PROXIMATE, MACRO ELEMENTAL AND GC-MS ANALYSIS OF *Sorbaria tomentosa*

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Abstract

Sorbaria tomentosa (Lindl.) Rehder of the family Rosaceae, is a wild, medicinal plant, native to the Himalayas. Proximate composition gives important information to assess the suitability of medicinal flora or their extracts taken orally by the trivial communities. In the current study, different proximate parameters like carbohydrate, ash, protein, moisture content and fat, along with carbon, hydrogen, nitrogen and sulphur were analyzed in whole plant of *S. tomentosa*. The results revealed the occurrence of considerable proportion of carbohydrates (52%) and protein (23.80%). Moisture, fat and ash contents were found in small amount i.e. 6.25%, 2.02% and 0.20%, respectively. Elemental analysis displayed the highest content of carbon (44.92%) followed by hydrogen (6.16%), nitrogen (5.17%) and sulphur (0.43%). GC-MS analysis of *n*-hexane fraction of *S. tomentosa* led to identification of five compounds *viz.* 3,13-dimethylpentadecanoic acid (**1**), 2,4-dimethyltetradecanoic acid (**2**), 2,4-heptadecadienoic acid; ethyl ester **(3),** 2-butyl cyclopropane dodecanoic acid **(4)** and heptadecanoic acid; ethyl ester **(5).** Further isolation and identification of active constituents in *S. tomentosa* could confirm the discovery of novel plant drugs.

Key words: Elemental analysis, Medicinal plant, Proximate parameters, *Sorbaria*

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INTRODUCTION

Traditional medicinal plants have been consumed in many regions of the world for a long time as they offer a cheap, safe and reliable substitute of chemical drugs. In addition to the pharmacologically significant, the phytochemicals of medicinal plant exhibit their own proximate composition consisting of primary metabolites like proteins, carbohydrates, and lipids in addition to secondary metabolites like akaloids, terpenoids, tannins, saponins, flavonoids, and cardiac glycosides (Bahadur *et al*., 2018). Primary metabolites play a vital role in fulfilling human needs for life processes and energy, while secondary metabolites can be utilized for synthesis of various antimicrobial and antifungal drugs. The connection between the elemental and proximate or phytochemical profiles in medicinal plants and its ethnomedicinal usage is regarded as a vital topic (Gebashe *et al*., 2020). Many plants species still need to be discovered for their elemental proportion in addition to proximate analysis profiles (Bahadur *et al*., 2018). Pakistan has distinctive recognition as having an extensive range of plants of almost 5700 species, including about 400–600 species of medicinally important plants, while some plants have been explored biochemically (Ahmad *et al*., 2007). On account of the increasing demand of herbal medicines, it is important to explore proximate composition and elemental analysis of scientifically ignored medicinal plants.

Genus *Sorbaria* belongs to the Rosaceae family, is a trivial Asiatic genus inhabitant to Pakistan. It contains four species, all of which are wild having medicinal as well as ornamental value even in Europe and Belgium (Verloove *et al*., 2014). *Sorbaria sorbifolia*, *S. kirilowii*, *S. grandiflora* and *S. tomentosa*, are the most common species of the genus and have been reported for antioxidant, anti-inflammatory, antitumor, analgesic and hepatoprotective potential (Xue-Wu *et al*., 2003; Xue-Wu *et al*., 2004; Park *et al*., 2011). *S. tomentosa* is native to Pakistan locally called as "Berre or Karhee" is a wild, medicinal, sprawling woody shrub and can therefore be grown in abundance with minimum maintenance (Hamayun *et al*., 2006). It is widely distributed in Swat District, Hindukush-Himalayan valleys of Gabral and Utror, Kaghan valley in Pakistan, also present in Afghanistan, Nepal and Tajikistan. In a natural plant vegetation, *S. tomentosa* proved a natural potential host for potato virus Y in north-west Hamalya in Hamachal Pradesh (Mehra *et al*., 2005). The plant has large fern-like leaves and creamy white flowers, and can grow up to 2 tall. All parts of *S. tomentosa* have ethnopharmacological importance being used in various ailments such as burns, asthma, skin rashes and wounds. Methanolic extract of *S. tomentosa* has shown antitumor effect and phytotoxicity activity, while ethanolic extract has marked antioxidant activity and stabilization potential for sunflower oil (Inayatullah *et al*., 2017). In the current study, proximate parameters like carbohydrate, protein, ash, fat and moisture were analyzed from the whole plant of *S. tomentosa*, while four elements including C (carbon), H (hydrogen), N (nitrogen) and S (sulphur) were also assayed. GC-MS analysis was performed to identify bioactive components of in hexane sub-fraction of the plant extract.

