INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

GC-MS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS AND SCREENING FOR ANTIBACTERIAL ACTIVITY OF THE METHANOL LEAF EXTRACT OF *AMARANTHUS VIRIDIS* LINN. AGAINST HUMAN PATHOGENIC BACTERIA

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ARTICLE INFO

Article history
Received 09/02/2017
Available online
14/03/2017

Keywords
Amaranthus Viridis,
Antibacterial Activity,
Gas Chromatography –
Mass Spectrometry Analysis,
Phytochemical Compounds.

ABSTRACT

The present study was aimed at determining phytochemical constituents with the aid of GC-MS technique and *in vitro* screening of pure methanol extracts of leaves from locally grown *Amaranthus viridis* plants for their antibacterial activity against human bacterial pathogens viz., *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The bioactive compounds present in the methanol extract fraction of *A. viridis* leaf were identified by GC-MS analysis which showed 50 peaks indicating the presence of fifty compounds. The results of the antibacterial activity proved the ability of the leaf extract of *A. viridis* to prevent the growth and survival of the test pathogens. Different bacterial species exhibited different sensitivities with variable extent towards the leaf extract. The order of activity against selected bacteria was *Escherichia coli* > *Staphylococcus aureus* > *Bacillus cereus* > *Pseudomonas aeruginosa* > *Klebsiella pneumoniae*. The methanol extract of *A. viridis* exerted maximum antibacterial activity against *E. coli*. The least activity was exhibited by *P. aeruginosa* and *K. pneumoniae* indicating their poor antibacterial activity. Medicinal plants are the backbone of traditional medicine and the antibacterial activity of plant extract is due to different bioactive substances in the extract with potential antimicrobial compounds.

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*Please cite this article in press as **Suneetha Devi .D** et al. GC-MS Analysis of Phytochemical Constituents and Screening for Aantibacterial Activity of the Methanol Leaf Extract of Amaranthus Viridis Linn. Against Human Pathogenic Bacteria. Indo American Journal of Pharmaceutical Research.2017:7(02).*

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 growth of pathogenic microbes because of their antimicrobial potential [1]. The medicinal value and multiple biological properties of several plants are defined by their phytochemical constituents [2].
Plants have provided a source of inspiration of novel drug compounds [3], as plant derived medicines made large contributions to human health and well-being. Plant derived medicines are widely used because they are relatively safer than the synthetic alternatives, as they are easily available and cheaper [4]. Many plant species have been evaluated for their antimicrobial activity in the past 20 years [5].

They provide us with key chemical structure for the development of new antimicrobial drugs and also as a phytomedicine [1] to be used for the treatment of disease. The active principles of many drugs found in plants are recognized as secondary metabolites [6, 7].

Many studies have been undertaken for determining different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both tropical and systemic microbial infections as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistant.

Because of the emerging development of drug resistance by pathogenic microorganisms against synthetic antibiotics; attention is now being shifted to extracts of biologically active components isolated from plant species commonly used as herbal medicine, as they may offer a new source of antibacterial, antifungal and antiviral activities [8]. The potential antimicrobial properties of plants are related to their ability to synthesize several secondary metabolites of relatively complex structures possessing antimicrobial activity [9, 10].

Amaranthus viridis L. belonging to Amaranthaceae family, is a fast growing herb mainly cultivated in Asia, Africa and Latin America [11]. *Amaranthus*, commonally known as Green amaranth, is a multinational genus of herbs. In the last decade, the amaranth is not only used in the common diet, but also in diet of people with celiac disease or allergies to typical cereals. The leaves are highly nutritious and the nutrients present in the leaves are carbohydrates, protein, vitamins and are rich in minerals such as calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese. Because of its high nutritional value, *A. viridis* is consumed more compared to other leafy vegetables.

Recently, drug resistance to human pathogenic bacteria has been reported worldwide. Medicinal plants are an expensive gift from nature as they are the sources of important 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 [12-15]. Therefore, characterization of extracts of medicinal plants is necessary due to its numerous benefits to science and society. Nevertheless, *Amaranthus* have received notably less research attention as vegetables than grain amaranths.

