Phytochemistry of Amaranthus viridis: GC-MS Analysis

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INTRODUCTION

The world is endowed with a rich diversity of medicinal plants. About 80% of the world's population uses herbs for medicinal purposes. Herbs have always been the principal form of medicine in the world. Some biologically active compounds isolated from herbs have been explored for the inhibition of the growth of pathogenic microbes because of their antimicrobial potential.

Amaranthus, commonly known as Green amaranth, is a multination annual genus of herbs. In the last decade, amaranth is not only used in the common diet but also in the diet of people with celiac disease or allergies to typical cereals.

Recently drug resistance to a human pathogenic organism has been reported worldwide. Medicinal plants are an expensive gift from nature as they are the sources of important than synthetic alternatives, as they are easily available and cheaper. Many plant species have been evaluated for their antimicrobial activity in the past 20 years.

They provide us with the key chemical structure for the development of new phytomedicine to be used for the treatment of disease. The active principles of many drugs found in plants are recognized as secondary metabolites. Amaranthus, commonly known as Green amaranth, is a multination genus of herbs. In the last decade, amaranth is not only used in the common diet but also in the diet of people with celiac disease or allergies to typical cereals.

ABSTRACT

Introduction: 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 with nutraceutical potentials. The herb was consumed as a leafy vegetable in various parts of India as a cheap and nutritive source of food materials in low economic society. Amaranthus Viridis (Amaranthaceae) widely distributed all over the world, growing under a wide range of climatic conditions and has been utilized as a medicinal herb in traditional Ayurveda medicine for the treatment of inflammation, ulcer, diabetes, asthma and hyperlipidemia.

Objective: The present research focused on evaluate the antioxidant and biological properties of Amaranthus Viridis.

Methods: The phytochemical compositions of the extract of leaves were studied using gas chromatography-mass spectroscopy (GC-MS), a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose was used.

Results: The GC-MS Analysis revealed 40 constituents of which many of the compounds were identified. The active principles with their retention time (RT), molecular weight (MW) and concentration (%) of the corresponding compounds were observed in the leaf extract. GC-MS chromatogram of the leaf extract of A. viridis belonging to the family Amaranthaceae showed 40 peaks which indicate the presence of forty compounds.

Conclusion: The current study suggests that the plant have a potent therapeutic activity and paves the way for the development of several treatment regimens based on compounds from this extract.

Key Words: Amaranthus Viridis, Ayurveda, Inflammation, Antioxidant, Treatment, Chromatography
therapeutic aids for alleviating human ailments. Gas Chromatography-Mass Spectrometry (GC-MS) is the best technique to identify the bioactive constituents of long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acid and nitro compounds. Therefore, characterization of extracts of medicinal plants is necessary due to its numerous benefits to science and society. Nevertheless, *Amaranthus* has received notably less research attention as vegetables than grain amaranths.

Hence, the present study was aimed at determining phytochemical constituents with the aid of the GC-MS technique and in vitro screening of pure extract of leaves from locally grown *Amaranthus Viridis* plants for their phytochemical study. The findings of this study provide important data on the bioactive substances of this underutilized vegetable, and thereby promoting their utilization in the food industry.

**MATERIALS AND METHODS**

**Collection of samples**
Fresh plant leaves of *Amaranthus Viridis* was collected and authenticated by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. A voucher specimen (Reg.No. PARC/2019/4054). The leaves were thoroughly washed through tap water and dried under shade for 3-5 days. The dried leaves were ground to a fine powder and stored in bags for further use.

**Preparation of extracts**
50 grams of dried powder of *Amaranthus Viridis* leaves were packed in a separate round bottom flask for sample extraction using 500 ml water. The extraction was conducted with 20 ml of water for 24 hours. At the end of the extraction, the crude extract was stored in refrigerator.

**Chemicals and reagents**
All chemicals and reagents were procured from certified suppliers and were of the highest analytical standard.

Gas Chromatography-Mass Spectroscopy

Gas chromatography-mass spectroscopy (GC-MS), a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose was used. The unknown organic compounds in the complex mixture can be determined by interpretation and also by matching the spectra with reference spectra.

