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Foliar anti-diabetic and antioxidant potential of a promising accession of *Amaranthus hypochondriacus* L.: GC-MS based evidences

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ABSTRACT

The present study makes an effort to investigate the foliar neutraceutical potential of a promising green accession of a seed amaranth (*Amaranthus hypochondriacus*, accession no. IC94661) based on anti-lipid peroxidation property, reducing power, metal chelating activity, hydroxyl radical scavenging property, antidiabetic factor and GC-MS based identification of hydroxyl containing phytochemicals. Methanol and aqueous fractions possessed exhibited better anti-lipid peroxidation, reducing, hydroxyl radical scavenging, and metal chelating properties in the experimental accession. Estimation of anti-diabetic factors from the young leaf extract also revealed significantly high α-glucosidase and α- amylase inhibition properties. When GC-MS study was carried out, it exhibited presence of several hydroxyls containing phytochemicals in the accession, some of which are having antioxidant properties. Taken as a whole, the data not only provide evidence of rich sources of marker antioxidant properties but also the availability of several phytochemicals with hydroxyls, in support of its rich pharmaceutical potential.

Keywords: Amaranth, Anti-diabetic factors, antioxidant properties, Phenolics, GC-MS.

INTRODUCTION

Oxidative stress, involving oxidative deterioration, is one of the major causative factors in the induction of aging, many degenerative and life style diseases including cancer, atherosclerosis, ischemic heart disease, diabetes mellitus, neuro-degenerative diseases etc, [1-4]. There are several evidences of of direct relationship between loss of redox homeostasis due to excess generation of ROS and damages of all major classes of macromolecules during cancer. In addition, oxidative stress is considered as one of the major factor for the induction of type II diabetes [5-6]. In view of rapid incidence of these diseases, the health costs of these degenerative diseases have increased alarmingly and solutions are urgently needed not only to combat the diseases but also to ease out the financial burdens of the patients.

In skirmishing those diseases, antioxidant therapy is gaining importance in recent times [2, 4, 7, 8]. Antioxidants have been shown to be extremely effective not only in the treatment but also in prevention of various health problems, including diabetes mellitus, cancer, atherosclerosis, neurodegenerative diseases, systemic and infectious diseases and natural ageing [1, 5-7, 9, 10]. Antioxidants are also added to food delay and prevent its oxidation, normally initiated by free radicals formed during the food's exposure to environmental factors such as air, light and temperature [11]. Further, the restrictions of the uses of several commercial synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertbutylhydroquinone (TBHQ) for their non-target side effects, the hunch for the identification of natural sources of antioxidant increased significantly [12]. Therefore, the current trend of research is to find out plant sources with natural antioxidant and anti-diabetic properties without side effects.

Several underutilized plants often exhibit their rich sources of secondary metabolites and offer as chemotherapeutic reservoirs. Amaranths, are of supreme importance, though underutilized, for their diverse use as green leafy vegetable (GLV), seed flour, natural dye and food supplement [13, 14]. The pseudocereal *Amaranthus hypochondriacus* is essentially recognized as a grain crop for their excellent nutritional and functional values associated with their seeds [13, 15]. Recent years rediscovered the potential of pseudocereal *A. hypochondriacus* as functional food for their excellent seed protein quantity and quality, availability of dietary fibers, lipid rich unsaturated fatty acids, vitamins, micronutrients etc. [16-18] It can also be projected as a multipurpose subsidiary food crop for the excellent nutritional

attributes and yield potential [13, 17].

Some workers reported presence of antioxidants like phenolic acids or flavonoids with along with good quality proteins fatty acids, fibres and vitamins from some selected leaf and grain amaranth [16-18]. Earlier studies also reported that seeds of these *Amaranthus* species exhibit antioxidant potential as confirmed by in-vitro radical scavenging assay [19]. But no detailed data were published on the foliar antioxidant potential of the seed amaranth assessed in terms of different antioxidant attributes, like reducing property, metal chelating property, anti-lipid peroxidation property, anti-diabetic factor etc. Moreover, in spite of the huge potential as functional food, the genetic diversity based analysis of nutritional attributes, particularly the antioxidant potential, anti-diabetic property of some important species like *Amaranthus hypochondriacus*, are not fully explored.