Hence, the present study was aimed at determining phytochemical constituents with the aid of GC-MS technique and *in vitro* screening of pure methanol extracts of leaves from locally grown *Amaranthus viridis* plants for their antibacterial activity against human bacterial pathogens. The findings of this study provide important data on the bioactive substances of these under utilised vegetable, and there by promote their utilization in food industry.

MATERIALS AND METHODS

Plant material

The seeds of *Amaranthus viridis* L. were obtained from the market, Hyderabad, Telangana, India and the plants were cultivated under natural conditions, identified and authenticated by Botanical Survey of India (BSI), Hyderabad, Telangana, India. Fully matured leaves from the plant were selected because there is maximum metabolism in fully matured leaves as compared to young leaves. The leaves were washed gently with distilled water for three to four times, shade dried at room temperature and coarsely crushed.

Preparation of Plant Extract

The coarsely crushed powdered samples were extracted using methanol by soxhlet apparatus. The extract was further concentrated using rotary evaporator under reduced pressure and stored at 4^{o}C in the refrigerator. Methanol extraction of the plant material was carried out by suspending 100 grams of *Amaranthus* powder in 1000 ml of 80% methanol (80:20, methanol: water, v/v).
The extraction was allowed to stand for 72 hours at room temperature. The extract was filtered first through muslin cloth, then through Whatman filter paper No.1 (125 mm) and dried using a rotary evaporator. This was transferred into sterile bottles and stored in the refrigerator until used.

Antimicrobial Activity

Test bacterial strains

In this study the test microorganisms used for antibacterial sensitivity testing included two Gram positive bacteria *Staphylococcus aureus and Bacillus cereus* and three Gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These microorganisms were obtained from the Department of Zoology, Osmania University, Hyderabad, Telangana, India.

All strains were maintained in Nutrient agar media at 4°C and activated on Mueller Hinton Agar plates 24 h prior to any antimicrobial test. The bacterial strains grown in Mueller Hinton Broth (MHB) for 24 h prior were used for antibacterial assays.

Antibacterial activity

Disc Diffusion Method

The antibacterial efficacy of the prepared methanol leaf extract of *Amaranthus viridis* was determined by using disc diffusion method. The bacterial strains were grown in Muller Hinton broth (Himedia, Mumbai, India) at 37^{o}C for 24 h, with constant agitation in orbital shaker. The cultures from the broth were aseptically swabbed on sterile Nutrient agar medium plates using sterile cotton swabs. The discs (6 mm in diameter) were loaded with 20 µg/ml, sample extracts (20 µg/disc) and placed on bacteria inoculated agar. The impregnated discs were placed on to the surface of NA plates previously seeded with the selected test organisms, along with discs containing solvent blanks separately. The plates were then incubated at 28±2^{o}C for 24 h. in upright position and were observed for inhibition zone. The diameter of the inhibition zone was measured in millimeters [16] which indicate antimicrobial activity. The activity assay was conducted in triplicates.

Phytochemical screening

The crude extracts were subjected to GC-MS analyses for the presence of different compounds. The analysis was performed at Central Analytical Facility Laboratory, University College of Technology, Osmania University, Hyderabad, Telangana, India.

Identification of bioactive compounds by GCMS

The purified methanol extract fractions were individually examined using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a mass spectrometer equipped with Elite-1 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 constant flow rate 1ml/min and an injection volume of 2µl was employed (injector temperature 25^{o}C; ion source temperature 28^{o}C).

The oven temperature was programmed from 100^{o}C (isothermal for 5 min) with a temperature of 4^{o}C / min to 24^{o}C with column flow rate of 1.21ml/min. The sample was run for 40 min with solvent out time of 9.50 min. Mass spectra were taken with scan interval of 0.6 seconds. Interpretation on mass spectrum was achieved by using data base of Wiley 22a LIB and MIST05S LIB for different bioactive compounds.

GCMS analysis of bioactive compounds from sample

The methanol leaf extract obtained from sample was subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. Some of the important features are summarized below.

