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Original Article

Metabolite Fingerprinting and Antioxidant Potential of Tartary Buckwheat- an Underutilized Pseudocereal crop from Kashmir Region

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ABSTRACT

Objective: Buckwheat is an underutilized pseudocereal crop used as a staple food especially in Himalayan regions. The aim of the present study was to evaluate the phytochemical screening, antioxidant potential and metabolite profiling of tartary buckwheat extract. **Methods:** Tartary buckwheat leaf samples were tested for total phenols, flavonoids and *in vitro* antioxidant potential in terms of total antioxidant activity, free radical scavenging (superoxide, hydrogen peroxide) and DPPH assay. GC-MS profiling was done to identify and quantify the various metabolites from the methanolic leaf and groat extract. **Results:** Preliminary phytochemical screening of methanolic extract revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, phlobatannins, coumarins, glycosides and anthoquinones. Methanolic extract exhibits higher TPC (28.32 ± 5.31 mg gallic acid equivalent g^{-1} DW) and TFC (25.18 ± 3.5 mg rutin equivalent g^{-1} DW). DPPH radical (EC_{50}=1.8 µg ml^{-1}) and H_2O_2 scavenging (EC_{50}=0.103 µg ml^{-1}) potential of tartary buckwheat leaf extract shows promising results. From GC-MS metabolite fingerprinting, over 111 and 24 metabolites were identified among leaf and groat extract respectively. The major compounds present in the extracts were fatty acids, hydrocarbons, steroids, terpenoids, esters, organic acids and aldehydes with excellent pharmaceutical properties. **Conclusion:** The tartary buckwheat extract were found to contain numerous metabolites with potent antioxidant and other pharmacological actions. Thus, tartary buckwheat could be a promising alternative in functional food sector to improve social well-being and neutraceutical to diminish malnutrition especially for the impoverished community.

Key words: Antioxidants, Metabolite fingerprinting, Tartary buckwheat, GC-MS, DPPH, FRAP.

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INTRODUCTION

Generation of free radicals in the form of active oxygen species (AOS) in biological system is a normal phenomenon. These AOS include; superoxide anions (O^{-}), hydrogen peroxide (H_2O_2), hydroxyl radicles (OH^{-}) and singlet oxygen ($_2O^1$).[1] Previously, AOS were considered as dangerous molecules which must be maintained at low levels in cells. However, this perception has been changed because these serve as important signaling molecules.[2] Sometimes these free radicles are produced to such an extent that the body's defence system is not able to expel them out and thus leads to oxidative stress.[3] Under such conditions these AOS cause damage to various cell organelles, cell death, DNA damage and gene mutation which often leads to chronic ailments like neurodegenerative diseases, cardiovascular dysfunctions, aging, weakening of immune system.[4] Earlier reports suggests that there exists strong association between dietary intake of these natural products and the disease prevention and such wonderful properties of these botanicals is due to the presence of secondary metabolites with healthcare properties.[5,6] Natural antioxidants are interesting green alternatives to artificial antioxidants because of the safety concerns and limitation of usage. Plants contain plethora of secondary metabolites (e.g, flavonoids, glycosides, terpenoids, tannins etc) with potential antioxidant properties and have an immense potential in pharmaceutical and food sectors.[7] Isolation and structural analysis of these secondary metabolites from medicinal plants is a main thrust of natural product chemistry to identify and evaluate their therapeutic potential. GC-MS is a robust approach for the qualitative and quantative analysis of metabolites of plant origin.[8]

Fagopyrum tataricum (tartary buckwheat) - a dicot pseudocereal belongs to family Polygonaceae is a potential candidate due to its high neutraceutical properties. It is the only pseudocereal that contains a well-known glycoside "rutin".[9] Rutin is known to serve as anti-hypertensive, anti-inflammatory, anti-carcinogenic and vasoconstrictive.[10] Other essential bioactive constituents of tartary buckwheat are phenols, fagopyrins, fagopyritols, resistant starch, dietary fibre, vitamins and lignans.[11] Buckwheat has attributed worldwide attention, especially from food scientists for its healthy effects over chronic diseases. In developing countries like India, majority of the population relay on traditional system of medicine besides due to the population explosion the current food production is not sufficient to cater the food crises so, it is the need of the hour to explore food crops that possess nutritional and medicine value. In view of the above facts, the current study was focussed to evaluate the phytochemical screening and the antioxidant potential of tartary buckwheat extracts by using various assays like FRAP, DPPH, RP, SOD, TPC and TFC. Besides, we performed metabolite profiling by GC-MS to identify and quantify the essential metabolites present in the extract of tartary buckwheat.

MATERIALS AND METHODS

Plant material

Seeds of *Fagopyrum tataricum* (buckwheat) were procured from Department of Botany, University of Kashmir, Hazratbal, Srinagar. Later these seeds were sown during the month of April-2014 in the Botanical garden of

Kashmir University. Harvesting of the leaf sample was done at the pre-flowering stage.

Collection and preparation of sample material

Fresh and healthy leaves of tartary buckwheat were collected and washed gently with distilled water (without squeezing) to remove debris and dust particles. The plant material is then air-dried under shade at room temperature for 15 days and ground into a powdered form using a surface sterilized mortar and pestle which was further used for extraction.

Solvent extraction procedure

Preparation of leaf extract was done in methanol and ethanol solvents following the protocol of Okogun.[12]

Phytochemical screening

Phytochemical analysis for antioxidants was done following the method of Bruneton.[13]

Estimation of TPC and TFC

The TPC was estimated by Folin-Ciocalteau reagent following the method of Malick and Singh.[14] TFC were investigated by a method described by Hung and Morita.[15] A gallic acid standard (R^2=0.998) was used to determine the TPC. For the determination of TFC, rutin was used as standard (R^2=0.99).

Antioxidant assays

Total reducing power

The reducing activity of the extracts was determined followed the protocol of Yen and Duh.[16]

Ferric Reducing Antioxidant Potential–FRAP assay.

FRAP assay was done using a modified protocol of Benzie and Strain[17] based on color (blue) development due to the reduction of the ferric iron (Fe^{3+}) to ferrous form(Fe^{2+}).