MATERIAL AND METHODS

Collection and extraction

Whole plant (root, stem and leaves) of *S.tomentosa* (No. GC. Bot. Herb. 816) were collected from Northern area, Kalam district of Khyber Pakhtun Khwa, Pakistan, dried in shade and pulverized in to fine powdered. The powdered material (1.98 g) was saturated in MeOH 95% (10 L × 2) at room temperature for seven days. Final extract solvent was evaporated by rotary evaporator at low temperature 50 ºC under reduced pressure, which resulted in dark brown gummy mass (190 g). The moisture, ash, fat, protein and carbohydrates content of *S. tomentosa* of the methanolic extract was determined by methods of AOAC (1990) and was noted in percentage(Catharina *et al*., 2013).

Determination of crude protein

Micro-Kjeldahl procedure was adopted to determine protein content by scheming nitrogen and multiplying to 6.25 factor. The plan sample (1 g) were taken in Pyrex digestion tube (250 mL) and mixed with conc. H_2SO_4 (30 mL), potassium sulphate (10 g) and copper sulphate (14 g). At low flame, this mixture was boiled using sand bath and the solution was made clear. The solution was transferred to Kjeldahl flask and further diluted with distilled water. The flask was joined with distillation assembly splash heads followed by addition of 100 mL of 40% caustic soda and granulated zinc (few pieces). The resultant liquid was then back-titrated against 0.01 M hydrochloric acid until the endpoint violet colour was reached and percentage nitrogen content was calculated as:

$$\frac{14 \times M \times Vt \times V100}{Weight\ of\ sample \times Va} \times 100$$

Where M is actual molarity of acid (HCl), V100 is the titre value (Cm^3) of HCl used, Vt is the total volume of the diluted digest, Va is the aliquot volume distilled. The value of nitrogen content was multiplied with 6.25 factor to calculate the crude protein content.

Determination of crude fat

In a Soxhelt extractor, 1.0 g pulverized plant sample was measured in round bottom flask (W_1), it was then extracted with diethyl ether solvent at 50 °C for 3 hours in a flask attached to the Soxhlet extractor, at reflux. The solvent residue was removed by filter paper (Whatmann No 40), the oil in round bottom flask was dried in oven and weighted (W_2). Following formula was used to determine the crude fat percentage.

$$\frac{W2 - W1}{Weight\ of\ sample} \times 100$$

Determination of carbohydrate content

Carbohydrate percentage was determined by subtracting the ash value, fat, protein and moisture from 100.

$$100 - (ash\% + fat\ \% + protien\% + moisture\ content\ \%)$$

Determination of energy content

The value of energy in the samples was estimated in kilojoule per hundred gram and calculated by adding up the values for carbohydrate, crude lipid and crude protein in the extract using following formula:

Energy value (KJ 100 g^{-1}) = (% crude protein × 6.736) + (% crude fat × 37.656) + % carbohydrate × 16.736)

C, H, N and S were calculated in percentage by C/S determinator (Model EMIA 820V).