GC-MS analysis of the sample was carried out using Shimadzu Make QP-2010 with nonpolar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40^{o}C and held for 3 min and the final temperature of the oven was 480^{o}C with rate at 10^{o}C [min.sup.-1]. 2 µL sample was injected with split less mode. Mass spectra was recorded over 35 - 650 amu range with electron impact ionization energy 70 eV. The total running time for a sample was 43.2 min. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

Identification of phytoconstituents

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns [17, 18]. The mass spectrum of the unknown component was compared with spectrum of known component stored in NIST library. Quantitative determinations were made by relating 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 min 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 AND DISCUSSION

The medicinal value of plants depends on the presence of phytoconstituents. *Amaranthus viridis* (L.) leaf extracts are found to be a vital source of useful bioactive substances. These bioactive compounds are involved in various biological functions such as communication, infection, reproduction and self- defense. In the present study, we have identified bioactive compounds present in the methanol extract fraction of *A. viridis* leaf by GC-MS analysis and summarized in Table 1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) of the corresponding compounds were observed in the leaf extract of *A. viridis* leaf and the total running time was 43.2 min. GC-MS chromatogram of the methanol leaf extract of *A. viridis* belonging to family Amaranthaceae showed 50 peaks which indicates the presence of fifty compounds (Figure 1). The spectra of the compounds were matched with Wiley 9.0 and National Institute of Standards and Technology libraries. The compounds detected are presented in Table 1.

Table 2: Table 1: Phytocomponents identified in the methanol leaf extract of A*maranthus viridis* (L.) by GC-MS analysis.