Superoxide radical scavenging activity

Superoxide radical scavenging activity of the leaf extracts was determined following the method of Fontana *et al*.[18]

H_2O_2 radical scavenging activity

The scavenging activity of the extracts towards hydrogen peroxide radicals was determined by the method of Ebrahimzadeh *et al*.[19]

DPPH assay

DPPH activity was measured by determining the hydrogen donating or radical scavenging ability of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical followed the method of Braca *et al*.[20]

Metabolite fingerprinting

Sample preparation for GC-MS analysis

The 0.2 g of dried extract powder of tartary buckwheat leaf and groat samples were dissolved in 10 ml of methanol solvent properly mixed and kept for 72 hrs, then filtered through 0.45 µm syringe filter (Millipore Corp., Bedford, MA, USA). 1 µl aliquot of the sample was then injected into the GC-MS port for the metabolite analysis (Shimadzu QP-2010 Plus with Thermal Desorption System TD 20). GC-MS analysis was performed according to the method of Dhar *et al*.[21]

Statistical analysis

Results are presented as mean ± standard deviation (SD) of three replicates. Data were subjected to analysis of variance using Graph pad prism 6.07 software and was considered significant at $p \leq 0.05$. IC_{50} values were calculated by using linear regression plots.

RESULTS

Phytochemical screening

Qualitative phytochemical analysis of tartary buckwheat leaf extract shows the presence of coumarins, alkaloids, flavonoids, phenols, tannins, saponins, phlobatannins, glycosides and anthoquinones (Table 1).

Total phenol and flavonoid contents

The present investigation reports that the total phenol content was high in the methanolic extract (28.32 ± 5.31 mg GAE g^{-1} dry powder) as compared to ethanolic extract (25.64 ± 3.41 mg GAE g^{-1} dry powder). Total flavonoid content follows the similar trend, methanolic extract contains 25.18 ± 3.5 mg RE g^{-1} dry fraction as compared to ethanolic extract (19.3 ± 2.7 mg RE g^{-1} dry fraction) (Figure 1A, B).

Antioxidant assays

Reducing power

The present result shows that reducing power increase in a concentration-dependent manner and is highest in the methanolic extract (0.50 ± 0.11 µg/ml) as compared to ethanolic extract (0.432 ± 0.2 µg/ml) (Figure 1C). The antioxidant present in the extract donates an electron to stabilize the radicals and also causes chain termination.[22] The capability of extract to exhibit the reducing power in this study may be due to the presence of antioxidant metabolites.

FRAP assay

The total antioxidant potential of the botanical extracts was calculated from their capability to reduce TPZR-Fe (III) complex to TPTZ-Fe (II). FRAP assay is a cost-effective approach and has become a valuable protocol to measure total antioxidant/ reducing power of the extract. In our study, the total antioxidant potential was higher in the methanolic extract (375.75 ± 36.74 µmol Fe-II/g DW) at 50 µg/ml concentration as compared to ethanolic extract (365.20 ± 40.12 µmol Fe-II/g DW) that signifies its high antioxidant potential (Figure 1D).

Superoxide radicle scavenging activity

Tartary buckwheat leaf extract shows a superoxide radicle scavenging activity ina dose-dependent manner (Figure 1E). Methanolic extract exhibits highest activity (88.05 ± 16.44 % inhibition) at 50 µg/ml concentration as compared to ethanolic extract (78.81 ± 15.76 % inhibition) over the same concentration. Besides, methanolic extract exhibits lowest EC_{50} value (EC_{50}=5.86 µg/ml) which means that the metabolites present in the methanolic extract are potent scavengers of $O_2^{\cdot-}$ radicles at low concentration.

Hydrogen peroxide radical scavenging activity

From the results, the H_2O_2 scavenging activity increases with increase in concentration of extract and are high in methanolic extract (98.59%) at 50 µg/ml concentration as compared to ethanolic extract (85%) over the same concentration (Figure 1F). The EC_{50} value of methanolic extract was found to be 0.103 µg/ml compared to ethanolic extract (24.54 µg/ml). The strong H_2O_2 scavenging activity of the buckwheat leaf extract may be due to the presence of bioactive constituentssuch as phenolic compounds and other metabolites (tannins, anthocyanins etc) which donates electron to H_2O_2 radicles thus neutralizing their effect.[23]

DPPH radicle scavenging activity

DPPH-radicle scavenging activity of tartary buckwheat is presented in Figure 1G. The results shows DPPH radicle scavenging activity of both the extracts was found to enhance in a dose-dependent manner. The methanolic extract shows high activity (93.84 ± 14.21) at the concentration of 40 µg/ml as compared to ethanolic extract (78.38 ± 12.11) over the same concentration. A higher DPPH radicle scavenging activity is linked with