Therefore, in this investigation an effort have been made to access the foliar antioxidant potential of different solvent extracts of a promising accession of *A. hypochondriacus* (Accession No IC94661) through investigation of standard markers of antioxidant potential (in terms of anti-lipid peroxidation property, reducing power, metal chelating activity, hydroxyl radical scavenging property, total antioxidant capacity, antidiabetic factor). The GC-MS study was also conducted simultaneously to assess and identify the presence of hydroxyl containing compounds with antioxidant properties of the experimental accessions of *A. hypochondriacus* (IC107144 and IC94661) to vouch qualitatively the antioxidant potential and neutraceutical value of the pseudo-cereal.

MATERIALS AND METHODS

Seeds of an accession of *Amaranthus hypochondriacus* L. (IC94661) were collected from National Bureau of Plant Genome Research (NBPGR), New Delhi, India, and were cultivated in Crop Research and seed Multiplication Farm, University of Burdwan, West Bengal, India, based on Standard Procedure.

Sample preparation for GC-MS study

20 grams of oven dried and powdered young leaves (35 day old) of each experimental material were extracted with 200 ml 95% ethanol for 12 hours and filtered through Whatmann filter paper # 1 (41cm) with 2 gram sodium salfate (before filtering, the filter paper was wetted with 95% ethanol containing sodium sulphate). The filtrate collected was concentrated by bubbling N_2 gas into the solution in a rotary vacuum evaporator. 2 µl of solution was taken for GC-MS study.

GC-MS Condition

Qualitative antioxidant profiling for hydroxyl containing flavonoids were done with Shimadzu GC-MS QP2010 system comprising a gas chromatograph interfused by a MS employing the following conditions: Fused silica column (30 x 0.25 mm) 1D x 1EMdf with 100% Dimethyl polysiloxane, operating in electron impact mode at 70ex, He (99.99%) as carrier gas at constant flow of 1 ml/min and injection volume of 0.5EI (split ratio 1:1); injector temperature 250°C, ion source temperature 280°C. Oven temperature was programmed from 110°C (isothermal for 2 mins) with an increase of 10°C/min, to 200°C,then 5°C to 280°C, ending with 9 mins isotherm at 280°C.

Assessment of Anti-diabetic factor

α-Amylase Inhibition Activity

For the extraction and estimation of α- amylase inhibition activity of the experimental plant tissue, the process of Wong *et al*. (2014) [20] was followed with slight modifications. Extract of plant sample was mixed with 0.3mL of 0.02M Sodium phosphate buffer (pH 6.9) and 100µL of α-Amylase solution (4.5 units/ mL/ min.). The mixture was incubated for 10 minutes at 25°C. Then 1% starch was added and incubated for 30 minutes at 25°C. The reaction was stopped by the addition of 1 mL of dinitrosalicylic acid reagent. The test tubes were then incubated in boiling water bath for 5 minutes and then cooled to room temperature. After that the reaction mixture was then diluted 10-fold times with distilled water and the absorbance was measured at 540nm. The reading were compared with the control (extract was replaced by buffer) and α-Amylase inhibition activity (%) was calculated.

α- Glucosidase Inhibition Activity

For the extraction and estimation of α- Glucosidase inhibition activity of the experimental plant tissue, the process of Wong *et al*. (2014) [20] was followed with slight modifications. 1mL extract was mixed with 1 mL 0.1M of phosphate buffer (pH 6.9) and 1mL α- Glucosidase solution (1unit/mL/min.) and incubated for 5 minutes at 25°C. After the pre-incubation,1mL of 5mM p-nitrophenyl-α-D-glucopyranoside solution was added and the reaction mixture was incubated for 10 minutes at 25°C. After the incubation absorbance was recorded at 405nm and α- Glucosidase inhibition (%) was calculated.

