Original Article

Journal of Phytology 2016, 8: 49-54
http://scienceflora.org/journals/index.php/jp/
doi: 10.19071/jp.2016.v8.3106

Metabolite profiling of tartary buckwheat - An underutilized neutraceutical crop of Kashmir Himalaya

Tanveer Bilal Pirzadah[1], Bisma Malik[1], Inayatullah Tahir[2], Reiaz Ul Rehman[1]*

[1]Department of Bioresources, University of Kashmir, Srinagar, Jammu and Kashmir, India, [2]Department of Botany, University of Kashmir, Srinagar, Jammu and Kashmir, India

Received: 18-08-2016
Accepted: 06-12-2016
Published: 15-12-2016

***Address for correspondence:**
Dr. Reiaz Ul Rehman, Department of Bioresources, University of Kashmir, Srinagar, Jammu and Kashmir, India.
Tel.: +91-9796546222.
E-mail: reiazrehman@yahoo.in

ABSTRACT

The aim of the present study was to explore the possible metabolites in the methanolic extract of root, stem, groat, and hull of the neutraceutical crop, *Fagopyrum tataricum* using gas chromatography–mass spectrometry (GC-MS) technique. From GC-MS metabolite profiling, over 90 different metabolites were identified among root, stem, groat, and hull extract. The most prevailing compounds were 3, 3', 4', 5, 7-pentahydroflavone-3-rhamnoglucoside (71.94%) in groat, 9, 12-octadecadienoic acid (49.38%) in root, 6-octadecanoic acid, a steric acid (70.46%) in hull and Cis-9-hexadecanal (13.38%) in stem. Present investigation reveals that *F. tataricum* is an excellent source of many metabolites such as fatty acids, hydrocarbons, steroids, terpenoids, esters, organic acids, and aldehydes with excellent pharmaceutical properties. These results suggest that tartary buckwheat could be a promising alternative in the functional food sector and neutraceutical to improve social well-being and diminish malnutrition.

KEY WORDS: Gas chromatography–mass spectrometry, metabolite profiling, tartary buckwheat

INTRODUCTION

Fagopyrum tataricum (tartary buckwheat) - A dicot pseudocereal belongs to family Polygonaceae is a potential candidate due to its high neutraceutical properties. Currently, buckwheat sprouts are used as a noval source of vegetables due to the presence of enormous neutraceutical properties (Liu *et al.,* 2008). In China, it is an old saying "People who love buckwheat live long" and "People who love buckwheat are healthy." In India, the flour prepared from buckwheat groats named as "kuttu ka atta" and is consumed by Hindus on particular fasting days, especially during "Navaratri, Ekadashi, Janamashthami, and Maha-Shivaratri." The studies on animal and humans have shown several health benefits, and thus it is being promoted as the functional food. It is the only pseudocereal that contains a well-known glycoside "rutin" (Jiang *et al.,* 2007). Rutin is known to serve as antihypertensive, anti-inflammatory, anti-carcinogenic, and vasoconstrictive (Landberg *et al.,* 2011; Sharma *et al.,* 2013). Buckwheat flour is gluten-free and is thus an important ingredient in diets or food products for people suffering from coeliac disease (Alvarez-Jubete *et al.,* 2010). Coeliac disease (also known as gluten-sensitive enteropathy) is a genetically determined disease of the small intestine linked with gluten intolerance. The buckwheat products are being produced for their medicinal properties such as "leaves" contain "antioxidants" is used for making tea, "groats" contain "fagopyritols" are used in soap industry. Other essential bioactive constituents of tartary buckwheat are phenols, fagopyrins, fagopyritols, resistant starch, dietary fiber, vitamins and lignans (Farooq *et al.,* 2015). 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. Gas chromatography–mass spectrometry (GC-MS) is a robust approach for the qualitative and quantitative analysis of metabolites of plant origin (Iordache *et al.,* 2009). In view of the above facts, the current study was focused to evaluate metabolite profiling by GC-MS to identify and quantify the phyto-chemotypes present in the extract of tartary buckwheat.