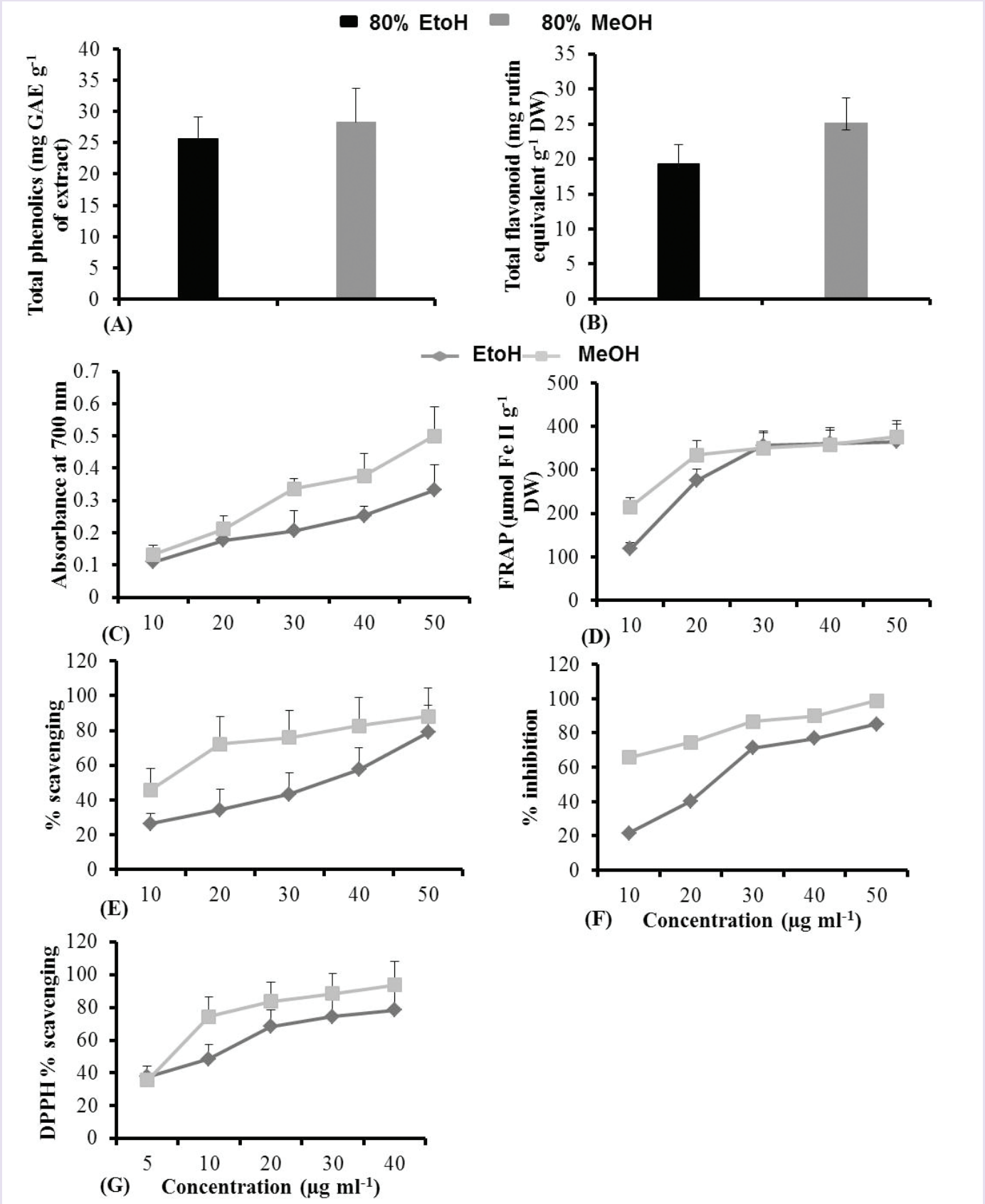

Figure 1: Estimation of total phenol (A), flavonoid content (B), reducing power (C), FRAP assay (D), SOD activity (E), H_2O_2 scavenging activity (F) and DPPH radicle scavenging activity (G) of methanolic and ethanolic leaf extract of tartary buckwheat. Data represents mean ± SD (n = 3). Significant at P<0.05.

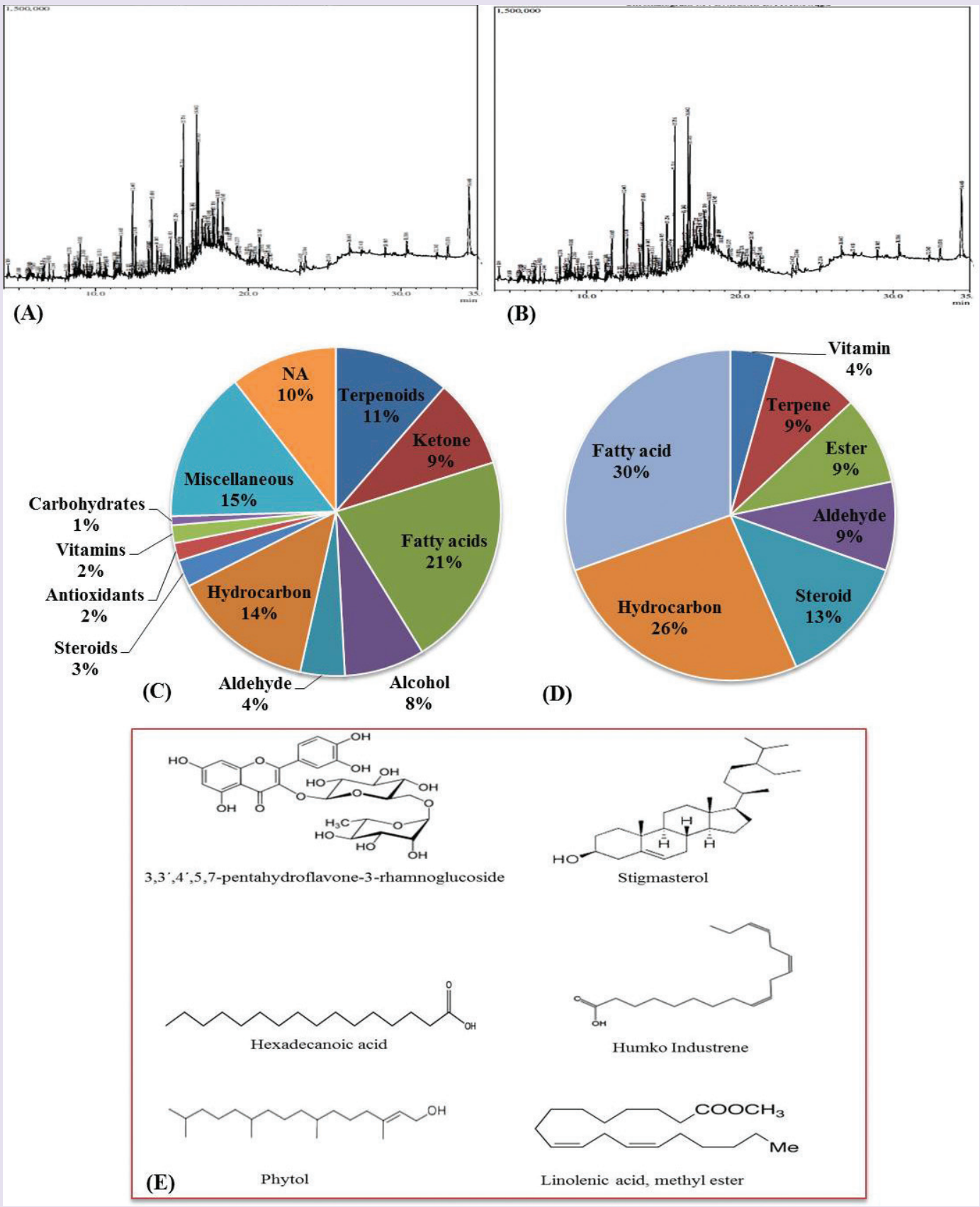

Figure 2: Shows GC-MS chromatograms (A, B), major phyto-chemotypes (C, D) and structure of major metabolics (E) of tartary buckwheat methanolic leaf and groat extract respectively.

a lower EC_{50} value (EC_{50}=1.8 µg/ml for methanolic extract; EC_{50}=11.13 µg/ml for ethanolic extract).