Determination of moisture content

An empty silica crucible was dried (105 °C) to a constant weight, and weighed (W_1). The pulverized plant sample (5.0 g) was weighed (W_2) in the crucible and dried at 105 °C to constant weight. After cooling, the crucible containing the plant sample was weighted (W_3), and the moisture content was calculated in percentage as:

$$\frac{W2 - W3}{W1 - W2} \times 100$$

Determination of ash content

For estimation of ash content, an empty silica crucible was dried (105 °C) to a constant weight, cool and weighed (W_1). Sample (5.0 g) was placed in silica crucible and weighted again (W_2). The sample was placed in muffle furnace and heated at 600 °C for 5 hours, then cool desiccator weighed again (W_3). Using following formula, % of ash content was calculated.

$$\frac{W2 - W3}{W1 - W2} \times 100$$

GC-MS analysis

GC-MS analysis of hexane fraction of whole plant extract was performed on gas chromatograph (Shimadzu GC-9A) equipped with capillary column (SPB-5) maintained with flame ionization detector at 220 °C. Carrier gas (N_2:1.0 mL min^{-1})

was adjusted at initial temperature at 50 °C for initial 5 min, followed by increase in temperature (5 ºC min^{-1}) up to 235 ºC and finally sustained for 5 min. A column (HP-5 with dimensions: 25 m × 0.22 mm and 0.25 µm df) was used to complete analysis of the fraction

RESULTS AND DISCUSSION

Medicinal plants hold a significant position in drug development, and among 265,000 species of plants only half of these are explored for medicinal purposes across the world. In developing countries, 80% of population rely on medicinal plants to treat different health issues (Johnsy *et al*., 2012), while in developed countries, 60% of the population uses these medicinal plants (Mussarat *et al*., 2014). In Pakistan, 600–700 plants are used to for medicinal purposes (Shinwari, 2010), which account only 10% of the total reported plant species (Shaheen *et al*., 2014). There are still many unexplored medicinal plants in Pakistan. In the present study, key proximate chemical composition of *S. tomentosa* was evaluated and its relationship to pharmacology and health benefits are discussed. Generally, carbohydrates, lipids, protein, fats, moisture content, ash content and energy value are taken as important parameters for assessing the proximate chemical composition of the medicinal plant. Carbohydrates are a source of energy and sustains basic brain physiology. Significance of protein as an enzymatic catalyst, growth control and cell differentiation is well-known. Fats give energy and support cell growth. Ash content not only facilitates the metabolic processes, it also helps in growth and development. Moisture content indicates storage/shelve life of the sample.

The results revealed that *S. tomentosa* plant was rich in contents of carbohydrates (52.26%) and protein (23.80%), low in moisture (6.25%) and fat (2.02%) (Table 1). Izhar *et al*. (2019) also reported the occurrence of carbohydrates in large quantities, while proteins and cardiac glycosides in little quantity in *S. tomentosa*. Begum *et al*. (2018) documented 63-74% carbohydrates, 0.85–4.37% protein, 4.07–7.70% fats, 6.01–17.67% ash and 10.27–15.50% moisture contents in medicinal plants including *Monotheca buxifolia*, *Geranium wallichianum* and *Saxifraga flagillaris*. The attributes of carbohydrate and protein in *S. tomentosa*are equal or even greater than many vegetables like potato, onion, pumpkin, ladyfinger and bitter gourd, where these content were reported in the range of 27.23–56.26% and 12.37–16.38%, respectively (Islam *et al*., 2013). High carbohydrate content of *S. tomentosa* could serve as the main energy donor, since crude protein and fat contribute only in small portion, while sufficient protein content may be helpful in fulfilling intake of dietary protein (0.8 g/kg of body weight) as prescribed in health guidelines (EFSA, 2012). Fat content may indicate therapeutic advantage in terms of preserving insulin (Nagao and Yanagita, 2010). The contents of carbohydrate, protein and fat may classify *S. tomentosa* as a valuable high energy plant source. Furthermore, the relatively high energy content of 1340 KJ/100 g DW could fulfill the daily calorie intake (Kumari *et al*., 2017). Altogether, the proximate chemical composition of *S. tomentosa* may contribute in providing high calorie diet in anorexia nervosa incidence (Garber*et al*., 2013). Rahman *et al*. (2016) also recognized *S. tomentosa* as the potential medicinal plant on the basis of its highest fidelity level and informant consensus factor, and recommend it for the treatment of digestive diseases, e.g. cholera, colon cancer, emetic, internal injuries, tumor and urine suppression. Over and above, low moisture content would likely to lessen the risk of contamination by microbial flora, hence increase shelf life (Idress *et al*., 2019).