MATERIALS AND METHODS

Plant material

Seeds of *F. 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 Kashmir University botanical garden. Harvesting of the leaf sample was done at the pre-flowering stage.

Metabolite Profiling

Preparation of metabolite extracts

Plant material was harvested in triplicates for GC-MS analysis. 200 mg washed-blot dried leaf was frozen in liquid nitrogen and pulverized in chilled mortar pestle and derivatized as mentioned in Desbrosses *et al.,* (2005). Samples were extracted in chloroform/acetonitrile/acetone solvents. Samples were placed in water bath shaker at 37°C for 10 min. The samples were extracted twice from same samples and solvent was subsequently pooled. The solvents were then vacuum dried to concentrate metabolites. Samples for GC-MS analysis were prepared in methanol and filtered through 0.45 µm filter.

GC-MS analysis

Mass spectrometric analysis of buckwheat extracts was carried out on GC-MS (Shimadzu 2010, Japan) gas chromatograph fitted with an AB-Wax column. Helium was used as the carrier gas. Sample (2.5 µl) was injected in the splitless mode. The chemical components of the extract were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds using the NIST05s.LIB. The identified compounds were catalogued in the form of metabolite library and used for result interpretation.

RESULTS

Metabolite profiling was done among different parts of tartary buckwheat by GC-MS analysis. The gas chromatograms of root, stem, groat, and hull of tartary buckwheat confirmed the presence of various interesting compounds with different retention times as illustrated in Figure 1a-d. These compounds were identified through mass spectrometry attached with GC and by comparing their mass fragmentation patterns with those in the NIST 2005 (National Institute of Standards and Technology, Gaithersburg, Maryland) database library having more than 62,000 patterns. The identified compounds and their retention time, molecular formula, molecular weight, peak area (%), category of compound and activities related with medicinal uses are given in Table 1a-d for root, stem, groat, and hull respectively. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical

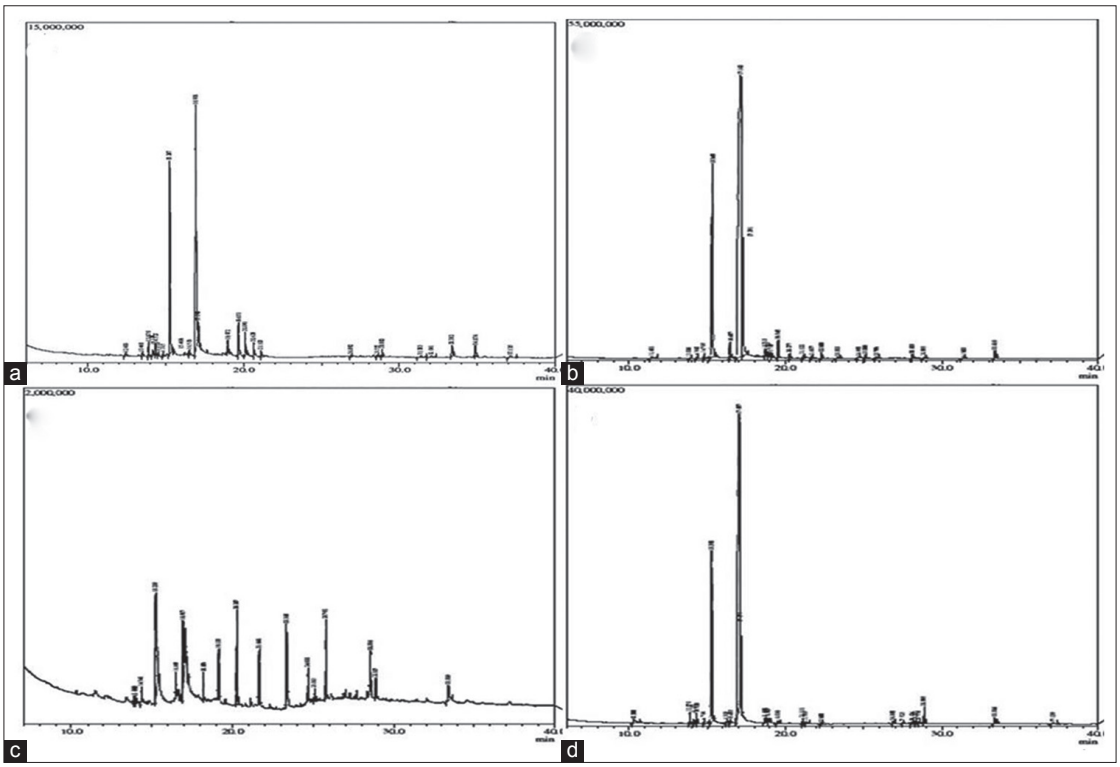

Figure 1: Gas chromatography–mass spectrometry chromatograms of tartary buckwheat methanolic extract, (a) Root, (b) stem, (c) groat and (d) hull samples

Table 1a: Chemo-metric profile of methanolic extract of tartary buckwheat root

RT	Peak area	Area (%)	Compound detected	Hit	SI	RI	CAS No	Molecular formula	MW
12.406	472,968	0.45	2,6,11-Trimethyldodecane	1	89	1320	31295-56-4	$C_{15}H_{32}$	212
13.448	232,435	0.22	Tetradecane	1	92	0	629-59-4	$C_{14}H_{30}$	198
13.879	1,369,383	1.29	Neophytadiene	5	88	0	504-96-1	$C_{20}H_{38}$	278
14.138	602,976	0.57	trans-Phytol	5	79	2045	150-86-7	$C_{20}H_{40}O$	296
14.327	1,033,046	0.97	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl) methyl]-, methyl ester	1	80	2203	10152-69-9	$C_{21}H_{38}O_2$	322
14.484	199,753	0.19	Tetradecane	3	88	0	29-59-4	$C_{14}H_{30}$	198
14.795	229,190	0.22	Hexadecanoic acid, methyl ester	1	91	0	112-39-0	$C_{17}H_{34}O_2$	270
15.247	25,559,743	24.09	Palmitic acid	9	90	1968	57-10-3	$C_{16}H_{32}O_2$	256
15.468	217,247	0.20	Tetradecane	1	90	0	629-59-4	$C_{14}H_{30}$	198
16.503	467,277	0.44	1,7,7-Trimethyl-3-phenethylidenebicyclo[2.2.1]heptan-2-one	1	68	1978	0-00-0	$C_{18}H_{22}O$	254
16.906	52,387,845	49.38	9,12-Octadecadienoic acid (Z, Z)-	1	95	2183	60-33-3	60-33-3	280
17.098	1,945,347	1.83	Octadecanoic acid	6	90	0	57-11-4	$C_{18}H_{36}O_2$	284
18.972	1,386,381	1.31	1-Hydroxy-6-(3-isopropenyl-cycloprop-1-enyl)-6-methyl-heptan-2-one	1	80	1649	0-00-0	$C_{14}H_{22}O_2$	222
19.651	4,634,848	4.37	1,3,6,10-cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-,	10	70	0	1898-13-1	$C_{20}H_{32}$	272
20.091	3,505,339	3.30	Podocarpa-8,11,13-trien-16-oic acid, 13-isopropyl-	3	75	2361	5155-70-4	$C_{20}H_{28}O_2$	300
20.639	2,005,102	1.89	Abietic acid	1	92	2265	514-10-3	$C_{20}H_{30}O_2$	302
21.105	769,657	0.73	Di-n-octyl phthalate	1	95	2832	117-84-0	$C_{24}H_{38}O_4$	390
26.892	245,243	0.23	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate	1	76	2813	7144-08-3	$C_{28}H_{45}ClO_2$	448
28.555	325,855	0.31	Cholesta-4,6-dien-3-ol, (3.beta.)-	1	83	2579	4214-69-8	$C_{27}H_{44}O$	384
28.882	1,217,254	1.15	Stigmast-5-en-3-OL, (3.BETA.,24S)-	1	983	0	83-47-6	$C_{29}H_{50}O$	414
31.383	601,198	0.57	Campesterol	3	86	2632	474-62-4	$C_{28}H_{48}O$	400
32.041	522,553	0.49	Stigmasterol	1	84	2739	83-48-7	$C_{29}H_{48}O$	412
33.382	2,655,283	2.50	Beta-Sitosterol	1	93	:2731	83-46-5	$C_{29}H_{50}O$	414
34.874	2,606,606	2.46	Lupeol	1	84	2848	545-47-1	$C_{30}H_{50}O$	426
37.135	895,663	0.84	Stigmast-4-en-3-one	1	85	0	1058-61-3	$C_{29}H_{48}O$	412