Metabolite fingerprinting

GC-MS chromatogram of methanolic extract of tartary buckwheat as per the aforementioned protocol exhibits several peaks indicating the presence of different metabolites in the extract (Figure 2A, B). The leaf and groat methanolic extract of tartary buckwheat revealed the presence of 111 (Table 2 Supplementary file) and 24 (Table 3) different metabolites respectively that were later characterized and identified using NIST library database. A representation of the chemical profile by groups of compounds is shown in Figure 2C, D. The major metabolites (> 3%) in the leaf extract were found to be 2-propenoic acid, tridecyl ester (4.47%), 5-Methyl-1-phenylbicycloheptane (3.69%), Linolenic acid, methyl ester (6.71%), phytol (5.22%) and stigmast-5-en-3-ol (9.81%) while as in the groat extract, the major metabolites found are, 3,3´,4´,5,7-pentahydroflavone-3-rhamnoglucoside (71.94%), n-Hexadecanoic acid (17.48%), Humko Industrene (5.20%) etc along with other metabolites also being reported (Figure 2E). Among flavonoids, rutin was found to be the major metabolite found in the groat. The major organic compounds present in the extracts were in the order of fatty acids > hydrocarbons > steroids > terpenoids > esters > organic acids > aldehydes > vitamins.

DISCUSSION

Phytochemical analysis is an essential parameter which provides basic information regarding medicinal importance of the plant extract. In the present investigation, phytochemical screening of chemical constituents of tartary buckwheat exhibits the presence of distinct metabolites such as coumarins, alkaloids, flavonoids, phenols, tannins, saponins, phlobatannins, glycosides and anthoquinones. Our results corroborates with earlier reports.[24] In the present study, the qualitative and quantitative estimation of total phenolic and flavonoid contents of tartary buckwheat revealed that methanolic extract exhibits the highest concentration of total phenol and flavonoid content. Our results are in accordance with earlier reports where total flavonoid and phenolic content are found in higher amount in different parts.[25,24] It has been reported that plants rich in phenolic and flavonoids could be a potential source of therapeutics against oxidative stress.[26] Reducing power is often an indicator of antioxidant activity. In reducing power, antioxidants present in the extract cause reduction of Fe^{3+} to Fe^{2+} this in turn can be observed by measuring the formation of Prussian blue at λ=700 nm. It is known that increasing absorbance means increasing reducing ability.[27] The present study shows that methanolic extract of tartary buckwheat possesses high reducing power. It is believed that the antioxidant property is primarily due to the redox potential[28] that plays a significant role in scavenging the free radicals. Our results are supported by various authors.[29-31] Technically, FRAP assay is simple to determine the total antioxidant potential and is a proven quantitative approach to determine potential of various phyto-foods.[32] Present results revealed that both the extracts of tartary buckwheat shows higher FRAP value, however it was more pronounced in methanolic extract as compared to ethanolic extract. Our results are in accordance with earlier reports of Jeong *et al.*[33] PMS-NADPH oxidation reaction system generates the superoxide radicals ($O_2^{\cdot-}$) which can be determined by their ability to reduce NBT. In the presence of plant extract, the absorbance at 560nm decreases indicating the ability of the plant extract to scavenge the $O_2^{\cdot-}$ present in the reaction mixture. In the present study, the $O_2^{\cdot-}$ radical scavenging activity of different fractions was increased dose-dependently. Low levels of IC_{50} values suggested that the chemical constituents found in the methanolic extract and its fractions are potent scavengers of superoxide radicals at low concentration. Superoxide radicle is regarded as a precursor of hydroxyl radicle ($OH^{\cdot}$) that is more dangerous and causes lipid peroxidation thus damages the cell membrane and often leads to apoptosis. The presence of potent electron quenching activity of tartary buckwheat extract may be due to the presence of various secondary metabolites especially phenols and flavonoids.[24] H_2O_2 itself is not very toxic to cellular system but sometimes it becomes toxic as it is directly involved in the production of $OH^{\cdot}$ radicals.[34] In this study methanolic extract exhibits strong potential to scavenge the H_2O_2 potential hazard indicating the antioxidant capacity of the plant. Our results coincides with Liu *et al.*[35] who compares the antioxidant potential of tartary and common buckwheat and concluded that tartary buckwheat possesses potent radical scavenging activity. DPPH assay is a cost-effective procedure to evaluate the antioxidant potential of botanical extract, where DPPH is consumed as a stable free radical. In the present study, DPPH radical scavenging ability of the tartary buckwheat leaf extract increases in a concentration-dependent manner. IC_{50} value of methanolic extract for DPPH radical was found lower as compared to ethanolic extract. Our results are in accordance with the studies on *Phodopyllum hexandrum*[36] and *Acalypha manniana*.[37]

Metabolite profiling of tartary buckwheat leaf and groat methanolic extract was done by GC-MS and a single metabolic profile can be thought of as a snapshot of the metabolic state of an organism at a given moment. In medicinal chemistry, metabolite profiling is of paramount importance to ascertain the chemo-typing of natural products that will not only allow us to scientifically determine but validate their traditional uses, pharmacological activities and therapeutic potential.[38] From the present investigation, the methanolic leaf and groat extract of tartary buckwheat revealed the presence of 135 metabolites. Rutin (3, 3', 4', 5, 7-pentahydroflavone-3-rhamnoglucoside-71.94%) a major flavonoid of buckwheat found in the groat extract possesses desirable physiological and biological properties such as anti-hypertensive, anti-carcinogenic, vasoconstrictive, anti-inflammatory properties.[10,11] Rutin is known to keep capillaries and arteries strong and flexible, besides it acts as a shield against gastric lesions, improve eyesight and hearing, protects against UV-light, X-rays and oxidative stress,[39,40] lowers plasma cholesterol and also suppresses gallstone formation.[41] Phytol (5.22%) found in the leaf extract is having anticancer, antioxidant, antitumor, diuretic and chemopreventative properties and used in vaccine formulation.[42,43] The other metabolites such as linoleic acid ester, 9-octadecanoic acid (Z)-, methyl ester is also having anti-inflammatory, anti-androgenic and anemiagenic properties.[44] The metabolite profiling by GC-MS from different botanicals that possess various pharmacological properties have been studied earlier that supports our current study.[45,46] The metabolites identified during GC-MS profiling were further investigated for their biological properties using Dr. Duke's database[47] that revealed that tartary buckwheat possess immense pharmaceutical properties, as the identified

Table 1: Preliminary phytochemical screening of Methanolic and ethanolic extracts of buckwheat species

Tests for metabolites	Methanolic extract	Ethanolic extract
Alkaloids	++	+
Anthraquinones	++	+
Glycosides	+	+
Coumarins	++	+
Flavonoids	++	+
Saponins	+	+
Phlobatannins	+	+
Tannins	++	+
Terpenoids	++	+
Phenols	++	+

+, present; ++ strong influence.