S No	RT	Name of the compound	Molecular formula	M W	Peak Area %
1	7.183	Cyclononasiloxane, octadecamethyl- $$	$C_{18}H_{54}O_9Si_9$	666	0.33
2	7.425	Dodecamethylcyclohexasiloxane $$ - A cyclic volatile methylsiloxane (cVMS) used in cosmetic and personal care products.	$C_{12}H_{36}O_6Si_6$	444	0.25
2	7.608	Monensin, methyl ester (CAS) Monensin methyl ester $$ 1,6-Dioxaspiro[4.5]decane-7-butyric acid, 2-[5-ethyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-(hydroxymethyl	$C_{37}H_{64}O_{11}$	684	0.32
4	7.748	3,3,5-Tributoxy-1,1,1,7,7,7-hexamethyl-5-(trimethylsiloxy)tetrasiloxane $$	$C_{21}H_{54}O_7Si_5$	558	0.23
5	8.597	Tetradecamethylcycloheptasiloxane $$	$C_{14}H_{42}O_7Si_7$	518	0.21
6	9.617	L-Threonine, N-[(2,4-dichlorophenoxy)acetyl]- $$ N.alpha.-(2,4-D)-L-Threonine $$	$C_{12}H_{13}C_{l2}NO_5$	321	0.16
7	9.933	Dibenz[a,c]cycloheptan-9-amine, 2,3,4-trimethoxy-N-acetyl- $$	$C_{20}H_{23}NO_4$	341	0.18
8	10.075	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyl-octasiloxane $$	$C_{16}H_{50}O_7Si_8$	578	0.22
9	11.854	Benzoic acid, 2,6-bis[(trimethylsilyl) oxy]-, trimethylsilyl ester $$ 2,6-Dihydroxybenzoic acid 3TMS $$	$C_{16}H_{30}O_4Si_3$	370	0.16
10	12.375	2-Cyclohexen-1-one, 3-methyl-6-(1-methyl ethenyl)- $$ p-Mentha-1,8-dien-3-one $$ Isopiperitenone $$	$C_{10}H_{14O}$	150	0.16
11	12.970	(4R,5R,6R,8R,8aS,12aS)-5,8,8a,11,12,12a-hexahydro-1,4,8-trihydroxy-5-methoxy-9,10,12a-trimethyl-3H-phenanthro[3,2-b]pyran-2(4H)-one $$	$C_{21}H_{26}O_6$	374	0.18
12	13.084	4-.alpha.,20-dimethyl-3-.beta.-dimethyl-... $$	$C_{29}H_{54}O_2SI$	462	0.34
13	13.137	Acetphenone 4-[1-adamantyl]-3- thiosemicarbazone $$	$C_{19}H_{25}N_3S$	327	0.31
14	13.201	3,4-Methylenedioxyphenyllactic acid, di-TMS $$	$C_{16}H_{26}O_5Si_2$	354	0.20
15	13.264	Benzoic acid, 3,5-dimethoxy-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester (CAS) 4-Hydroxy-3,5-Dimethoxybenzoic Acid-Ditms Syringic Acid-Ditms $$ Trimethylsilyl 3,5	$C_{15}H_{26}O_5SI_2$	342	0.31
16	13.338	di-3-(1-Phenyl-1-methylethyl)phenyl amine $$	$C_{30}H_{31}N$	405	0.46
17	13.467	4-.alpha., 20-dimethyl-3-.beta.-dimethyl-... $$	$C_{29}H_{54}O_2SI$	462	1.16
18	13.516	Silane, trimethyl(triphenylethenyl)- $$	$C_{23}H_{24}Si$	328	0.58
19	13.567	Heptamethyl-Phenyl-Cyclotetrasiloxane $$	$C_{13}H_{26}O_4SI_4$	358	0.88
20	13.661	Pregnan-20-one, 3-(acetyloxy)-5,6:16,17-diepoxy-, (3.beta.,5.alpha.,6.alpha.,16.alpha.)- $$ 5.alpha.-Pregnan-20-one, 5,6.alpha.:16.alpha.,17-diepoxy-3.beta.-hydroxy-, acetate $$	$C_{23}H_{32}O_5$	388	1.18
21	13.724	[5-(3-Methoxymethoxy-10,13-dimethyl-2,3,4,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-17-yl)-hex-1-ynyl]-trime $$	$C_{30}H_{48}O_2Si$	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 $$ Pyrimido[4,5-d] pyrimidine-2,4(1H,3H)-dione, 7-[(4-chlorophenyl)imino]- 6,7	$C_{21}H_{18}CLN_5O_2$	407	3.14
23	14.167	(+,-)-3.beta.-(acetyloxy)-3-ethynyl-1,2,3,4,4a.beta.,12a.beta.-hexahydro-6,11-dihydroxy-7-methoxy-1.alpha.-(trimethylsilyl)-5,12-naphthacenedione $$	$C_{26}H_{28}O_7SI$	480	5.67
24	14.274	Silane, [1,3,5-benzenetriyltris(oxy)]tris[trimethyl- (CAS) Phloroglucinol TriTMS $$ 1,3,5-Trihydroxybenzene 1,3,5-Tritms $$ 1,3,5-Trihydroxybenzene 3TMS $$	$C_{15}H_{30}O_3SI_3$	342	2.76
25	14.349	1,2-Diphenyltetramethyldisilane $$ Disilane,1,1,2,2-tetramethyl-1,2-diphenyl- $$	$C_{16}H_{22}Si_2$	270	4.98
26	14.453	[Bis(trimethylsilyl)methyl]diphenylphosphine $$ Phosphine, [bis(trimethylsilyl)methyl]diphenyl- (CAS)	$C_{19}H_{29}PSI_2$	344	5.59
27	14.588	4H-1-Benzopyran-4-one, 2-(2,6-dimethoxy phenyl)-5,6-dimethoxy- (CAS) Zapotin $$ 7187202001 Zapoti $$ Flavone, 2',5,6,6'-tetramethoxy- $$	$C_{19}H_{18}O_6$	342	2.46
28	14.653	3,5,8-Trioxanonane, 4-methyl-6-oxiranyl-9-phenyl- $$	$C_{15}H_{22}O_4$	266	1.70
29	14.708	2-Isopropylphenol-Trimethylsilyl-Ether $$	$C_{12}H_{20}OSI$	208	2.73
30	14.856	Anisuric acid, di-TMS $$	$C_{16}H_{27}NO_4Si_2$	353	1.52
31	14.970	Cyclohexa-1,4-Diene, 1,3,6-Tris(Trimethylsilyl)- $$	$C_{15}H_{32}SI_3$	296	1.23
32	15.007	Propylparaben TMS Ether $$	$C_{13}H_{20}O_3SI$	252	0.73
33	15.233	(Z)-1-[(1',1'-dimethylethyl)diphenylsilyl]-3-trimethylsilyloxyprop-1-ene $$ (Z)-3-[(1',1'-dimethylethyl)diphenylsilyl]-1-trimethylsilyloxyprop-1-ene $$ Silane, [[3-[(1,1-dimethylethyl)diphenylsilyl]	$C_{22}H_{32}OSI_2$	368	2.37
34	15.342	Benzophenone, 2-(trimethylsiloxy)- (CAS) Trimethylsilyl ether of o-hydroxybenzophenone $$	$C_{16}H_{18}O_2SI$	270	3.50
35	15.434	Prosta-5,10,13-trien-1-oic acid, 15-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-9-oxo-, methyl ester, (5Z,13E,15S)- $$	$C_{27}H_{46}O_4Si$	462	2.19
36	15.508	Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester (CAS) 2-Hydroxybenzoic	$C_{13}H_{22}O_3SI_2$	282	5.32