MW: Molecular weight

Table 1b: Chemo-metric profile of methanolic extract of tartary buckwheat stem

RT	Peak area	Area (%)	Compound detected	Hit	SI	RI	CAS No	Molecular formula	MW
13.89	89,198	0.63	Neophytadiene	1	85	0	504-96-1	$C_{20}H_{38}$	278
13.996	75,025	0.53	6-dodecanone	1	84	0	0-00-0	$C_{12}H_{18}O$	178
14.341	269,777	1.92	Phthalic acid, butyl undecyl ester	1	87	2732	0-00-0	$C_{23}H_{36}O_4$	376
15.235	3,227,563	22.94	n-Hexadecanoic acid	1	93	1968	57-10-3	$C_{16}H_{32}O_2$	256
16.495	242,234	1.72	9-Octadecenoic acid (Z)-, methyl ester	1	91	2085	112-62-9	$C_{19}H_{36}O_2$	296
16.917	1,883,261	13.38	cis-9-Hexadecenal	1	90	1808	56219-04-6	$C_{16}H_{30}O$	238
18.183	288,971	2.05	Tetradecane	1	92	0	629-59-4	$C_{14}H_3O$	198
19.135	505,276	3.59	Hexadecane	4	92	1612	544-76-3	$C_{16}H_{34}$	226
20.267	1,435,637	10.2	Docosane	2	91	0	629-97-0	$C_{22}H_{46}$	310
21.648	903,323	6.42	Hexadecane	1	92	1612	544-76-3	$C_{16}H_{34}$	226
23.345	1,501,914	10.67	Eicosane	7	91	2009	112-95-8	$C_{20}H_{42}$	282
24.688	426,369	3.03	Hexadecane	1	94	1612	544-76-3	$C_{16}H_{34}$	226
25.083	161,829	1.15	Squalene	1	91	2914	7683-64-9	$C_{30}H_{50}$	410
25.792	1,224,144	8.7	Eicosane	1	94	2009	112-95-8	$C_{20}H_{42}$	282
28.556	764,645	5.43	Cholesta-4,6-dien-3-ol, (3.beta.)-	1	86	2579	14214-69-8	$C_{27}H_{44}O$	384
28.887	450,339	3.2	3-Bromocholest-5-ene	1	81	0	516-91-6	$C_{27}H_{45}Br$	448
33.389	622,233	4.42	Beta-Sitosterol	1	88	2731	83-46-5	$C_{29}H_{50}O$	414

MW: Molecular weight, RT: Reaction time

databases. In the present study, the methanolic root extract of tartary buckwheat revealed the presence of 25 different metabolites belongs to various compound types. Among them, the most prevailing major compounds were n-hexadecanoic acid (24.09%) and 9, 12-octadecadienoic acid (49.38%). Quantitative phytochemical analysis of the stem extract showed the presence of seventeen different organic compounds. The major metabolites among them were n-hexadecanoic acid a fatty acid (22.94%), Cis-9-hexadecanal an unsaturated aldehyde (13.38%), docosane (10.67%), eicosane (10.67%), hexadecane (6.42%), and a phytosterols cholesta-4, 6-diene-3-ol (5.43%). Phytochemical investigation of the groat extract of tartary buckwheat showed 24 bioactive constituents. Among them, the major metabolites are 3, 3', 4', 5, 7-pentahydroflavone-3-rhamnoglucoside (71.94%) a major flavonoid of buckwheat, n-hexadecanoic acid (17.48%) and Humko Industrene (5.2%). The chemo-profiling of hull extract revealed 24 compounds. In this account, n-hexadecanoic acid, a palmitic acid

