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

Tanveer Bilal Pirzadah1, Bisma Malik1, Inayatullah Tahir2, Reiaz Ul Rehman1*

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

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, Janamashtami, 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, “groots” 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](image-url)
In the present study, the methanolic root extract of tartary buckwheat revealed the presence of 25 different metabolites belonging 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 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-Trimethylidodecane          | 1   | 89 | 320 | 13295-56-4 | C_{32}H_{64}     | 212 |
| 13.448 | 232,435   | 0.22     | Tetradecane                        | 1   | 92 | 0  | 629-59-4  | C_{16}H_{34}     | 198 |
| 13.879 | 1,369,383 | 1.29     | Neophytadiene                      | 5   | 88 | 0  | 504-96-1  | C_{18}H_{36}     | 278 |
| 14.138 | 602,976   | 0.57     | trans-Phytol                       | 5   | 79 | 2045| 150-86-7 | C_{14}H_{26}O     | 296 |
| 14.327 | 1,033,046 | 0.97     | Cyclopropanonoanoic acid, 2-[(2-butylcyclopropyl] methyl]-, methyl ester | 1   | 80 | 2203| 10152-69-9 | C_{16}H_{30}O      | 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_{16}H_{32}O     | 270 |
| 15.247 | 25,559,743| 24.09    | Palmitic acid                      | 9   | 90 | 1968| 57-10-3   | C_{16}H_{32}O     | 256 |
| 15.468 | 217,247   | 0.20     | Tetradecane                        | 1   | 90 | 0  | 629-59-4  | C_{16}H_{32}O     | 198 |
| 16.503 | 467,277   | 0.44     | 1,7,7-Trimethyl-3-phenethylidenebicycl[2.2.1]heptan-2-one | 1  | 68 | 1978| 0-00-0   | C_{21}H_{38}O     | 280 |
| 16.906 | 52,387,845| 49.38    | 9,12-Octadecadienoic acid (Z, Z)-  | 1   | 95 | 2183| 60-33-9   | C_{18}H_{36}O     | 280 |

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_{16}H_{32}O   | 278 |
| 13.996 | 75,025    | 0.53     | 6-dodecanoic acid                  | 1   | 84 | 0  | 0-00-0   | C_{12}H_{24}O   | 187 |
| 14.341 | 269,777   | 1.92     | Phthalic acid, butyl undecyl ester | 1   | 87 | 2732| 0-00-0   | C_{13}H_{22}O   | 376 |
| 15.235 | 3,227,563 | 22.94    | n-Hexadecanoic acid                | 1   | 93 | 1968| 57-10-3   | C_{16}H_{32}O   | 256 |
| 16.495 | 242,234   | 1.72     | 9-Octadecanoic acid (Z)-, methyl ester | 1  | 91 | 2085| 112-62-9 | C_{17}H_{36}O   | 296 |
| 16.917 | 1,883,261 | 13.38    | cis-9-Hexadecanal                 | 1   | 90 | 1808| 56219-04-6| C_{18}H_{36}O   | 238 |
| 18.183 | 288,971   | 10.2     | Tetradecane                        | 1   | 92 | 0  | 629-59-4 | C_{16}H_{32}O   | 198 |
| 19.135 | 505,276   | 3.59     | Hexadecane                         | 4   | 92 | 1612| 544-76-3 | C_{18}H_{36}O   | 228 |
| 20.267 | 1,435,637 | 10.2     | Docosane                           | 2   | 91 | 0  | 629-97-0 | C_{24}H_{48}O | 310 |
| 21.648 | 903,323   | 6.42     | Hexadecane                         | 1   | 92 | 1612| 544-76-3 | C_{18}H_{36}O   | 226 |
| 23.345 | 1,501,914 | 10.67    | Eicosane                           | 7   | 91 | 2009| 112-95-8 | C_{20}H_{42}O | 282 |
| 24.688 | 426,369   | 3.03     | Hexadecane                         | 1   | 94 | 1612| 544-76-3 | C_{18}H_{36}O   | 226 |
| 25.083 | 161,829   | 1.15     | Squalane                           | 1   | 91 | 2914| 7683-64-0| C_{30}H_{60}O | 410 |
| 25.792 | 1,229,144 | 8.7      | Eicosane                           | 1   | 94 | 2009| 112-95-8 | C_{20}H_{42}O | 282 |
| 28.556 | 764,645   | 5.43     | Cholesta-4,6-dien-3-ol, (3.beta.,- | 1   | 86 | 2579| 14214-69-8| C_{30}H_{50}O | 394 |
| 28.887 | 450,339   | 3.2      | 3-Bromocholest-5-ene               | 1   | 81 | 0  | 516-91-6 | C_{30}H_{50}O | 448 |
| 33.389 | 622,233   | 4.42     | Beta-Sitosterol                    | 1   | 88 | 2731| 83-46-5 | C_{30}H_{50}O | 414 |