Assessment of Antioxidative properties

Metal chelating property

For the estimation of metal chelating property of experimental plant tissue, the process of Lin *et al*. (2009) [21] was followed with slight modifications. Shortly, 1 ml of different solvent extracts (extraction procedure described earlier) was added to a solution of .02 ml 2 mM ferrous chloride and .04 ml 5 Mm ferrozine. The mixture was vigorously shaken and incubated for 10 mins. Absorbance was taken at 562 nm. Metal chelating activity was expressed as:

$$\text{Activity (\%)}: [Ac- As / Ac] \times 100$$

where Ac= Absorbance of control, As= Absorbance of sample

Reducing power

For the estimation of reducing power of experimental plant tissue the process of Lin *et al* (2009) [21] was followed with slight modifications shortly, 1 g of dry powder was extracted with 50 ml of distilled water at 70°C under reflux for 4 hours and then centrifuged for 3000 rpm for 10 mins. 25 ml of supernatant was taken and added with 200 Mm sodium- phosphate buffer (pH 6.6) and .1% potassium ferricyanide. The mixture was incubated for 20 mins at 50°C and then added with .25ml 10% TCA. Subsequently the mixture was centrifuged at 3000 rpm for 10 mins. Supernatant was collected and mixed with deionised water and 1% ferric chloride solution. The mixture was kept for 10 mins and absorbance was taken at 700 nm. Reducing power was

expressed as activity (%).

Activity (%): [Ac- As/ Ac] x 100

Where, Ac= Absorbance of control, As= Absorbance of sample respectively.

Hydroxyl radical (OH•) scavenging activity

Hydroxyl radical scavenging capacity of methanolic extract of leaf sample was determined according to the method of Jan *et al*. (2013) [22] with slight modifications. The assay mixture(sample dialuted with phosphate buffer 10mM, pH 7.4,1ml of 2.8 mM 2-deoxy-ribose, 20µM FeCl3 and 100 µM EDTA, 200µM H_2O_2 and 300µM ascorbic acid) was incubated at37º C for 1h. Then 1ml of 2.8% TCA, 1ml of 1% TBA and .1 ml 50 mM NaOH were added. The reaction mixture was heated in aboiling water bath for 15 min. The absorbance was recorded at 532nm.The hydroxyl radical scavenging capacity was calculated according to equation:

Activity (%): [Ac- As/ Ac] x 100

Where, Ac= Absorbance of control, As= Absorbance of sample respectively.

Anti-lipid peroxidation assay in linoleic acid system

For the estimation of anti-lipid peroxidation assay experimental plant tissue the process of Amabye (2015) [23] was followed with slight modifications The antioxidant activity of two different accessions of *Amaranthus hypochondriacus* extracts were determined by measuring the oxidation of linoleic acid. 5 mg of different solvent extracts were added separately to a solution of linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 10 mL of 0.2 M Sodium Phosphate buffer (pH=7). The mixture was made up to 25 mL with distilled water and incubated at 40°C up to 360 hours. Extent of oxidation was measured by peroxide value applying thiocyanate method. Briefly, 10 mL of ethanol (75% v/v), 0.2 mL of an aqueous solution of Ammonium thiocyanate (30% w/v), 0.2 mL of sample solution and 0.2 mL of ferrous chloride ($FeCl_2$) solution (20 mM in 3.5% HC1; v/v) added sequentially. After 3 min of stirring, the absorption was measured at 500 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc. and Tokyo, Japan). A negative control contained all reagents with exception of extracts. Synthetic antioxidants Butylated Hydroxytoluene (BHT) (also we can use ascorbic acid) was used as positive control. The maximum per oxidation level was observed at 360 h (15 days) in the sample that possesses no antioxidant component percent inhibition of linoleic acid oxidation was calculated with the following equation: % inhibition of Linoleic acid peroxidation: [1- Change in absorbance of treated sample / Change in absorbance of Control sample] x 100.