Table 2: GC-MS chromatogram analysis of methanolic extract of tartary buckwheat

Peak ID	R. Time	Peak Area	Area (%)	Compound detected	Major group	Hit	SI	Ret Index	CAS No	Mol. Formula	Mol. Wt.
1	4.309	138620	0.66	Linalool	Terpenoid	1	89	1082	78-70-6	$C_{10}H_{18}O$	154
2	5.009	32647	0.15	2,6-Dimethyl-6-nitro-2-hepten-4-one	Ketone	1	81	1334	73583-56-9	$C_9H_{15}NO_3$	185
3	5.536	22622	0.11	Pentanedioic acid, 2,4-dimethyl-, dimethyl ester	Fatty acid	1	75	1122	2121-68-8	$C_9H_{16}O_4$	188
4	5.607	26342	0.12	4-terpineol	Terpenoid	1	79	0	562-74-3	$C_{10}H_{18}O$	154
5	5.703	115188	0.55	3-Dodecanol	Alcohol	1	85	1376	10203-30-2	$C_{12}H_{26}O$	186
6	5.79	62722	0.3	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin		1	74	1507	108511-85-9	$C_{13}H_{22}O_3$	226
7	5.947	24854	0.12	NA	NA	NA	NA	NA	NA	NA	NA
8	6.248	45907	0.22	Cyclopentane, 2-methyl-1-methylene-3-(1-methylethenyl)-		1	75	954	56710-83-9	$C_{10}H_{16}$	136
9	6.506	36855	0.17	NA	NA	NA	NA	NA	NA	NA	NA
10	6.578	110288	0.52	Cuminal	Aldehyde	1	87	1230	122-03-2	$C_{10}H_{12}O$	148
11	6.674	136250	0.64	Anthranilic acid linyl ester	Fatty acid	1	91	2157	7149-26-0	$C_{17}H_{23}NO_2$	273
12	6.98	169779	0.8	6-OCTEN-1-OL, 3,7-DIMETHYL-, FORMATE	Fatty acid	1	92	0	105-85-1	$C_{11}H_{20}O_2$	184
13	7.244	85284	0.4	PHENOL, 2-METHYL-5-(1-METHYLETHYL)-	Terpenoid	1	83	0	499-75-2	$C_{10}H_{14}O$	150
14	8.109	55476	0.26	Natural Rhodinol acetylated	Terpenoid	1	89	1302	150-84-5	$C_{12}H_{22}O_2$	198
15	8.278	236761	1.12	PHENOL, 2-METHOXY-4-(2-PROPENYL)-	Terpenoid	1	92	0	97-53-0	$C_{10}H_{12}O_2$	164
16	8.608	21497	0.1	alpha-cubebene	Terpenoid	1	79	0	17699-14-8	$C_{15}H_{24}$	204
17	8.647	35193	0.17	NA						$C_{15}H_{24}$	
18	8.753	174301	0.82	1-IODO-2-METHYLUNDECANE	Hydrocarbon	1	84	0	73105-67-6	$C_{12}H_{25}I$	296
19	8.867	28484	0.13	Oxalic acid, butyl 6-ethyloct-3-yl ester	Fatty acid	1	73	1818	0-00-0	$C_{16}H_{30}O_4$	286
20	8.915	121168	0.57	AIDS-003250	Aldehyde	1	93	0	124-25-4	$C_{14}H_{28}O$	212
21	9.01	296582	1.4	1H-CYCLOPROP[E]AZULENE,		1	91	0	489-40-7	$C_{15}H_{24}$	204
22	9.26	111494	0.53	Caryophyllene	Terpenoid	1	86	1494	87-44-5	$C_{15}H_{24}$	204
23	9.446	126752	0.6	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-,		1	87	1403	17334-55-3	$C_{15}H_{24}$	204
24	9.531	33099	0.16	NA						$C_{15}H_{24}$	
25	9.63	24407	0.12	NA							
26	9.708	37015	0.18	NA							
27	9.777	35465	0.17	Alcohol C-11	Alcohol	1	93	0	112-42-5	$C_{11}H_{24}O$	172

Continued..

28	10.224	26020	0.12	NA	NA	NA	NA	NA	NA	NA
29	10.311	137677	0.65	Phenol, 3,5-bis(1,1-dimethylethyl)-	Terpenoid	1	90	1555	1138-52-9	NA	206
30	10.523	35634	0.17	1-Bicyclo[3.3.1]non-6-en-3-yl-2-methylpropan-1-one	Ketone	1	72	1395	103258-73-7	$C_{13}H_{20}O$	192
31	10.67	126987	0.6	1-Undecanol	Alcohol	1	93	0	112-42-5	$C_{11}H_{24}O$	172
32	10.743	58622	0.28	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	Ketone	1	81	1426	15356-74-8	$C_{11}H_{16}O_2$	180
33	11.248	136944	0.65	1-Pentadecene	Hydrocarbon	1	95	1502	13360-61-7	$C_{15}H_{30}$	210
34	11.326	71915	0.34	1-CHLOROHEXADECANE	Hydrocarbon	1	77	0	01-03-4860	$C_{16}H_{33}Cl$	260