MW: Molecular weight, RT: Reaction time
(17.84%), 6-octadecanoic acid, a steric acid (70.46%), 3-bromocholesterol-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**

In natural 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

| 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 | C10H24O3Si2 | 248 |
| 13.886 | 451,651 | 0.04 | Neophytadiene | 1 | 91 | 0 | 504-96-1 | C9H16O | 152 |
| 14.33 | 830,534 | 0.08 | Phthalic acid, butyl undecyl ester | 1 | 83 | 0 | 0-00-0 | C23H36O4 | 376 |
| 14.795 | 2,131,738 | 0.2 | Palmitic acid, methyl ester | 1 | 96 | 0 | 112-39-0 | C17H34O2 | 270 |
| 15.349 | 181,793,266 | 17.48 | n-Hexadecanoic acid | 1 | 95 | 0 | 57-10-3 | C16H32O2 | 256 |
| 16.487 | 10,870,431 | 1.05 | Emery oleic acid ester | 1 | 94 | 0 | 112-62-9 | C19H36O2 | 296 |
| 17.142 | 748,276,764 | 71.94 | 3,3,4,5,7-pentahydroflavone-3-rhamnoglucoside | 1 | 90 | 0 | 60-33-3 | C18H32O2 | 280 |
| 17.291 | 54,111,517 | 5.2 | Humko industriene | 1 | 95 | 2 | 629-97-0 | C18H36O2 | 284 |

| 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 | C6H10O5 | 162 |
| 13.878 | 2,554,758 | 0.57 | Neophytadiene | 1 | 93 | 0 | 504-96-1 | C9H16O | 152 |
| 14.137 | 1,037,982 | 0.23 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 3 | 91 | 2045 | 102680-53-7 | C20H40O | 296 |
| 14.328 | 2,486,302 | 0.56 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 1 | 92 | 2045 | 102680-53-7 | C20H40O | 296 |
| 14.796 | 294,955 | 0.07 | Hexadecanoic acid, methyl ester | 1 | 94 | 0 | 112-39-0 | C16H32O2 | 270 |
| 15.292 | 79,505,393 | 17.84 | 6-Octadecanoic acid | 1 | 95 | 1999 | 368-66-4 | C18H36O2 | 284 |
| 16.203 | 505,780 | 0.11 | Heptadecanoic acid | 1 | 90 | 0 | 506-12-7 | C17H34O2 | 270 |
| 16.485 | 1,198,206 | 0.27 | 8-Octadecenoic acid, methyl ester | 1 | 92 | 2085 | 2345-29-1 | C18H36O2 | 296 |
| 17.057 | 313,939,760 | 70.46 | 6-Octadecanoic acid, (Z)- | 1 | 92 | 2175 | 57-10-3 | C18H34O2 | 282 |
| 17.175 | 15,825,243 | 3.55 | Octadecanoic acid | 1 | 95 | 0 | 57-11-4 | C18H36O2 | 284 |
| 18.698 | 999,975 | 0.22 | Oleic acid | 1 | 90 | 0 | 112-80-1 | C18H36O2 | 282 |
| 19.53 | 785,144 | 0.18 | Hexadecanal/palmitaldehyde | 1 | 95 | 0 | 629-80-1 | C16H32O | 240 |
| 21.111 | 1,858,202 | 0.42 | Di-octyl phthalate/Dinopol NOP | 1 | 95 | 2832 | 117-84-0 | C20H40O2 | 302 |
| 21.333 | 1,170,472 | 0.26 | 9-Octadecanoic acid (Z)- | 1 | 91 | 1968 | 57-10-3 | C18H34O2 | 282 |
| 22.3 | 275,832 | 0.06 | Hexadecanal | 1 | 92 | 0 | 629-80-1 | C18H36O2 | 240 |
| 26.892 | 896,339 | 0.2 | 3-bromocholest-5-ene | 1 | 85 | 0 | 516-91-6 | C27H45Br | 448 |
| 27.523 | 599,466 | 0.13 | Cholest-5-en-3-ol, (3.beta.,24S)- | 1 | 83 | 0 | 83-47-6 | C29H50O | 414 |
| 28.385 | 1,005,075 | 0.1 | Ergost-5-en-3-ol | 1 | 90 | 0 | 0-00-0 | C29H50O | 400 |
| 33.41 | 7,485,646 | 0.72 | Gamma-Sitosterol | 1 | 94 | 2731 | 83-46-5 | C29H50O | 414 |

MW: Molecular weight, RT: Reaction time
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 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 nutraceutical and functional food sector to diminish malnutrition and improve social well-being especially for the impoverished community.

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