Statistical analysis

For statistical analysis of data, standard error was calculated using three replicate of independent contents. Further, extractability data were also analysed for ANOVA test. The means of the significant differences were separated using Fisher's least significant test for difference at the 0.05 level of probability.

RESULT

The different functional antioxidant attributes, like $OH^{\cdot}$ radical scavenging property, metal chelating activity, reducing property and anti-lipid peroxidation property of methanol extract and other solvent fractions (ethyl acetate, n-hexane, chloroform and aqueous) obtained from leaves of two experimental accessions of *Amaranthus hypochondriacus* are shown in Table 1. However, the highest expression of all antioxidant attributes was noted from methanol extracts for the both the accessions, whereas residual aqueous fraction gave the minimum for both the accessions (Table 1).

This scavenging hydroxyl radical, the most potent ROS, is extremely important for protection of biological systems. In this study, methanolic leaf extracts of the experimental accessions exhibit highest OH radical scavenging property, corroborating well the data of reducing property (which indirectly reflect radical scavenging ability) of leaf extracts of the experimental accessions (Table1).

Table 1 also shows the reducing power of the experimental accession by measuring the Fe^{3+} to Fe^{2+} transitions in presence of foliar extracts. Reducing power simply reflects the antioxidant function either by donating electrons or by forming radical chain breaking reaction. Present study showed the highest reducing power in methanolic leaf extract for the accession IC47434 substantiating well with the data of DPPH radical scavenging and $OH^{\cdot}$ radical scavenging properties.

Transition metal ions, particularly Fe stimulate Fenton reaction and accelerate lipid peroxidation through conversion of hydroperoxides into alkyl and peroxyl radicals and hence perpetuate further the chain reaction of membrane lipid peroxidation. According to our result, the methanolic leaf extract of the experimental accession of *A. hypochondriacus* exhibit highest metal chelating property than other solvent extracts, corroborating again our earlier data of other antioxidant properties of leaf samples i.e, reducing power, $OH^{\cdot}$ radical scavenging properties and DPPH radical scavenging properties (Table 1).

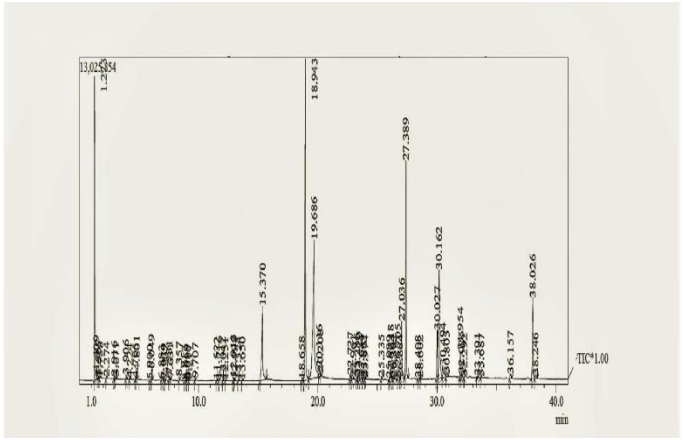
Anti-lipid peroxidation of experimental plant sample was determined by inducing oxidation of linoleic acid as model system. Linoleic acid was incubated in oxidizing atmosphere with or without plant sample and subsequently the oxidizing value was measured by estimating the peroxide value applying thiocyanate method. The result of anti-lipid peroxidation assay for the methanolic leaf extracts of experimental accessions showed highest inhibition percentage for the accession IC47434. So, like other attributes of antioxidant potential tested for the foliar extracts of the experimental accession of *A. hypochondriacus* ($OH^{\cdot}$ radical scavenging properties and DPPH radical scavenging properties, metal chelating property, reducing power), anti-lipid peroxidation property strongly corroborate the fact that the accession no IC47434 possess significant antioxidant attributes.