**Preparation of extract**
The extract of the leaves was analyzed using Gas Chromatography Mass Spectroscopy for the identification of the phytochemical compounds present. A solvent blank analysis was first conducted using 1 μl of absolute ethanol. Then 1 μl of the reconstituted extract solution was employed for GC-MS analysis as previously described with modifications.

**Procedure**

**Identification of bioactive compounds by GCMS**
The purified extract fractions were individually examined using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a mass spectrometer equipped with Elite-I fused silica capillary column. For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium was used as carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2μl was employed.

The sample was run for 40 minutes with a solvent out time of 9.50 minutes. Mass spectra were taken with a scan-interval of 0.6 seconds. Interpretation of the mass spectrum was achieved by using the database for different bioactive compounds.

**GCMS analysis of bioactive compounds from the sample**
The leaf extract was subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds.

**Identification of phytoconstituents**
Interpretation of the mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. NIST library was used to calculate the mass spectrum. Quantitative determination was made by respective peak areas to TIC areas from the GC-MS. The principle name, molecular weight, retention time and peak area percentage of the test materials was ascertained. The column temperature was programmed from 75 - 260°C (rate = 6°C/min) with the lower and upper temperatures being held for 3 and 10 minutes respectively. Total GC running time was 43.2 min. The GC injector and MS transfer line temperatures were set at 280°C and 290°C respectively. All analysis was done in the split-less mode.

Helium (99.9%) was used as a carrier gas (flow rate = 1.0 ml/min) and an injection volume of one μl was used for analysis. Major and essential compounds were identified by their retention times and mass fragmentation patterns.

**RESULTS**

Phenolic compounds can be defined as a large series of chemical constituents possessing at least one aromatic ring, bearing hydroxyl and other sub-constituents. GC-MS analysis is the most used method for the identification of plant phenolic...
DISCUSSION

The plants contain large amounts of secondary metabolites that exert a wide range of biological activities on physiological systems. It was also reported that the activities of some plant constituents with compound nature of alkaloids, flavonoids, palmitic acid (hexadecanoic acid, ethyl ester and n-hexadecanoic acid, unsaturated fatty acid and linoleic (docosatetraenoic acid and octadecatrienoic acid) as antimicrobial, antioxidant, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistaminic, antieczemic, immunomodulatory and anticancer activity, as well as further research is necessary to identify and purify the active compounds responsible for therapeutic activity, as well as the unidentified compounds.

Secondary metabolites of the plants attract beneficially and repel harmful organisms, serve as phytoprotectants and respond to environmental changes. In general, the phytochemical contents (Table 1) were by the previous reports for some of the vegetables. These phytoconstituents are responsible for various pharmacological actions of the leaves of the plant.

Benzolic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester (24.09%) is found as the major compound and forty nine minor compounds such as Nonamethyl, Phenyl, Cyclopentasiloxane (6.10%), (+,−)-3-beta.-(acetylxy)-3-ethynyl-1,2,3,4,4a, beta,12a (5.67%), [Bis (trimethylsilyl) methyl] diphenyl phosphine $$ Phosphine (5.59%), Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester (5.32%), 1,2-Diphenyl tetramethyldisilane $$ Disilane,1,1,2,2-tetramethyl-1,2-di-phenyl (4.98%), Benzophenone, 2-(trimethyloxil)- (CAS) Trimethylsilyl ether of O-hydroxybenzophenone (3.50%), 7-(P-Chlorophenyl)limino-6-(P-tolyl)-1,3-dimethyl-2,4 di- oxo-1,2,3,4,6,7- hexahydro pyramid [4,5-d] pyramidine (3.14%), 4-α.20-dimethyl-3-.beta.-dimethyl-.. (2.84%), Silane, [1,3,5-benzenetetrtlris (oxy)] tri [trimethyl- (CAS) Phloroglucinol tris MS (2.76%), 2-Isopropyl phenol Tri- methylsilyl- Ether (2.73%), 4H-1-Benzopyran-4-one, 2-(2,6-dimethoxyphenyl)- 5,6-dimethoxy- (CAS) Zapotin $$ Flavone (2.46%), (Z)-1-[(1’,1’-dimethylethyl)diphenylsilyl]-3-trimethylsilyloxprop-1-ene (2.37%), Prosta-5,10,13-trien-1-oic acid, 15-[(1,1-dimethylethyl) dimethyl ethylsilyl] oxyl-9-oxo- (2.19%), Silane, trimethyl (triphenylethenyl)- $$ (2.18%) and the remaining compounds peak area ranged from 1.84% to 0.16%