		Acid-DiTMS $$ Bistrimethylsilyl Salicylic Acid $$ Trimethylsilyl O-Trimethylsilylsalicylate			
37	15.636	Benzoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl	$C_{13}H_{22}O_3Si_2$	282	24.09
38	15.880	Nonamethyl, Phenyl-, Cyclopentasiloxane $$	$C_{15}H_{32}O_5SI_5$	432	0.17
39	21.767	1-Trimethylsilyloxy-2-Trimethylsilylamino-3-(4'-Methoxyphenyl) Propanone $$	$C_{16}H_{29}NO_3SI_2$	339	0.21
40	17.027	Nonamethyl, Phenyl-, Cyclopentasiloxane	$C_{15}H_{32}O_5Si_5$	432	6.10

RT- retention time, MW- molecular weight

Antibacterial activity

The disc diffusion method is widely employed to check the susceptibility of chemical components of herbal extracts against selected bacterial strains and is a reliable method too. The results of inhibitory effect of methanol leaf extract of *A. viridis* on the pathogenic bacteria tested are shown in Table 2. The present results of antibacterial activity proved the ability of the leaf extract of *A. viridis* to prevent the growth and survival of the test pathogens.

The results showed that different bacterial species exhibit different sensitivities towards the leaf extract. The extract was found to be inhibitory to all bacterial isolates but with variable extent. The order of activity against selected bacteria was *Escherichia coli* > *Staphylococcus aureus* > *Pseudomonas aeruginosa* > *Klebsiella pneumoniae* > *Bacillus cereus*. The methanol extract of *A. viridis* exerted a maximum antibacterial activity against *E. coli.*

In the present study, the *A. viridis* (L) leaf methanol extract effectively inhibited all bacteria tested. The zone inhibition against tested bacteria was medium to nil indicating the methanol extract exhibited less activity against *P. aeruginosa* and *K. pneumoniae.* The results of the present study indicate that the antibacterial activity varies according to type of bacteria used for the study. The least activity was exhibited by *P. aeruginosa* and *K. pneumoniae* with smallest zone of inhibition (Table 2) indicating their poor antibacterial activity. The antibacterial activity of tested *A. viridis* was compared with the standard drug streptomycin. The standard antibiotic tested, showed higher zone of inhibition indicating highest activity.

The methanol extract of *A. viridis* achieved maximum zone of inhibition with *E. coli* compared to others. The differences in the antibacterial activity of leaf extract might be due to the phytochemical components present in the extract.

Medicinal plants play a central role not only as traditional medicines but also as commercial commodities meeting the demand of distant markets. Literature indicates that medicinal plants are the backbone of traditional medicine and the antibacterial activity of plant extract is due to different chemical agents in the extract with potential antimicrobial compounds [19-22].

Table 3. Antibacterial activity of methanol leaf extract of *Amaranthus viridis* against selected human pathogenic bacteria.

S No	Name of the microorganisms	Zone of inhibition (mm)	Streptomycin (mm)
1	*Staphylococcus aureus*	15.7	14.3
2	*Bacillus cereus*	0.00	12.5
3	*Pseudomonas aeruginosa*	7.8	16.8
4	*Klebsiella pneumoniae*	4.6	18.5
5	*Escherichia coli*	19.6	15.3