Table 2: Cont'd

Peak ID	R. Time	Peak Area	Area (%)	Compound detected	Major group	Hit	SI	Ret Index	CAS No	Mol. Formula	Mol. Wt.
35	11.4	45377	0.21	Tert-amyl bromide	Hydrocarbon	1	71	730	507-36-8	$C_5H_{11}Br$	150
36	11.472	55289	0.26	Fumaric acid, octyl 3-phenylpropyl ester	Fatty acid	1	75	2528	0-00-0	$C_{21}H_{30}O_4$	346
37	11.559	77124	0.36	3A(1H)-AZULENOL, 2,3,4,5,8,8A-HEXAHYDRO-6,8A-DIMETHYL-3-(1-METHYLETHYL[3R-(3. ALPHA.,3A.ALPHA.,8A.ALPHA.)]-	Alcohol	1	85	0	465-28-1	$C_{15}H_{26}O$	222
38	11.665	325932	1.54	Isopropyl laurate	Fatty acid	1	94	1615	10233-13-3	$C_{15}H_{30}O_2$	242
39	12.207	29628	0.14	BICYCLO[3.1.1]HEPT-2-ENE-2-METHANOL, 6,6-DIMETHYL-	Alcohol	1	61	0	515-00-4	$C_{10}H_{16}O$	152
40	12.384	28350	0.13	Triplal 1 (IFF)	Aldehyde	1	73	0	0-00-0	$C_9H_{14}O$	138
41	12.443	944473	4.47	Tridecyl acrylate	Fatty acid	1	92	1769	08-04-3076	$C_{16}H_{30}O_2$	254
42	12.638	501088	2.37	5,9-Tetradecadienedioic acid, 5,6,9,10-tetramethyl-, dimethyl ester	Fatty acid	1	67	2268	0-00-0	$C_{20}H_{34}O_4$	338
43	12.774	48228	0.23	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	Ketone	1	73	1673	0-00-0	$C_{15}H_{22}O$	218
44	12.917	59514	0.28	BENZENE, (3-NITROPROPYL)-	Hydrocarbon	1	73	0	22818-69-5	$C_9H_{11}NO_2$	165
45	13.027	40797	0.19	2-THIA-ADAMANTANE-4,8-DIOL	Alcohol	1	64	0	0-00-0	$C_9H_{14}O_2S$	186
46	13.167	9882	0.05	NA	NA	NA	NA	NA	NA	NA	NA
47	13.33	46711	0.22	NA	NA	NA	NA	NA	NA	NA	NA
48	13.487	153908	0.73	2(4H)-BENZOFURANONE, 5,6,7,7A-TETRAHYDRO-6-HYDROXY-4,4,7A-TRIMETHYL-	Ketone	1	86	0	06-02-5989	$C_{11}H_{16}O_3$	196
49	13.526	78013	0.37	1-Heptadecene	Hydrocarbon	1	94	1701	6765-39-5	$C_{17}H_{34}$	238
50	13.66	212541	1.01	Cyclopropaneacrylic acid, 3-carboxy-.alpha.,2,2-trimethyl-, dimethyl ester, trans-(+)-	Fatty acid	1	59	1433	22467-83-0	$C_{12}H_{18}O_4$	226
51	13.698	399752	1.89	2-BUTENSAEURE, 4-(1-METHOXYCARBONYLETHYL)-CYCLOPROPYL	Fatty acid	3	57	0	0-00-0	$C_{12}H_{18}O_4$	226
52	13.967	15933	0.08	NA	NA	NA	NA	NA	NA	NA	NA
53	14.045	207424	0.98	Neophytadiene	Fatty acid	1	92	0	504-96-1	$C_{20}H_{38}$	278
54	14.185	32805	0.16	4,4-DIMETHYL-3-(3-METHYL-2-BUTENYLIDENE)-2,7-OCTANEDIONE	Ketone	1	72	0	0-00-0	$C_{15}H_{24}O_2$	236

Continued..

55	14.311	211415	1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Alcohol	1	68	2045	102608-53-7	$C_{20}H_{40}O$	296
56	14.426	26765	0.13	Isopentyl picolinic acid	Fatty acid	1	77	1591	49751-50-0	$C_{11}H_{15}NO_2$	193
57	14.489	83523	0.4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Alcohol	1	89	2045	102608-53-7	$C_{20}H_{40}O$	296
58	14.632	32865	0.16	HEXADECANE	Hydrocarbon	1	82	0	544-76-3	$C_{16}H_{34}$	226
59	14.758	27295	0.13	Ketone, methyl 2,4,5-trimethylpyrrol-3-yl	Ketone	1	72	1303	19005-95-9	$C_9H_{13}NO$	151
60	14.825	20333	0.1	2-phenyltridecane	Hydrocarbon	1	88	0	4534-53-6	$C_{19}H_{32}$	260
61	14.915	343511	1.63	Metholene-2216	Fatty acid	1	90	1878	112-39-0	$C_{17}H_{34}O_2$	270
62	15.254	587129	2.78	Morfax		1	67	2400	95-32-9	$C_{11}H_{12}N_2OS_3$	284