The results from the current study indicate that methanol leaf extract of the *Amaranthus Viridis* tested by GC-MS analysis contained various types of compounds with potential pharmacological activity. The presence of various bioactive compounds justifies the use of *Amaranthus viridis* for various ailments by traditional practitioners. From GC-MS data, identification of more compounds in their extract and it previously reported that these compounds have antibacterial, antifungal, antioxidant and anticancer activity but further research should be made to isolate and purification of natural products in their extract.

CONCLUSION

The edible plant species *Amaranthus viridis* from the underutilized plant family had a rich amount of valuable ingredients that are beneficial for health. Further research work is required in more details about in vitro and in vivo investigations to establish which components of the extract are biologically active in terms of activity. The isolation of components from this readily available plant resource and its utilization as natural agents could be of high economic value. Hence, the identified plant components using GC-MS can be used as a tool for the identification of adulterants. The current pioneering study suggests that the extract is a potent therapeutic agent. It paves the way for the development of several treatment regimens based on this extract. Also, further research is necessary to identify and purify the active compounds responsible for therapeutic activity, as well as the unidentified compounds.

ACKNOWLEDGEMENT

We are thankful to the Department of Biochemistry, Kongunadu College, Coimbatore, Tamil Nadu, and India for providing facilities to carry out research work. The authors acknowledge the immense help received from the scholars whose articles are cited and included in references to this manuscript. The authors are also grateful to authors/ editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed

Conflict of Interest: Authors declare no conflict of interest.

Source of Funding: None
Table 1: Phytocomponents identified in the leaf extract of *Amaranthus viridis* by GC-MS analysis