GC-MS method was employed for the ethanolic extract of young leaf tissue of accession no IC94661 for testing availability of hydroxyl rich phytochemical constituents. The acidic fraction was silyated and subjected to GC-MS investigation. GC-MS data identified sixty nine compounds from leaf extracts of accession no IC94661 based on library data (NIST and WILEY) of corresponding compounds (Fig. 1). The ethanolic leaf extract of the experimental accession IC94661 of *A. hypochondriacus* showed 14 major phenolic constituents, as: 4H-pyran-4-one,2 hydroxy(peak area 0.07%), / 4H-pyran-4-one,2,3 dihydro-3,5- (peak area 0.17%), / 3-heptanol (peak area 0.03%), / 2-methoxy-4-vinyl phenol (peak area 0.35%), / 1-tridecene (peak area 0.03%), / Benzaldehyde,2-hydroxy-1-propenyl)-2 (peak area 0.06%), /

4-(1E)-3- hydroxyl-1-propenyl)-2 (peak area 0.06%) / 13-Heptadecyn-1-ol (peak area 0.06%), / Phytol isomer (peak area 17.7%), / Hexadecanoic acid,2-hydroxyl (peak area 0.05%), / Cycloheptadecanol (peak area 0.68%), / Hexadecanoic acid,2-hydroxyl-1-(1,1,6-10 Dodecatrien-3-ol,3 (peak area 3.82%), / Gama-tocopherol (peak area 0.27%), / Gama-tocopherol-beta-D-mannose (peak area 5.46%) respectively.

Table 1: Estimation of, anti-lipid peroxidation, metal chelating activity, reducing and hydroxyl radical scavenging properties and anti-diabetic factors of foliar extract and different solvent fractions of a promising accession of *Amaranthus hypochondriacus* L., Accession no. IC94661 [Results are mean of three replicates ± SE. Different super indexes indicate significant differences within a column, compared by Fishers least significant difference test ($p<0.05$)].

Accession No. of *Amaranthus hypochondriacus* L.	Extract	Anti-lipid peroxidation property (% inhibition g-1 dm)	Metal chelating activity (% g-1 dm)	Reducing property (% g-1 dm)	Hydroxyl radical scavenging property(% g-1 dm)	Anti-diabetic factor	
						% Inhibition of *α*-amylase	% Inhibition of *α*-glucosidase
IC94661	Methanol	33±0.46 a	24.4±0.72 a	20..1±0.70a	22.2±0.90a	NA	NA
	Ethyl acetate	18.2±1.50b	17.4±1.75b	18.8±0.55b	18.0±0.70a	NA	NA
	n-hexane	10.8.49c	9.5±1.70c	10.8±0.75c	9.0±0.60d	NA	NA
	Chloroform	9.6±0.90d	17.8±1.20b	16.1±0.80b	17.0±0.60c	NA	NA
	Aqueous fraction	20.5±1.25c	17.0±1.30b	24.5±0.70b	20.0±0.80b	85.66	2.846



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	1.273	1.233	1.800	54922711	17.92	12120397	19.67	4.53	S	ETHANOL
2	1.409	1.383	1.458	198523	0.06	123246	0.20	1.61	T	BUTANAL, 3-METHYL-
3	1.567	1.500	1.600	414843	0.14	116273	0.19	3.57	T	PROPANOIC ACID, 2-OXO-, M
4	1.625	1.600	1.658	153583	0.05	56243	0.09	2.73	TV	Pentanoic acid, 3-methyl-
5	1.758	1.658	1.792	195230	0.06	30154	0.05	6.47	TV	2-FURANMETHANOL
6	2.274	2.217	2.342	311481	0.10	69992	0.11	4.45		1,2,3-PROPANETRIOL
7	2.916	2.875	2.967	230018	0.08	109197	0.18	2.11		BENZENEACETALDEHYDE
8	3.071	2.967	3.150	215958	0.07	81223	0.13	2.66	V	4H-PYRAN-4-ONE, 2-HYDROX
9	3.906	3.858	4.008	510507	0.17	199165	0.32	2.56		4H-Pyran-4-one, 2,3-dihydro-3,5-
10	4.224	4.183	4.267	87452	0.03	35527	0.06	2.48		4-AMINO-1,2,5-OXADIAZOLE-
11	4.705	4.667	4.758	152047	0.05	50523	0.08	3.01		2,3-DIHYDRO-BENZOFURAN
12	4.801	4.758	4.883	824163	0.27	363433	0.59	2.27	V	2-Furancarboxaldehyde, 5-(hydro
13	5.900	5.875	6.008	81589	0.03	20579	0.03	3.96		3-HEPTANOL
14	6.049	6.008	6.158	1071278	0.35	421389	0.68	2.54	V	2-METHOXY-4-VINYLPHENOI
15	6.883	6.858	7.050	65107	0.02	24585	0.04	2.65		D-Allose
16	7.079	7.050	7.117	101981	0.03	60628	0.10	1.68		1-TRIDECENE
17	7.207	7.175	7.258	69289	0.02	32254	0.05	2.15		Benzaldehyde, 4-(acetyloxy)-3-m