Table 1c: Chemo-metric profile of methanolic extract of tartary buckwheat groat

RT	Peak area	Area (%)	Compound detected	Hit	SI	RI	CAS No	Molecular formula	MW
11.481	2,582,193	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	451,651	0.04	Neophytadiene	1	91	0	504-96-1	$C_{20}H_{38}$	278
14.33	830,534	0.08	Phthalic acid, butyl undecyl ester	1	83	2732	0-00-0	$C_{23}H_{36}O_4$	376
14.795	2,131,738	0.2	Palmitic acid, methyl ester	1	96	1878	112-39-0	$C_{17}H_{34}O_2$	270
15.349	181,793,266	17.48	n-Hexadecanoic acid	1	95	1968	57-10-3	$C_{16}H_{32}O_2$	256
16.487	10,870,431	1.05	Emery oleic acid ester 2301	1	94	2085	112-62-9	$C_{19}H_{36}O_2$	296
17.142	748,276,764	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	54,111,517	5.2	Humko industrene	7	90	2167	57-11-4	$C_{18}H_{36}O_2$	284
18.713	5,197,954	0.5	Oleic acid	1	92	2175	112-80-1	$C_{18}H_{34}O_2$	282
18.923	1,731,356	0.17	Eicosanoic acid	8	89	2366	506-30-9	$C_{20}H_{40}O_2$	312
19.543	6,944,741	0.67	Octadecanal	1	95	1999	638-66-4	$C_{18}H_{36}O$	268
20.277	1,889,036	0.18	Docosane	2	92	0	629-97-0	$C_{22}H_{46}$	310
21.122	1,399,060	0.13	Di-n-octyl phthalate	1	93	2832	117-84-0	$C_{24}H_{38}O_4$	390
22.306	3,637,248	0.35	Octadecanal	1	95	1999	638-66-4	$C_{18}H_{36}O$	268
23.353	1,596,835	0.15	Octacosane	2	92	2804	630-02-4	$C_{28}H_{58}$	394
24.692	888,545	0.09	Eicosane	1	91	2009	112-95-8	$C_{20}H_{42}$	282
25.086	1,111,051	0.11	Squalene	1	95	2914	7683-64-9	$C_{30}H_{50}$	410
28.105	2,623,968	0.25	Gamma.-Tocopherol	1	90	3036	7616-22-0	$C_{28}H_{48}O_2$	416
28.893	1,085,525	0.1	Stigmast-5-en-3-ol, (3.beta.,24S)-	1	83	0	83-47-6	$C_{29}H_{50}O$	414
31.385	1,005,075	0.1	Ergost-5-en-3-ol	1	90	0	0-00-0	$C_{28}H_{48}O$	400
33.41	7,485,646	0.72	Gamma.-Sitosterol	1	94	2731	83-47-6	$C_{29}H_{50}O$	414