| S No | RT  | Name of the compound                                                                 | Molecular formula | MW  | Peak Area |
|------|-----|--------------------------------------------------------------------------------------|-------------------|-----|-----------|
| 1    | 7.483 | Cyclononasiloxane, octadecamethyl- $$                                              | C_{16}H_{32}O_{14}Si_{8} | 666 | 0.33      |
| 2    | 7.425 | Dodecamethylcyclohexasiloxane $$ - A cyclic volatile methylsiloxane (cVMS) used in cosmetic and personal care products. | C_{16}H_{32}O_{14}Si_{8} | 444 | 0.25      |
| 3    | 7.608 | Monensin, methyl ester (CAS) Monensin methyl ester $$ 1,6-Dioxaspiro[4.5]decan-7-butryic acid, 2-[5-ethyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-(hydroxymethyl) | C_{18}H_{34}O_{14}Si_{8} | 684 | 0.32      |
| 4    | 7.748 | 3,3,5-Trisubloy-1,1,1,7,7,7-hexamethyl-5-(trimethylsiloxy)tetrasiloxane $$          | C_{18}H_{34}O_{14}Si_{8} | 558 | 0.23      |
| 5    | 8.597 | Tetradecamethylcycloheptasiloxane $$                                             | C_{16}H_{32}O_{14}Si_{8} | 518 | 0.21      |
| 6    | 9.617 | L-Threonine, N-[(2,4-dichlorophenox)acetyl]- $$ N.alpha.- (2,4-D)- L-Threonine $$ | C_{16}H_{32}O_{14}Si_{8} | 321 | 0.16      |
| 7    | 9.933 | Dibenzen[a,c]cycloheptan-9-amine, 2,3,4-trimethoxy-N-acetyl- $$                     | C_{18}H_{34}O_{14}Si_{8} | 341 | 0.18      |
| 8    | 10.075 | 1,3,3,5,7,7,9,9,11,13,15,15-Hexadecamethyl-octasiloxane $$                       | C_{16}H_{32}O_{14}Si_{8} | 578 | 0.22      |
| 9    | 11.854 | Benzoi acid, 2,6-bis[(trimethylsilyl) oxy]-, trimethylsilyl ester $$                | C_{16}H_{32}O_{14}Si_{8} | 370 | 0.16      |
| 10   | 12.375 | 2-Cyclohexen-1-one, 3-methyl-6-(1-methyl ethenyl)- $$ p-Metha-1,8-dien-3-one $$    | C_{16}H_{32}O_{14}Si_{8} | 150 | 0.16      |
| 11   | 12.970 | (4R,s,6R,8R,8aS,12aS)-5,8,8a,11,12a-hexahydro-1,4,8-trihydroxy-5-methoxy-9,10,12a-trimethyl-3H-phenanthro[3,2-b] pyran-2(4H)-one $$ | C_{18}H_{34}O_{14}Si_{8} | 374 | 0.18      |
| 12   | 13.084 | 4-.alpha.,20-dimethyl-3-.beta.-dimethyl-… $$                                      | C_{16}H_{32}O_{14}Si_{8} | 462 | 0.34      |
| 13   | 13.137 | Acetphenone 4-[1-adamantyl]-3-.thiosemicarbazone $$                              | C_{16}H_{32}O_{14}Si_{8} | 327 | 0.31      |
| 14   | 13.201 | 3,4-Methylenedioxyphenylactic acid, di-TMS $$                                      | C_{16}H_{32}O_{14}Si_{8} | 354 | 0.20      |
| S No | RT  | Name of the compound                                                                 | Molecular formula | MW  | Peak Area |
|------|-----|--------------------------------------------------------------------------------------|-------------------|-----|-----------|
| 15   | 13.264 | Benzoic acid, 3,5-dimethoxy-4-{[(trimethylsilyl)oxy]-, trimethylsilyl ester (CAS) | C_{6}H_{4}O_{2}S_{15} | 342 | 0.31      |
| 16   | 13.338 | di-3-(1-Phenyl-1-methylthyl)phenyl amine $$                                      | C_{7}H_{11}NO      | 205 | 0.46      |
| 17   | 13.467 | 4.-alpha., 20-dimethyl-3.-beta.-dimethyl-... $$                                      | C_{27}H_{30}N_{2}  | 346 | 1.16      |
| 18   | 13.516 | Silane, trimethyl[(triphenylethyl]- $$                                             | C_{27}H_{42}Si     | 328 | 0.58      |