Among the elements, the role of carbon is known in regulating body physiology, and in the formation of proteins, carbohydrates and fats. Nitrogen is basic element (macronutrient) in amino acids and nucleic acids such as DNA and RNA. The hydrogen helps to create water and body hydrated, and the sulphur is necessary for the synthesis of certain key proteins. Elemental analysis of *S. tomentosa* exhibited high proportion of carbon (44.92%) followed by hydrogen (6.16%), nitrogen (5.17%) and sulphur (0.42%). The findings are in accordance with Maiti *et al*. (2015) and Wang *et al*. (2016), where higher amount of C (45 to 55%) were reported in other herbs, shrubs and tree species. Anjum *et al*. (2019) also C: H: S: N in the range of 35–46%, 5-6%, 0.04–0.62%, 132–4.93% in different medicinal plants (*Sophora mollis* and *Peganum harmala*) collected from Balochistan, Pakistan. Misra *et al*. (2018) also reported C: H: N: S in 33: 6: 5: 1 in aquatic herb *Monochoria hastata*. Hence, the elemental analysis of *S. tomentosa* may be helpful in the exploitation of its role in pharmacology.

From hexane fraction of *S. tomentosa*, five compounds 3,13-dimethylpentadecanoic acid (**1**), 2,4-dimethyltetradecanoic acid (**2**), 2,4-heptadecadienoic acid; ethyl ester (**3**), 2-butylcyclopropane dodecanoic acid (**4**) and heptadecanoic acid; ethyl ester (**5**) were identified by GC-MS analysis (Table 3; Fig. 1). 3,13-dimethylpentadecanoic acid is fatty acid, and has been documented in many medicinal plants including *Aegle marmelos* (Ariharan *et al*., 2015), *Indoneesiella echioides* (Elaiyaraja *et al*., 2016) and *Shuteria involucrata* (Senthamizh *et al.,* 2018). The odd chain fatty acid have been reported to play role in reducing risk for type 2 diabetes (Forouhi *et al*., 2014), while it acts as an antioxidant, antifungal and antimicrobial agents (Elaiyaraja *et al*., 2018). Tetradecanoic acid is used in antibiotics (Agoramoorthy *et al*., 2007), and this compound was also recorded in methanolic leaf extract of *Catharanthus roseus* and *Moringa oleifera* (Syeda and Riazunnisa, 2020). 2,4-heptadecadienoic acid; Et ester and heptadecanoic acid; Et ester and cyclopropane dodecanoic acid have been also found to present in the mixture of plants extract (Rajaduri *et al*., 2018) and these compounds are used in the preparation of antibiotics as well (Agoramoorthy *et al*., 2007).

Elements	**Quantity** (%)
Carbon (C)	44.92
Hydrogen (H)	6.159
Nitrogen (N)	5.1668
Sulphur (S)	0.427

Table 1: Proximate Composition of *Sorbaria tomentosa* whole plant.

CONCLUSIONS

The study showed that *S. tomentosa* has high content of carbohydrate and protein, low content of fats and moisture, and sufficient amount of macronutrients. GC-MS profile revealed fatty acids as the major compound bioactive compounds in the hexane fraction of the plants. These results support the use of *S. tomentosa* as effective and safe candidate in pharmaceutical utilization.

