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

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## 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, anticarcinogenic, and vasoconstrictive (Landberg et al., 2011; Sharma et al., 2013). Buckwheat flour is glutenfree 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 preflowering 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  $37^{\circ}$ 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  $(2.5 \ \mu$ l) 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
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RT	Peak area	Area (%)	Compound detected	Hit	SI	RI	CAS No	Molecular	MW
								formula	
12.406	472,968	0.45	2,6,11-Trimethyldodecane	1	89	1320	31295-56-4	C15H32	212
13.448	232,435	0.22	Tetradecane	1	92	0	629-59-4	C14H30	198
13.879	1,369,383	1.29	Neophytadiene	5	88	0	504-96-1	C_0H_38	278
14.138	602,976	0.57	trans-Phytol	5	79	2045	150-86-7	C <sub>20</sub> H <sub>40</sub> O	296
14.327	1,033,046	0.97	Cyclopropanenonanoic acid, 2-[(2-butylcyclopropyl) methyl]-, methyl	1	80	2203	10152-69-9	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>	322
14.484	199,753	0.19	Tetradecane	3	88	0	29-59-4	C14H20	198
14.795	229,190	0.22	Hexadecanoic acid, methyl ester	1	91	0	112-39-0	C, H, O,	270
15.247	25,559,743	24.09	Palmitic acid	9	90	1968	57-10-3	C, H, Q	256
15.468	217,247	0.20	Tetradecane	1	90	0	629-59-4	Č,4H_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	C18H220	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	C18H3602	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 <sub>14</sub> H <sub>22</sub> O <sub>2</sub>	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	Č <sub>20</sub> H <sub>32</sub>	272
20.091	3,505,339	3.30	Podocarpa-8,11,13-trien-16-oic acid, 13-isopropyl-	3	75	2361	5155-70-4	C20H28O2	300
20.639	2,005,102	1.89	Abietic acid	1	92	2265	514-10-3	C <sub>20</sub> H <sub>30</sub> O <sub>2</sub>	302
21.105	769,657	0.73	Di-n-octyl phthalate	1	95	2832	117-84-0	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390
26.892	245,243	0.23	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate	1	76	2813	7144-08-3	C <sub>28</sub> H <sub>45</sub> CIO <sub>2</sub>	448
28.555	325,855	0.31	Cholesta-4,6-dien-3-ol, (3.beta.)-	1	83	2579	4214-69-8	C <sub>27</sub> H <sub>44</sub> O	384
28.882	1,217,254	1.15	Stigmast-5-en-3-0L, (3.BETA.,24S)-	1	983	0	83-47-6	C <sub>29</sub> H <sub>50</sub> O	414
31.383	601,198	0.57	Campesterol	3	86	2632	474-62-4	C <sub>28</sub> H <sub>48</sub> O	400
32.041	522,553	0.49	Stigmasterol	1	84	2739	83-48-7	C <sub>29</sub> H <sub>48</sub> O	412
33.382	2,655,283	2.50	Beta-Sitosterol	1	93	:2731	83-46-5	C <sub>29</sub> H <sub>50</sub> 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 <sub>29</sub> H <sub>48</sub> 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 , H ,	278
13.996	75,025	0.53	6-dodecanone	1	84	0	0-00-0	C, H, O	178
14.341	269,777	1.92	Phthalic acid, butyl undecyl ester	1	87	2732	0-00-0	C_3 H_60	376
15.235	3,227,563	22.94	n-Hexadecanoic acid	1	93	1968	57-10-3	C, H, O	256
16.495	242,234	1.72	9-Octadecenoic acid (Z)-, methyl ester	1	91	2085	112-62-9	C10H302	296
16.917	1,883,261	13.38	cis-9-Hexadecenal	1	90	1808	56219-04-6	C16H30	238
18.183	288,971	2.05	Tetradecane	1	92	0	629-59-4	C H O	198
19.135	505,276	3.59	Hexadecane	4	92	1612	544-76-3	C16H34	226
20.267	1,435,637	10.2	Docosane	2	91	0	629-97-0	C, H 46	310
21.648	903,323	6.42	Hexadecane	1	92	1612	544-76-3	C16H34	226
23.345	1,501,914	10.67	Eicosane	7	91	2009	112-95-8	C_0 H_2	282
24.688	426,369	3.03	Hexadecane	1	94	1612	544-76-3	C16H34	226
25.083	161,829	1.15	Squalene	1	91	2914	7683-64-9	C 10 H 50	410
25.792	1,224,144	8.7	Eicosane	1	94	2009	112-95-8	C_0 H_2	282
28.556	764,645	5.43	Cholesta-4,6-dien-3-ol, (3.beta.)-	1	86	2579	14214-69-8	C 27 H 40	384
28.887	450,339	3.2	3-Bromocholest-5-ene	1	81	0	516-91-6	C <sub>27</sub> H <sub>45</sub> Br	448
33.389	622,233	4.42	Beta-Sitosterol	1	88	2731	83-46-5	C <sub>29</sub> H <sub>50</sub> 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 chemoprofiling of hull extract revealed 24 compounds. In this account, n-hexadecanoic acid, a palmitic acid