18	7.531	7.458	7.600	764947	0.25	193375	0.31	3.96		GUANOSINE
19	7.631	7.600	7.675	178178	0.06	87959	0.14	2.03	V	Benzaldehyde, 2-hydroxy-6-methy
20	8.357	8.308	8.433	404266	0.13	138567	0.22	2.92		1,6-Anhydro-.beta.-D-glucopyran
21	8.817	8.792	8.942	49462	0.02	18232	0.03	2.71		2-Cyclopenten-1-one, 2,3,4-trimet
22	8.969	8.942	9.008	82483	0.03	46163	0.07	1.79	V	Phosphoric acid, diethyl octyl este
23	9.086	9.008	9.125	58817	0.02	33826	0.05	1.74	V	2(4H)-BENZOFURANONE, 5,6,
24	9.217	9.175	9.258	79191	0.03	39585	0.06	2.00		DECANOIC ACID
25	9.707	9.658	9.750	197981	0.06	93682	0.15	2.11		Z-5-Nonadecene
26	11.522	11.483	11.600	143790	0.05	46381	0.08	3.10		4-((1E)-3-Hydroxy-1-propenyl)-2
27	11.716	11.675	11.783	142510	0.05	56888	0.09	2.51		Tetradecanoic acid
28	12.042	12.008	12.083	71002	0.02	36911	0.06	1.92		2(4H)-BENZOFURANONE, 5,6,
29	12.251	12.208	12.300	103110	0.03	47111	0.08	2.19		1-HEXADECENE
30	12.913	12.858	12.967	467004	0.15	149953	0.24	3.11		2,6,10-TRIMETHYL,14-ETHYLI
31	12.998	12.967	13.067	177545	0.06	61109	0.10	2.91	V	2-PENTADECANONE, 6,10,14-
32	13.324	13.275	13.392	174452	0.06	58950	0.10	2.96		13-HEPTADECYN-1-OL
33	13.650	13.608	13.725	173199	0.06	57845	0.09	2.99		2,6,10-TRIMETHYL,14-ETHYLI
34	15.370	15.150	15.708	19373575	6.32	2942703	4.78	6.58		l-(+)-Ascorbic acid 2,6-dihexadec
35	18.658	18.608	18.725	234790	0.08	75288	0.12	3.12		11,14,17-Eicosatrienoic acid, meth
36	18.943	18.800	19.233	54355594	17.73	12882679	20.91	4.22		PHYTOL ISOMER
37	19.686	19.233	20.333	53211452	17.36	5512797	8.95	9.65	SV	9,12,15-OCTADECATRIEN-1-O
38	20.116	20.042	20.183	1094438	0.36	298755	0.48	3.66	T	Octadecanoic acid
39	20.208	20.183	20.325	334356	0.11	114443	0.19	2.92	TV	ETHYL (9Z,12Z)-9,12-OCTADE
40	22.727	22.667	22.800	536893	0.18	156574	0.25	3.43		N1-Isopropyl-2-methyl-1,2-propa
41	22.961	22.800	23.025	166057	0.05	55588	0.09	2.99	V	HEXADECANOIC ACID, 2-HYI
42	23.286	23.233	23.342	317448	0.10	97830	0.16	3.24		2-Piperidinobicyclo[3.3.1]nonan-9
43	23.420	23.342	23.508	658550	0.21	150897	0.24	4.36	V	2-Piperidinobicyclo[3.3.1]nonan-9
44	23.644	23.508	23.750	444089	0.14	77130	0.13	5.76	V	Pentadecanal-