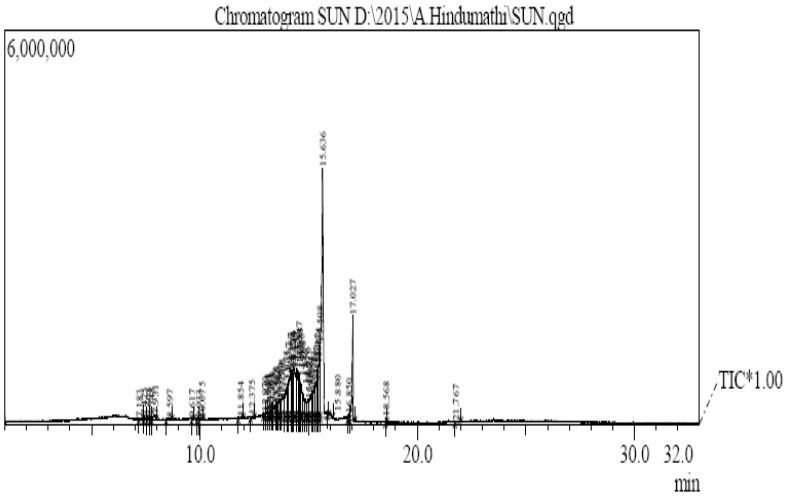

Figure 1: GC-MS Chromatogram of methanol leaf extract of *Amaranthus viridis* (L.).

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-hexadecaonoic acid), unsaturated fatty acid and linolenic (docosatetraenoic acid and octadecatrienoic acid) as antimicrobial, antioxidant, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic, immunomodulatory and anticoronary [23].

Secondary metabolites of the plants attract beneficial and repel harmful organisms, serve as phytoprotectants and respond to environmental changes. In general, the phytochemical contents (Table 1) were in accordance with the previous reports for some of the vegetables [24-26]. These phytocompounds are responsible for various pharmacological actions of the leaves of the plant.

Benzoic 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.-(acetyloxy)-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-diphenyl (4.98%), Benzophenone, 2-(trimethylsiloxy)- (CAS) Trimethylsilyl ether of O-hydroxybenzophenone (3.50%), 7-(P-Chlorophenyl)imino-6-(P-tolyl)-1,3-dimethyl-2,4 dioxo-1,2,3,4,6,7- hexahydro pyramid [4,5-d] pyramidine (3.14%), 4-.alpha.,20-dimethyl-3-.beta.-dimethyl-.. (2.84%), Silane, [1,3,5-benzenetriyltris (oxy)] tris [trimethyl- (CAS) Phloroglucinol trit MS (2.76%), 2-Isopropyl phenol- Trimethylsilyl- Ether (2.73%), 4H-1-Benzopyran-4-one, 2-(2,6-dimethoxyphenyl)- 5,6-dimethoxy- (CAS) Zapotin $$ Flavone (2.46%), (Z)-1-[(1',1'-dimethylethyl)diphenylsilyl]-3-trimethylsilyloxyprop-1-ene (2.37%), Prosta-5,10,13-trien-1-oic acid, 15-[[(1,1-dimethylethyl) dimethyl ethylsily]oxy]-9-oxo- (2.19%), Silane, trimethyl (triphenylethenyl)- $$ (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 *A. viridis* (L) tested by GC-MS analysis contained various types of compounds with potential pharmacological activity against bacterial pathogens tested. The presence of various bioactive compounds justifies the use leaves of *A. 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 has antibacterial, antifungal, antioxidant and anticancer activity but further researches should be made to isolation and purification of natural products in their extract.

CONCLUSION

From the study it was concluded that the edible plant species *Amaranthus viridis* from underutilized plant family had a rich amount of valuable ingredients that are beneficial for health and also has antibacterial activity.

Further research is required work in more detail *in vitro* and *in vivo* investigations to establish which components of the extract are biologically active in terms of antibacterial activity against disease causing human pathogenic bacteria. The isolation of bioactive components from this readily available natural resource and their utilization as potential natural antibacterial agents could be of high economic value.

ACKNOWLEDGEMENTS

Dr. D. Suneetha Devi is very thankful to UGC, New Delhi, India, for providing Grant-in-Aid under Minor Research Project No. 4725/14 (SERO/UGC). I also thank principal, the management of Sarojini Naidu Vanitha Maha Vidyalaya, Exhibition Grounds and Head Department of Zoology for providing the laboratory facitlities.

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