MW: Molecular weight, RT: Reaction time

Table 1d: Chemo-metric profile of methanolic extract of tartary buckwheat hull

RT	Peak area	Area (%)	Compound detected	Hit	SI	RI	CAS No	Molecular formula	MW
10.298	4,237,454	0.95	Beta.-D-Glucopyranose, 1,6-anhydro-	1	87	1404	498-07-7	$C_6H_{10}O_5$	162
13.878	2,554,758	0.57	Neothytadiene	1	93	0	504-96-1	$C_{20}H_{38}$	278
14.137	1,037,982	0.23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3	91	2045	102608-53-7	$C_{20}H_{40}O$	296
14.328	2,486,302	0.56	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1	92	2045	102608-53-7	$C_{20}H_{40}O$	296
14.796	294,955	0.07	Hexadecanoic acid, methyl ester	1	94	0	112-39-0	$C_{17}H_{34}O_2$	270
15.292	79,505,393	17.84	n-Hexadecanoic acid	1	95	1968	57-10-3	$C_{16}H_{32}O_2$	256
16.203	505,780	0.11	Heptadecanoic acid	1	90	0	506-12-7	$C_{17}H_{34}O_2$	270
16.485	1,198,206	0.27	8-Octadecenoic acid, methyl ester	1	92	2085	2345-29-1	$C_{19}H_{36}O_2$	296
17.057	313,939,760	70.46	6-Octadecenoic acid, (Z)-	1	92	2175	593-39-5	$C_{18}H_{34}O_2$	282
17.175	15,825,243	3.55	Octadecanoic acid	1	95	2167	57-11-4	$C_{18}H_{36}O_2$	284
18.698	999,975	0.22	Oleic acid	1	90	2175	112-80-1	$C_{18}H_{34}O_2$	282
18.907	1,782,083	0.4	Eicosanoic acid	1	92	2366	506-30-9	$C_{20}H_{40}O_2$	312
19.53	785,144	0.18	Hexadecanal/palmitaldehyde	1	95	0	629-80-1	$C_{16}H_{32}O$	240
21.111	1,858,202	0.42	Di-n-octyl phthalate/Dinopol NOP	1	95	2832	117-84-0	$C_{24}H_{38}O_4$	390
21.333	1,170,472	0.26	9-Octadecenoic acid (Z)-	1	89	0	112-80-1	$C_{18}H_{34}O_2$	282
22.3	275,832	0.06	Hexadecanal	1	92	0	629-80-1	$C_{16}H_{32}O$	240
26.892	896,339	0.2	3-bromocholest-5-ene	1	85	0	516-91-6	$C_{27}H_{45}Br$	448
27.523	599,466	0.13	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate	2	83	2813	7144-3-08-3	$C_{28}H_{45}ClO_2$	448
28.104	663,135	0.15	2H-1-benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl	1	86	0	7616-22-0	$C_{28}H_{48}O_2$	416
28.331	1,084,813	0.24	Beta-Sitosterol	3	83	2731	83-46-5	$C_{29}H_{50}O$	414
28.554	980,174	0.22	Cholesta-4,6-dien-3-ol, (3.beta.)	1	87	2579	14214-69-8	$C_{27}H_{44}O$	384
28.895	7,037,290	1.58	3-bromocholest-5-ene	1	83	0	516-91-6	$C_{27}H_{45}Br$	448
33.386	4,564,437	1.02	Beta-Sitosterol	3	83	2731	83-46-5	$C_{29}H_{50}O$	414

MW: Molecular weight, RT: Reaction time

(17.84%), 6-octadecanoic acid, a steric acid (70.46%), 3-bromocholest-5-ene, a phytosterols (1.58%) was the major phytochemical on the basis of quantity. A representation of the chemical profile by groups of compounds in each part is shown in Figure 2a-d.

DISCUSSION

Innatural product chemistry, metabolite profiling is an important approach to ascertain the chemo-typing of natural products that will allow us to scientifically determine and validate their traditional uses, pharmacological activities, and therapeutic potential (Belkacem *et al.*, 2013). In the present study, we have determined the bioactive constituents from the tartary buckwheat by GC-MS chemometrics profiling. The present investigation reveals that the methanolic extract of root, stem, groat, and hull parts of tartary buckwheat altogether showed the presence of 90 metabolites. Among them, resource allocation was found more toward roots that constitute