Table 2: Cont'd

Peak ID	R. Time	Peak Area	Area (%)	Compound detected	Major group	Hit	SI	Ret Index	CAS No	Mol. Formula	Mol. Wt.
63	15.393	207781	0.98	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1-methyl-, methyl ester [1S-(1.alpha.,4a.alpha.,10a.beta.)]-	Fatty acid	1	60	1950	57378-56-0	$C_{17}H_{22}O_2$	258
64	15.45	13552	0.06	Desoxyisosteviol semicarbazone	Terpenoid	1	61	2979	0-00-0	$C_{20}H_{31}N_3O_3$	361
65	15.565	196325	0.93	1-Nonadecene	Hydrocarbon	1	95	1900	18435-45-5	$C_{19}H_{38}$	266
66	15.716	534841	2.53	1-METHYL-5-PHENYLBICYCLO[3.2.0]HEPTANE	Hydrocarbon	1	72	0	0-00-0	$C_{14}H_{18}$	186
67	15.756	779975	3.69	5-Methyl-1-phenylbicyclo[3.2.0]heptane	Hydrocarbon	2	72	1462	0-00-0	$C_{14}H_{18}$	186
68	15.981	142581	0.67	1-Pentadecyne	Hydrocarbon	1	82	1510	765-13-9	$C_{15}H_{28}$	208
69	16.18	31818	0.15	NA	NA	NA	NA	NA	NA	NA	NA
70	16.276	40182	0.19	2-methyl quinoline	Terpenoid	1	79	0	91-63-4	$C_{10}H_9N$	143
71	16.34	486746	2.3	1-METHYL-5-PHENYLBICYCLO[3.2.0]HEPTANE	Hydrocarbon	2	70	0	0-00-0	$C_{14}H_{18}$	186
72	16.432	286130	1.35	13-Hexyloxacyclotridec-10-en-2-one	Ketone	1	91	2325	127062-51-5	$C_{18}H_{32}O_2$	280
73	16.572	304292	1.44	9,12-octadecatrienoic acid, methyl ester	Fatty acid	1	90	2093	2462-85-3	$C_{19}H_{34}O_2$	294
74	16.642	1417396	6.71	Linolenic acid, methyl ester	Fatty acid	1	92	2101	301-00-8	$C_{19}H_{32}O_2$	292
75	16.743	1103503	5.22	Phytol	Terpenoid	1	93	2045	150-86-7	$C_{20}H_{40}O$	296
76	16.99	400537	1.9	(Z,Z)-6,9-CIS-3,4-EPOXY-NONADECADIENE		1	87	0	0-00-0	$C_{19}H_{34}O$	278
77	17.06	19052	0.09	Bicyclo[3.2.2]non-2-ene, 2-phenyl-		1	60	1627	0-00-0	$C_{15}H_{18}$	198
78	17.207	170397	0.81	4,5-Diphenylocta-1,7-diene(dl)		1	72	2014	0-00-0	$C_{20}H_{22}$	262
79	17.254	65750	0.31	Bicyclo[3.2.2]non-2-ene, 2-phenyl-		1	64	1627	0-00-0	$C_{15}H_{18}$	198
80	17.418	142319	0.67	1-Nonadecene	Hydrocarbon	1	96	1900	18435-45-5	$C_{19}H_{38}$	266
81	17.483	147977	0.7	4,5-Diphenylocta-1,7-diene(dl)		1	72	2014	0-00-0	$C_{20}H_{22}$	262
82	17.566	58817	0.28	Nonadecyl acetate		1	92	2276	0-00-0	$C_{21}H_{42}O_2$	326
83	17.709	389793	1.84	2(5H)-Oxepinone, 3-tert-butyl-7-phenyl-	Ketone	1	73	1996	0-00-0	$C_{16}H_{18}O_2$	242
84	17.785	370671	1.75	Benzoic acid N'-(2,2,7,7-tetramethyl-tetrahydro-bis[1,3]dioxolo[4,5-b;4',5'-d]pyran-5-carbonyl)-hydrazide	Fatty acid	1	57	3091	0-00-0	$C_{19}H_{24}N_2O_7$	392
85	18.007	476171	2.25	6-Benzoyl-6-ethyl-4,4-dimethyl-2-oxo-3-oxa-bicyclo[3.1.0]hexane-1-carboxylic acid ethyl ester		1	70	2464	0-00-0	$C_{19}H_{22}O_5$	330

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86	18.115	23503	0.11	Citric acid, trimethyl ester	Fatty acid	1	57	1509	1587-20-8	$C_9H_{14}O_7$	234
87	18.225	127361	0.6	Oxalic acid, cyclobutyl dodecyl ester	Fatty acid	1	87	2168	0-00-0	$C_{18}H_{32}O_4$	312
88	18.345	532693	2.52	2,6-Dimethyl-4-nitro-3-phenyl-cyclohexanone	Ketone	1	68	1991	101328-12-5	$C_{14}H_{17}NO_3$	247
89	18.604	60894	0.29	SPIRO[2,5-METHANO-1-BENZOXEPIN-10,2'-OXIRANE], TRICHOTHEC-9-EN-8-ONE DERIV		1	55	0	51481-10-8	$C_{15}H_{20}O_6$	296
90	18.822	47304	0.22	Ethyl 2-benzamido-2-[(3-ethyl-3-oxetanyl)methoxy]-3,3,3-trifluoropropionate	Fatty acid	1	67	2365	339352-46-4	$C_{18}H_{22}F_3NO_5$	389
91	19.275	95122	0.45	1-Nonadecene	Hydrocarbon	1	90	1900	18435-45-5	$C_{19}H_{38}$	266
92	19.461	28724	0.14	D-galactitol-1-thioheptyl	Carbohydrate	1	63	0	0-00-0	$C_{13}H_{28}O_5S$	296

Table 2: Cont'd

Peak ID	R. Time	Peak Area	Area (%)	Compound detected	Major group	Hit	SI	Ret Index	CAS No	Mol. Formula	Mol. Wt.
93	19.95	72390	0.34	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-		1	68	3350	0-00-0	$C_{30}H5_2O_2$	444
94	20.128	164450	0.78	8-Hexadecenal, 14-methyl-, (Z)-	Aldehyde	1	77	1843	60609-53-2	$C_{17}H_{32}O$	252
95	20.3	52939	0.25	NA							
96	20.498	85796	0.41	Oxalic acid, 3,5-difluorophenyl tetradecyl ester	Fatty acid	1	75	2569	0-00-0	$C_{22}H_{32}F_2O_4$	398
97	20.745	428583	2.03	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Fatty acid	1	88	2498	23470-00-0	$C_{19}H_{38}O_4$	330
98	20.951	64607	0.31	ANTIOXIDANT 425	Antioxidant	1	72	0	0-00-0	$C_{25}H_{36}O_2$	368
99	21.219	114232	0.54	Cyclohexanecarboxylic acid, 3-phenylpropyl ester	Fatty acid	1	62	1919	70275-61-5	$C_{16}H_{22}O_2$	246
100	21.348	205863	0.97	1,2-BENZENEDICARBOXYLIC ACID	Fatty acid	1	92	0	117-81-7	$C_{24}H_{38}O_4$	390
101	21.487	25957	0.12	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-	Antioxidant	1	67	2987	88-24-4	$C_{25}H_{36}O_2$	368
102	23.435	223777	1.06	7-Tetradecenal, (Z)-	Aldehyde	1	78	1609	65128-96-3	$C_{14}H_{26}O$	210
103	23.744	308021	1.46	1-PENTACONTANOL	Alcohol	1	77	0	40710-43-8	$C_{50}H_{102}O$	718
104	25.274	34221	0.16	Adogen 73	Peptide	1	77	2228	301-02-0	$C_{18}H_{35}NO$	281
105	26.643	216190	1.02	DOTRIACONTANE	Acyclic Alkane	1	87	0	544-85-4	$C_{32}H_{66}$	450
106	27.41	29745	0.14	NA							
107	28.985	125496	0.59	.beta.-Tocopherol	Vitamin	1	82	3036	148-03-8	$C_{28}H_{48}O_2$	416
108	30.386	244929	1.16	dl-.alpha.-Tocopherol	Vitamin	1	88	3149	10191-41-0	$C_{29}H_{50}O_2$	430
109	32.343	77848	0.37	NA	NA	NA	NA	NA	NA	NA	NA
110	33.078	297213	1.41	Stigmasterol	Steroids	1	80	2739	83-48-7	$C_{29}H_{48}O$	412
111	34.469	2072122	9.81	STIGMAST-5-EN-3-OL, (3.BETA.)-	Steroids	1	90	0	83-46-5	$C_{29}H_{50}O$	414

