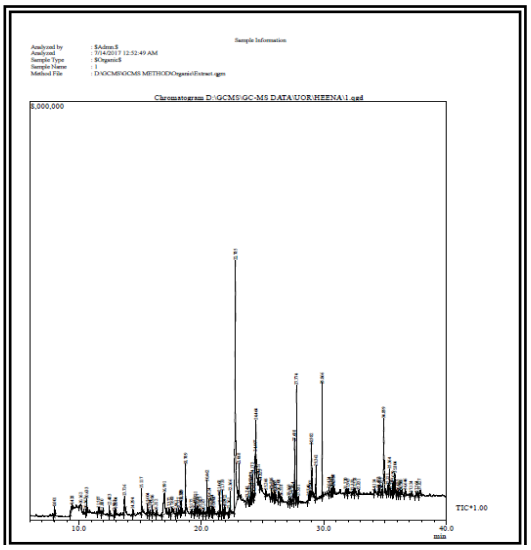


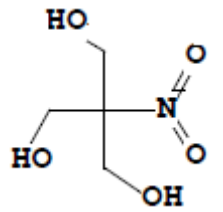
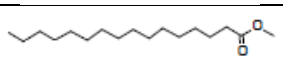
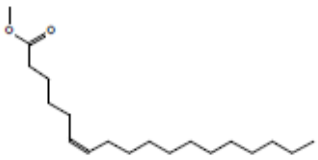
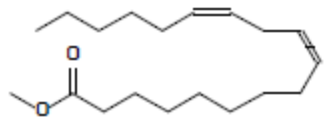
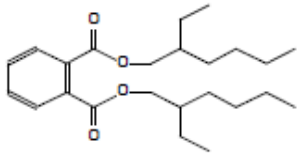
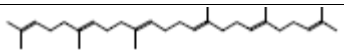
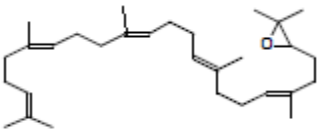
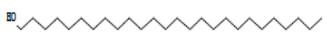
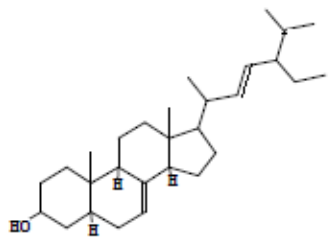
Figure 1: GC-MS chromatogram of methanolic extract of *Amaranthus hybridus*

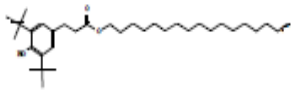
DISCUSSION

GC-MS analysis of the methanolic extract of the plant shows many bioactive compounds. Among entire compounds which are shown in plant sample, squalene is the most prevailing compound (34.29%). The squalene (triterpene) is a phenolic compound which shows antimicrobial activity[13], anticancer, chemo-preventive, pesticide, anti-tumor[14], anti-oxidant[15] and sunscreen properties. Other compounds like n-Hexadecanoic acid keep antibacterial and antifungal properties[16].9, 12-octadecadienoic acid (Z, Z)-, methyl ester displays anti-cancer[17] property while chondrillasterol is cytotoxic[18] in nature. Due to occurrence of numerous bioactive compounds, *Amaranthus hybridus*is beneficial for phytopharmaceutical works and was used for various ailments by traditional experts.

Current investigation provided indication to approve the existence of various medicinally important bioactive compounds or phytochemicals that has got biological importance and it justifies their use in the traditional medicines for the treatment of different diseases.

Table 2: Phytochemical identification of *Amaranthus hybridus* in methanolic extract by GC-MS peak report TIC

No.	RT	Peak area %	Compound's name	Molecular formula	Molecular weight	Structure of the chemical
1	12.205	5.70	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	151	
2	19.215	2.82	n-Hexadecanoic acid	$C_{17}H_{34}O_2$	270	
3	20.819	1.25	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	296	
4	21.332	1.18	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	
5	24.154	1.15	Pentacosane	$C_{25}H_{52}$	352	
6	25.473	2.70	Pentacosane	$C_{25}H_{52}$	352	
7	25.937	1.01	Bis (2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	
8	27.100	3.48	Tetracontane	$C_{40}H_{82}$	562	
9	29.015	6.19	Pentatriacontane	$C_{35}H_{72}$	492	
10	30.365	5.07	Tetracontane	$C_{40}H_{82}$	562	
11	30.505	34.29	Squalene	$C_{30}H_{50}$	410	
12	31.109	1.38	Hexacosane, 1-iodo-	$C_{26}H_{53}I$	492	
13	31.594	4.32	Hexatriacontane	$C_{36}H_{74}$	506	
14	31.990	1.37	2,2-Dimethyl-3-[(3E,7E,11E,15E)-3,7,12,16,20-PENTAME	$C_{30}H_{50}O$	426	
15	33.031	1.91	Hexatriacontane	$C_{36}H_{74}$	506	
16	34.870	1.61	1-Hexacosanol	$C_{26}H_{54}O$	382	
17	39.247	6.29	Chondrillasterol	$C_{29}H_{48}O$	412	

18	50.269	8.13	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy	$C_{35}H_{62}O_3$	530	

CONCLUSION

GC-MS analysis of the methanolic extract of *Amaranthus hybridus* relates the presence of pharmaceutically valued bioactive compounds like flavonoids, tannins, terpenoid and phytosterols. Extant study enhances the pharmacological profile of the plant in the field of traditional medicine.

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