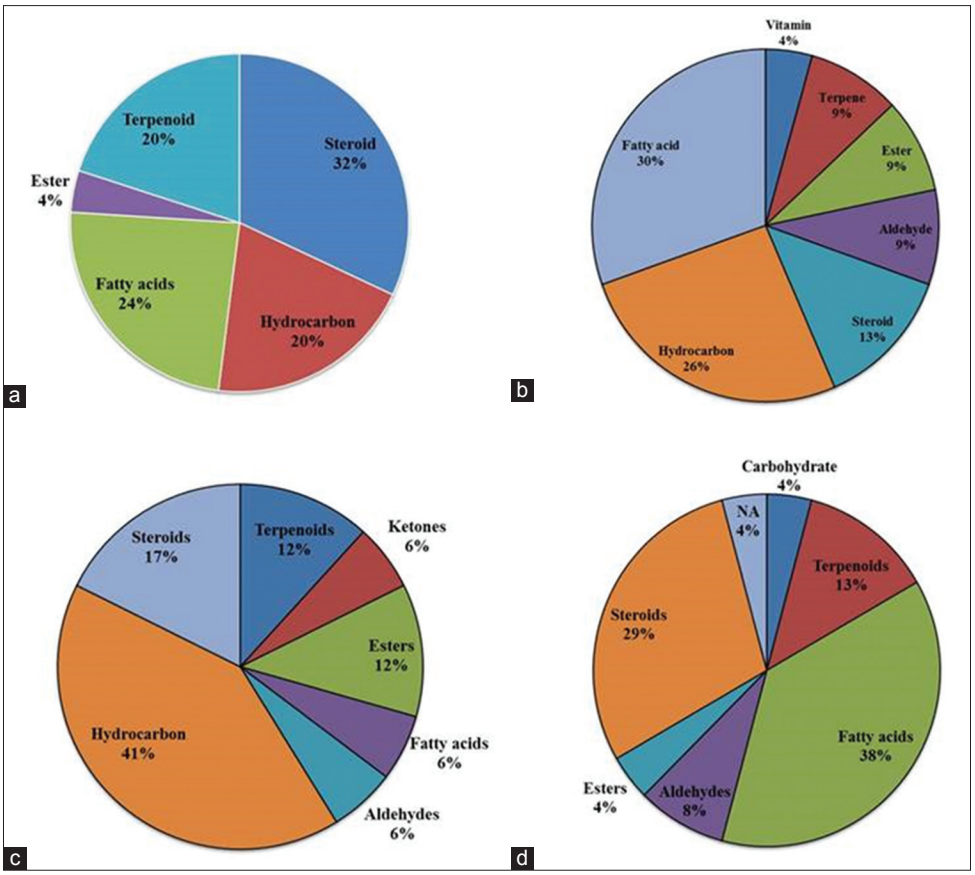

Figure 2: Major phyto-chemotypes identified during metabolite fingerprinting of tartary buckwheat methanolic, (a) root, (b) stem, (c) groat and (d) hull extract

12.43% followed by hull and groat (each 11.94%) and stem (8.45%) (Figure 3). All these compounds identified by GC-MS analysis were further investigated for their biological activities in Dr. Duke's database (Duke, 2012) which revealed that they possess a diverse range of positive pharmacological activities (Table 1). Eventually, in the present study, we have found terpenoids, phytosterols, hydrocarbons, fatty acids, antioxidants, vitamins, esters and carbohydrates as the major group of phyto-chemotypes in the extracts which are extremely beneficial for improving human health. These compounds have a good range of pharmacological and therapeutic potential and could also be responsible for the high antioxidant capacities of tartary buckwheat. 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 (as reviewed in Pirzadah *et al.,* 2013; Farooq *et al.,* 2016). 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 ultraviolet light, X-rays

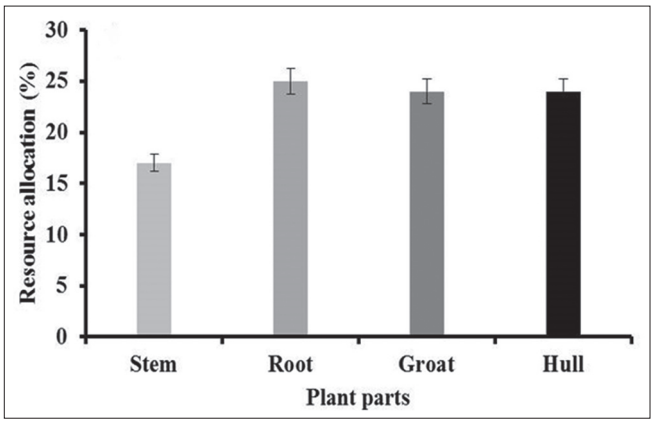

Figure 3: Resource allocation among different parts of tartary buckwheat

and oxidative stress (Gong *et al.,* 2010; Giménez-Bastida and Zielinski, 2015), lowers plasma cholesterol and also suppresses gallstone formation (Kuntic *et al.,* 2011). Phytol (5.22%) found in the leaf extract is having anticancer, antioxidant, antitumor, diuretic and chemopreventative properties and used in vaccine formulation (Sen, 2012; Prabhadevi *et al.,* 2012). The other metabolites such as linoleic acid ester, 9-octadecanoic acid (Z) and methyl

ester is also having anti-inflammatory, anti-androgenic and anemiagenic properties (Singh *et al.,* 2008).