| 19   | 13.567 | Heptamethyl-Pheny1-Cycloadasiloxane $$                                             | C_{27}H_{42}O_{6}  | 358 | 0.88      |
| 20   | 13.661 | Pregnan-20-one, 3-(acetoxy)-5, 6:16, 17-diepoxy-, (3.beta.-5. alpha., 6.alpha., 16.alpha.)- $$ | C_{27}H_{30}O_{16} | 388 | 1.18      |
| 21   | 13.724 | [5.- (3-Methoxymethoxy-10,13-dimethyl-2,3,4,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthen-17-yl)-hex-1-ynyl]-trime $$ | C_{45}H_{44}Si     | 468 | 1.11      |
| 22   | 13.975 | 7-(P-Chlorophenyl)imino-6-(P-tolyl)-1,3-dimethyl-2,4-dioxo-1,2,3,4,6,7-hexahydro pyrimido [4,5-d] pyrimidine $$ | C_{27}H_{22}ClNO_{7} | 407 | 3.14      |
| 23   | 14.167 | (+.-)-3.beta.- (acetoxy)-3-ethyl-1,2,3,4,4a.beta.-12a.beta.-hexahydro-6,11-dihydroxy-7-methoxy-1,2-alpha.- (trimethylsilyl)-5,12-naphthacenenedione $$ | C_{30}H_{25}O_{4}  | 480 | 5.67      |
| 24   | 14.274 | Silane, [1,3,5-benzenetriyltris(ox)]tris[trimethyl- (CAS) Phloroglucinol TriTMS $$ | C_{30}H_{30}O_{9}  | 342 | 2.76      |
| 25   | 14.349 | 1,2-Diphenyltetramethylsilane $$ Disilane,1,2,2,2-tetramethyl-1,2-diphenyl- $$      | C_{27}H_{30}Si     | 270 | 4.98      |
| 26   | 14.453 | [Bis(trimethylsilyl)methyl] diphenylphosphone $$ Phosphate, [bis(trimethylsilyl)methyl] diphenyl- (CAS) | C_{27}H_{30}PS_{2} | 344 | 5.59      |
| 27   | 14.588 | 4H-isobenzopyran-4-one, 2-(2,6-dimethoxy phenyl)-5,6-dimethoxy- (CAS) Zapotin $$ | C_{20}H_{16}O_{6}  | 342 | 2.46      |
| 28   | 14.653 | 3,5,8-Trioxanonane, 4-methyl-6-oxiranyl-9-phenyl- $$                                | C_{24}H_{22}O_{4}  | 266 | 1.70      |
| 29   | 14.708 | 2-Isopropylphenol-Trimethylsilyl-Ether $$                                          | C_{24}H_{24}OS      | 208 | 2.73      |
| 30   | 14.856 | Anisic acid, di-TMS $$                                                             | C_{16}H_{10}NO_{4}Si | 353 | 1.52      |
| 31   | 14.970 | Cyclohexa-1,4-Diene, 1,3,6-Tris(Trimethylsilyl)- $$                                  | C_{16}H_{18}Si_{3} | 296 | 1.23      |
| 32   | 15.007 | Propylparaben TMS Ether $$                                                          | C_{16}H_{18}O_{2}  | 252 | 0.73      |
| 33   | 15.233 | (Z)-1-[(1'-3-dimethyl)diethylsilyl]-3-trimethylsilyloxyprop-1-ene $$ (Z)-3-[1'-3'-dimethyl)diethylsilyl]-1-trimethylsilyloxyprop-1-ene $$ Silane, [(3-[1-(1-dimethylthethyl)diethylsilyl] | C_{27}H_{26}O_{2}S_{2} | 368 | 2.37      |
| 34   | 15.342 | Benzoephone, 2-(trimethylsilyloxy)- (CAS) Trimethylsilyl ether of 0-hydroxybenzophenone $$ | C_{26}H_{24}O_{2}S_{2} | 270 | 3.50      |
| 35   | 15.434 | Prosta-5,10,13-trien-1-0ic acid, 15-[(1,1-dimethylthethyl)dimethylsilyl][oxy]-9-oxo-, methyl ester, (5Z,15E,15S)- $$ | C_{27}H_{28}O_{2}Si | 462 | 2.19      |
| 36   | 15.508 | Benzoic acid, 2-[(trimethylsilyloxy)-, trimethylsilyl ester (CAS) 2-Hydroxybenzoic Acid-DiTMS $$ Bistrimethylsilyl Salicylic Acid $$ | C_{27}H_{28}O_{2}SiS_i | 282 | 5.32      |
| 37   | 15.636 | Benzoic acid, 2-[(trimethylsilyloxy)-, trimethylsilyl | C_{27}H_{28}O_{2}SiS_i | 282 | 24.09     |
| 38   | 15.880 | Nonamethyl, Phenyl-, Cyclopentasiloxane $$                                          | C_{27}H_{28}O_{2}SiS_i | 432 | 0.17      |
| 39   | 21.767 | 1-Trimethylsilyloxy-2-Trimethylsilylamino-3-(4'-Methoxyphenyl) Propanone $$         | C_{31}H_{28}NO_{2}SiS_i | 339 | 0.21      |
| 40   | 17.027 | Nonamethyl, Phenyl-, Cyclopentasiloxane $$                                          | C_{27}H_{28}O_{2}SiS_i | 432 | 6.10      |