Table 3: Chemo-metric profile of methanolic extract of tartary buckwheat groat

R. Time	Peak Area	Area (%)	Compound detected	Hit	SI	RI	CAS No.	Mol. Formula	Mol. Wt.
11.481	2582193	0.25	Butanoic acid 4-(Trimethylsilyl) oxy- Trimethylsilyl ester	1	70	0	55133-95-4	$C_{10}H_{24}O_3Si_2$	248
13.886	451651	0.04	Neophytadiene	1	91	0	504-96-1	$C_{20}H_{38}$	278
14.33	830534	0.08	Phthalic acid, butyl undecyl ester	1	83	2732	0-00-0	$C_{23}H_{36}O_4$	376
14.795	2131738	0.2	Palmitic acid, methyl ester	1	96	1878	112-39-0	$C_{17}H_{34}O_2$	270
15.349	181793266	17.48	n-Hexadecanoic acid	1	95	1968	57-10-3	$C_{16}H_{32}O_2$	256
16.487	10870431	1.05	Emery oleic acid ester 2301	1	94	2085	112-62-9	$C_{19}H_{36}O_2$	296
17.142	748276764	71.94	3,3′,4′,5,7-pentahydroflavone-3-rhamnoglucoside	1	90	2183	60-33-3	$C_{18}H_{32}O_2$	280
17.291	54111517	5.2	Humko Industrene	7	90	2167	57-11-4	$C_{18}H_{36}O_2$	284
18.713	5197954	0.5	Oleic Acid	1	92	2175	112-80-1	$C_{18}H_{34}O_2$	282
18.923	1731356	0.17	Eicosanoic acid	8	89	2366	506-30-9	$C_{20}H_{40}O_2$	312
19.543	6944741	0.67	Octadecanal	1	95	1999	638-66-4	$C_{18}H_{36}O$	268
20.277	1889036	0.18	Docosane	2	92	0	629-97-0	$C_{22}H_{46}$	310
21.122	1399060	0.13	Di-n-octyl phthalate	1	93	2832	117-84-0	$C_{24}H_{38}O_4$	390
22.306	3637248	0.35	Octadecanal	1	95	1999	638-66-4	$C_{18}H_{36}O$	268
23.353	1596835	0.15	Octacosane	2	92	2804	630-02-4	$C_{28}H_{58}$	394
24.692	888545	0.09	Eicosane	1	91	2009	112-95-8	$C_{20}H_{42}$	282
25.086	1111051	0.11	Squalene	1	95	2914	7683-64-9	$C_{30}H_{50}$	410
28.105	2623968	0.25	gamma.-Tocopherol	1	90	3036	7616-22-0	$C_{28}H_{48}O_2$	416
28.893	1085525	0.1	Stigmast-5-en-3-ol, (3.beta.,24S)-	1	83	0	83-47-6	$C_{29}H_{50}O$	414
31.385	1005075	0.1	Ergost-5-en-3-ol	1	90	0	0-00-0	$C_{28}H_{48}O$	400
33.41	7485646	0.72	gamma.-Sitosterol	1	94	2731	83-47-6	$C_{29}H_{50}O$	414

metabolites possess potent anti-diabetic, anti-hypertensive, antioxidant, anti-carcinogenic, hypocholesterolemic and 5-α-reductase inhibitor activity. Thus from the metabolite fingerprinting reveals that the tartary buckwheat can be used as an important source of neutraceutical food.

CONCLUSION

From the current study, it is concluded that tartary buckwheat possesses a potent antioxidant potential as revealed by its higher scavenging activity. Methanolic extract exhibits higher activity, thus could be the optimal solvent for the extraction of bioactive constituents. Metabolite fingerprinting of tartary buckwheat revealed its potential in the neutraceutical and functional food sector to diminish malnutrition and improve social well-being especially for the impoverished community.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION USED

AOS: Active oxygen species; **BHT:** Butylated hydroxytoluene; **DPPH:** 2, 2-diphenyl-1-picrylhydrazyl; **EDTA:** Ethylenediamine tetracetic acid; **EC:** Effective concentration; **FRAP:** Ferric Reducing Antioxidant Potential; **GC-MS:** Gas chromatography–mass spectrometry; **NADH:** Nicotinamide adenine dinucleotide; **NBT:** Nitro blue tetrazolium; **NIST:** National Institute of Standards and Technology; **PMS:** Phenazine methosulphate; **SOD:** Superoxide radical; **TBA:** Thiobarbituric acid; **TPC:** Total phenol content; **TFC:** Total flavonoid content; **TPTZ:** 2,4,6-tripyridyl-s-triazine.

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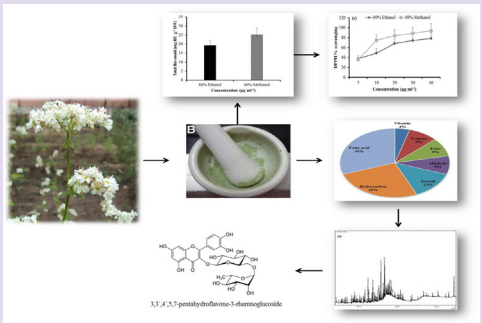
PICTORIAL ABSTRACT



SUMMARY

- Leaves of methanol extract of tartary buckwheat effectively scavenged free radicals in different models like hydrogen peroxide radical, antioxidant assay by inhibition of superoxide anion radical, 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH), total antioxidant activity (FRAP and reducing power) and reducing ability at different concentrations and showed its potent antioxidant activity.
- Further, GC-MS profiling of leaf and groat samples revealed the presence of many important secondary metabolites, thus specifies its significant role in the functional food sector.

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