45	23.867	23.750	23.925	278886	0.09	77043	0.13	3.62	V	1,2-BENZENEDICARBOXYLIC
46	23.974	23.925	24.025	117758	0.04	41303	0.07	2.85		4,8,12,16-Tetramethylheptadecan-
47	25.335	25.283	25.400	308317	0.10	108016	0.18	2.85		PHOSPHORIC ACID, 2-ETHYL
48	25.989	25.942	26.042	183229	0.06	55998	0.09	3.27		Benzedrex
49	26.118	26.042	26.217	1800147	0.59	500936	0.81	3.59	V	CYCLOHEXANEETHANAMIN
50	26.264	26.217	26.317	179879	0.06	52723	0.09	3.41	V	(R)-(-)-14-Methyl-8-hexadecyn-1-
51	26.382	26.317	26.458	348893	0.11	94704	0.15	3.68	V	Trichloroacetic acid, tridec-2-ynyl
52	26.705	26.633	26.850	2072836	0.68	477245	0.77	4.34		Cycloheptadecanol
53	26.884	26.850	26.925	139840	0.05	47913	0.08	2.92	V	HEXADECANE, 2,6,10,14-TETF
54	27.036	26.925	27.258	11696417	3.82	2285226	3.71	5.12	V	Hexadecanoic acid, 2-hydroxy-1-(
55	27.389	27.258	27.600	30704028	10.02	8670059	14.07	3.54	V	1,2-Benzenedicarboxylic acid, mo
56	28.408	28.375	28.575	139593	0.05	32227	0.05	4.33		.lambda.-Cyhalothrin
57	28.602	28.575	28.658	76293	0.02	31359	0.05	2.43	V	Heneicosane
58	30.027	29.917	30.067	8439235	2.75	1948463	3.16	4.33		9,12-Octadecadienoic acid (Z,Z)-,
59	30.162	30.067	30.425	29660434	9.68	4331060	7.03	6.85	V	Methyl (Z)-5,11,14,17-eicosatetra
60	30.494	30.425	30.717	3654785	1.19	468996	0.76	7.79	V	Octadecanoic acid, 2,3-dihydroxy
61	30.803	30.717	30.942	792481	0.26	125809	0.20	6.29	V	Oxirane, hexadecyl-
62	31.954	31.875	32.008	3273557	1.07	1066104	1.73	3.07		2,6,10,14,18,22-Tetracosahexaene
63	32.025	32.008	32.117	285843	0.09	117693	0.19	2.43	V	1,2-BENZENEDICARBOXYLIC
64	32.292	32.250	32.317	34992	0.01	13042	0.02	2.68		5,9,13-Pentadecatrien-2-one, 6,10
65	33.391	33.333	33.458	191886	0.06	57339	0.09	3.35		NONADECANE
66	33.687	33.633	33.733	128644	0.04	40867	0.07	3.15		1,6,10-DODECATRIEN-3-OL, 3,
67	36.157	36.083	36.283	837732	0.27	182711	0.30	4.59		.gamma.-Tocopherol
68	38.026	37.883	38.175	16728084	5.46	3233394	5.25	5.17		.alpha.-Tocopherol- beta -D-mann
69	38.246	38.175	38.417	852462	0.28	112087	0.18	7.61	V	.alpha.-Tocopherolquinone
				306527590	100.00	61618236	100.00			