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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	C10H203Si2	248
13.886	451,651	0.04	Neophytadiene	1	91	0	504-96-1	C _ H _ 8	278
14.33	830,534	0.08	Phthalic acid, butyl undecyl ester	1	83	2732	0-00-0	C23H3604	376
14.795	2,131,738	0.2	Palmitic acid, methyl ester	1	96	1878	112-39-0	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
15.349	181,793,266	17.48	n-Hexadecanoic acid	1	95	1968	57-10-3	C16H3202	256
16.487	10,870,431	1.05	Emery oleic acid ester 2301	1	94	2085	112-62-9	C19H3602	296
17.142	748,276,764	71.94	$3,3\times,4\times,5,7$ -pentahydroflavone-3-rhamnoglucoside	1	90	2183	60-33-3	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280
17.291	54,111,517	5.2	Humko industrene	7	90	2167	57-11-4	C18H3602	284
18.713	5,197,954	0.5	Oleic acid	1	92	2175	112-80-1	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
18.923	1,731,356	0.17	Eicosanoic acid	8	89	2366	506-30-9	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312
19.543	6,944,741	0.67	Octadecanal	1	95	1999	638-66-4	C18H360	268
20.277	1,889,036	0.18	Docosane	2	92	0	629-97-0	C <sub>22</sub> H <sub>46</sub>	310
21.122	1,399,060	0.13	Di-n-octyl phthalate	1	93	2832	117-84-0	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390
22.306	3,637,248	0.35	Octadecanal	1	95	1999	638-66-4	C <sub>18</sub> H <sub>36</sub> O	268
23.353	1,596,835	0.15	Octacosane	2	92	2804	630-02-4	C <sub>28</sub> H <sub>58</sub>	394
24.692	888,545	0.09	Eicosane	1	91	2009	112-95-8	C <sub>20</sub> H <sub>42</sub>	282
25.086	1,111,051	0.11	Squalene	1	95	2914	7683-64-9	C <sub>30</sub> H <sub>50</sub>	410
28.105	2,623,968	0.25	GammaTocopherol	1	90	3036	7616-22-0	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	416
28.893	1,085,525	0.1	Stigmast-5-en-3-ol, (3.beta.,24S)-	1	83	0	83-47-6	C_9H_0	414
31.385	1,005,075	0.1	Ergost-5-en-3-ol	1	90	0	0-00-0	C28H480	400
33.41	7,485,646	0.72	GammaSitosterol	1	94	2731	83-47-6	C <sub>29</sub> H <sub>50</sub> 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	BetaD-Glucopyranose, 1,6-anhydro-	1	87	1404	498-07-7	C_H_0_5	162
13.878	2,554,758	0.57	Neothytadiene	1	93	0	504-96-1	Č <sub>20</sub> H <sub>38</sub>	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 <sub>20</sub> H <sub>40</sub> O	296
14.796	294,955	0.07	Hexadecanoic acid, methyl ester	1	94	0	112-39-0	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
15.292	79,505,393	17.84	n-Hexadecanoic acid	1	95	1968	57-10-3	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256
16.203	505,780	0.11	Heptadecanoic acid	1	90	0	506-12-7	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
16.485	1,198,206	0.27	8-Octadecenoic acid, methyl ester	1	92	2085	2345-29-1	C19H3602	296
17.057	313,939,760	70.46	6-Octadecenoic acid, (Z)-	1	92	2175	593-39-5	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
17.175	15,825,243	3.55	Octadecanoic acid	1	95	2167	57-11-4	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284
18.698	999,975	0.22	Oleic acid	1	90	2175	112-80-1	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
18.907	1,782,083	0.4	Eicosanoic acid	1	92	2366	506-30-9	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312
19.53	785,144	0.18	Hexadecanal/palmitaldehyde	1	95	0	629-80-1	C_6H_20	240
21.111	1,858,202	0.42	Di-n-octyl phthalate/Dinopol NOP	1	95	2832	117-84-0	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390
21.333	1,170,472	0.26	9-Octadecenoic acid (Z)-	1	89	0	112-80-1	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282
22.3	275,832	0.06	Hexadecanal	1	92	0	629-80-1	C <sub>16</sub> H <sub>32</sub> O	240
26.892	896,339	0.2	3-bromocholest-5-ene	1	85	0	516-91-6	C <sub>27</sub> H <sub>45</sub> Br	448
27.523	599,466	0.13	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate	2	83	2813	7144-3-08-3	C28H45CIO2	448
28.104	663,135	0.15	2H-1-benzopyran-6-ol,	1	86	0	7616-22-0	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	416
			3,4-dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl						
28.331	1,084,813	0.24	Beta-Sitosterol	3	83	2731	83-46-5	C29H50	414
28.554	980,174	0.22	Cholesta-4,6-dien-3-ol, (3.beta.)	1	87	2579	14214-69-8	C <sub>27</sub> H <sub>44</sub> O	384
28.895	7,037,290	1.58	3-bromocholest-5-ene	1	83	0	516-91-6	C <sub>27</sub> H <sub>45</sub> Br	448
33.386	4,564,437	1.02	Beta-Sitosterol	3	83	2731	83-46-5	C <sub>29</sub> H <sub>50</sub> 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 antihypertensive, anti-carcinogenic, vasoconstrictive, antiinflammatory 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.

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