CONCLUSION

From the present investigation, metabolite profiling 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.

ACKNOWLEDGMENTS

RUR thankfully acknowledges the financial support from University of Kashmir under Seed Money Grant.

REFERENCES

Alvarez-Jubete L, Arendt EK, Gallagher E. Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. Trends Food Sci Tech 2010;21:106-13.

Giménez-Bastida JA, Zielinski H. Buckwheat as a functional food and its effects on health. J Agric Food Chem 2015;63:7896-913.

Belkacem N, Djaziri R, Lahfa F, El-Haci IA, Boucherit Z. Phytochemical screening and *in vitro* antioxidant activity isolated bioactive compounds from *Tridax procumbens* Linn. Pak J Biol Sci 2013;16:1971-7.

Desbrosses GG, Kopka J, Udvardi MK. *Lotus japonicus* metabolic profiling. Development of gas chromatography-mass spectrometry resources for the study of plant-microbe interactions. Plant Physiol 2005;137:1302-18.

Farooq S, Rehman RU, Pirzadah TB, Malik B, Dar FA, Tahir I. Cultivation, agronomic practices and growth performance of buck wheat. Molecular Breeding and Nutritional Aspects of Buckwheat. Amsterdam: Elsevier; 2016. p. 299-320.

Gong G, Qin Y, Huang W, Zhou S, Yang X, Li D. Rutin inhibits hydrogen peroxide-induced apoptosis through regulating reactive oxygen species mediated mitochondrial dysfunction pathway in human umbilical vein endothelial cells. Eur J Pharmacol 2010;628:27-35.

Iordache A, Culea M, Gherman C, Cozar O. Characterization of some plant extracts by GC–MS. Rom Nucl Instrum Methods Phys Res 2009;267:338-42.

Jiang P, Burczynski F, Campbell C, Pierce G, Austria JA, Briggs CJ. Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum* and *F. homotropicum* and their protective effects against lipid peroxidation. Food Res Int 2007;40:356-64.

Kuntic V, Filipovic I, Vujic Z. Effects of rutin and hesperidin and their Al(III) and Cu(II) complexes on *in vitro* plasma coagulation assays. Molecules 2011;16:1378-88.

Landberg R, Sun Q, Rimm EB, Cassidy A, Scalbert A, Mantzoros CS, *et al*. Selected dietary flavonoids are associated with markers of inflammation and endothelial dysfunction in U.S. Women. J Nutr 2011;141:618-25.

Liu CL, Chen YS, Yang JH, Chiang BH. Antioxidant activity of tartary (*Fagopyrum tataricum* (L.) Gaertn.) and common (*Fagopyrum esculentum* Moench) buckwheat sprouts. J Agric Food Chem 2008;56:173-8.

Pirzadah TB, Malik B, Tahir I, Rehman RU. Buckwheat: An intarospective and Future Perspective in Kashmir Himalayas. Proceedings of the 12th International Symposium on Buckwheat, Laško, August 21-25 Pernica: *Fagopyrum*; 2013. p. 212-5.

Prabhadevi V, Sahaya SS, Johnson M, Venkatramani B, Janakiraman N. Phytochemical studies on *Allamanda cathartica* L. Using GC-MS. Asian Pac J Trop Biomed 2012;2:550-4.

Sen BA. Chemical composition of methanol extract of the leaves of *Melia azedarach* L. Asian J Pharm Clin Res 2012;5:42-5.

Sharma S, Ali A, Ali J, Sahni JK, Baboota S. Rutin: Therapeutic potential and recent advances in drug delivery. Expert Opin Investig Drugs 2013;22:1063-79.

Singh S, Nair V, Jain S, Gupta YK. Evaluation of anti-inflammatory activity of plant lipids containing alpha-linolenic acid. Indian J Exp Biol 2008;46:453-6.