Figure 1: GC-MS spectrum and validation data of identified compounds of *Amaranthus hypochondriacus* L., Accession no. IC94661.

DISCUSSION

Here we have assessed the neutraceutical value of a promising accession of *A. hypochondriacus* through assessment of important antioxidant related attributes or important biomarker for antioxidant potential. In fact, the neutraceutical and medicinal properties of the plants have been explored in the existing scientific progress across the world on the basis of availability of compounds with antioxidant properties with no side effect and economic viability [7, 24-26]. Among these naturally occurring antioxidants, phenolics and other hydroxyl containing phytochemicals deserve special attention for its unique ability to scavenge ROS [12, 27, 28]. These compounds are important constituents of the plants and their radical scavenging ability is due to their –OH groups [27-29]. Another significant mechanism through which phenolic compound exerts their functional antioxidant purpose is via metal chelation and restriction of accessibility of transition type of metal ions for participation in Fenton type reactions, necessary for the production of the most potent ROS hydroxyl radical [28].

Since, due to extremely complex nature of phytochemicals, it is not wise to use a single method for evaluating antioxidant potential of plant extract [30, 31], we have used four different biomarkers like $OH^{\cdot}$ radical scavenging property, metal chelating activity, reducing property and anti-lipid peroxidation property to validate the neutraceutical property of the leaf extracts from the experimental accession of *A. hypochondriacus*. Based on that, our result unequivocally identified the experimental accession green accession IC94661 rich in its neutraceutical value and may be projected as a potential subsidiary food crop.

Phenolic acids, flavonoids, tannins and other hydroxyl rich compounds might be held responsible as the basis for the better metal chelating, $OH^{\cdot}$ radical scavenging and reducing properties [20, 31-34]. Significantly superior capacity of leaf extract of the experimental accession IC94661 to scavenge excess ROS and restore redox homeostasis is directly related to inhibition of lipid peroxidation and prevention of hydroxyl radical formation through metal chelation apart from basic chain breaking free radical reactions [20, 33, 35]. In fact, metal chelating, $OH^{\cdot}$ radical scavenging and reducing properties of the tissue extracts mainly reflect their non-enzymatic antioxidant competence [33, 35, 36-38].

Additionally, to substantiate the data of quantitative antioxidant competence of leaf extract, GC-MS based method was used for assessing the availability of –OH phenolic compounds present in the ethanolic leaf extract of the experimental accession. The results indicated presence of several hydroxyl rich phytochemical constituents from ethanolic extract of foliar tissue of the experimental accession. Several which contain hydroxyls are found to be present in the accession, which might be responsible for better radical scavenging effects and antioxidant potentials of the accession no. IC94661, corroborating well with the data of functional antioxidant potential [32, 34]. Since these hydroxyl-containing phytochemicals act as a free radical terminators, exhibiting medicinal activity as well as important physiological function, the presence of these phytochemicals in the accession IC94661 is a significant finding of the present study. The presence of those phytochemicals with -OH groups as well as their qualitative diversified nature is also a noteworthy outcome in the present study. Moreover, the method of extraction of hydroxyl rich phenolics from mature leaf tissues of the experimental accessions of *A. hypochondriacus* for GC-MS analysis is simple, rapid and sensitive and can be exploited efficiently for the identification of active principles in herbs for pharmaceutical and food industry. It is evident from the validation table (showing 13 major compounds) that all GC-MS separated fractions have complex chemical composition, in spite of the fact that some peaks remain unidentified because of lack of library data of the corresponding compounds. Most of these phytochemicals are rich in -OH and may therefore be used as terminators of free radical chain reaction or may chelate transition metal ions required for Fenton type reaction for the generation of more toxic ROS [27, 28, 32].

CONCLUSION

The knowledge of the rich foliar antioxidant potential (assessed in terms of anti-lipid peroxidation property, reducing power, metal chelating activity, hydroxyl radical scavenging property, anti-diabetic factor) of the pseudocereal *A. hypochondriacus* L. (accession no. IC94661) will maximize the effective and practical utilization of specific amaranth germplasm. GC-MS based identification of several hydroxyl-rich phytochemical having antioxidant properties (phenolic compounds) in ethanolic leaf extracts of the green experimental accession is also a novel finding of the present study.

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