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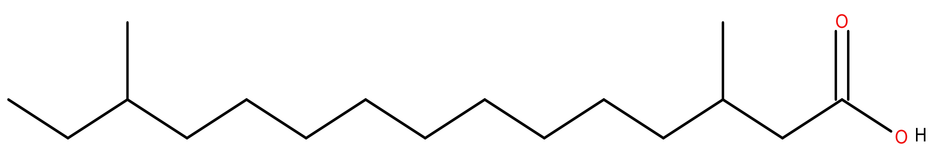
Table 2: Elemental analysis of *Sorbaria tomentosa* whole plant.

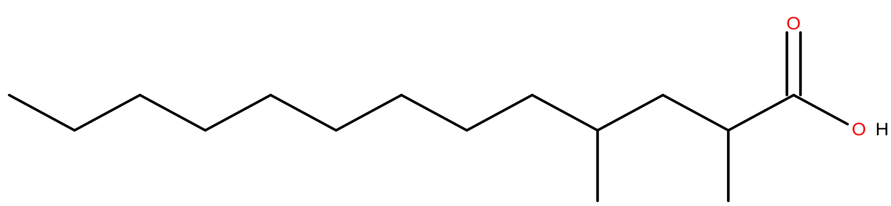
Parameters	**Quantity** (%)
Moisture	6.25
Ash	0.20
Fat	2.02
Protein	23.80
Carbohydrates	52.0
Energy content	1340 KJ 100 g^{-1}

Table 3: Bioactive components identified in the *n*-hexane fraction of *Sorbaria tomentosa* and their general biological activities (Duke, 2007).

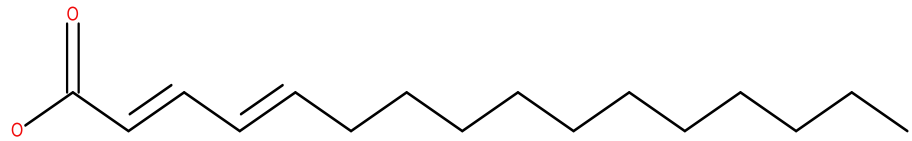
No.	Retention time (min)	Compounds	Molecular formula	MW	Peak area (%)	Biological activity
1	10.91	3,13-Dimethylpentadecanoic acid	$C_{17}H_{34}O_2$	270	24.20	Antioxidant, Allergenic, anesthetic, antibacterial, antifungal, anticancer, antimutagenic, antipeptic, antiseptic, antispasmodic
2	11.07	2,4-Dimethyltetradecanoic acid	$C_{16}H_{32}O_2$	256	6.61	Antioxidant, hypercholesterolemic, cancer-preventive, cosmetic
3	11.83	2,4-Heptadecadienoic acid; ethyl ester	$C_{19}H_{34}O_2$	294	14.32	Antioxidant
4	11.87	2-Butylcyclopropanedodecanoic acid	$C_{19}H_{36}O_2$	296	51.8	Antioxidant
5	12.01	Heptadecanoic acid; ethyl ester	$C_{19}H_{38}O_2$	298	3.07	Antioxidant



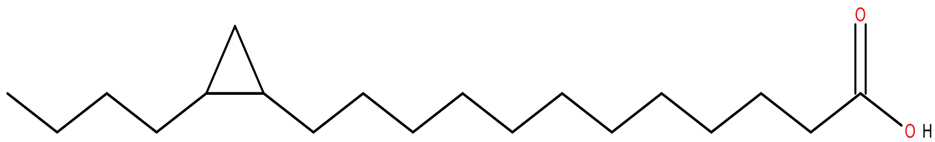
(**1**)



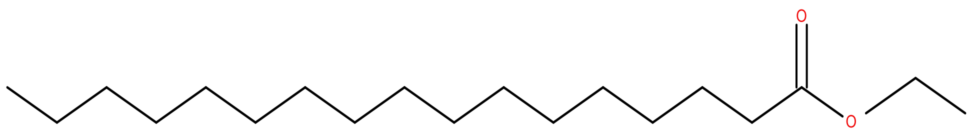
(**2**)



(**3**)



(**4**)



(**5**)

Fig. 1: Components identified in the hexane oily fraction of *Sorbaria tomentosa.*

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