

Original article

Biochemical and nutritional attributes of *Stevia rebaudiana* grown in Pakistan

Muhammad Farhan Jahangir Chughtai[1], *Imran Pasha*[1], *Masood Sadiq Butt*[1], *Muhammad Asghar*[2]

[1] National Institute of Food Science and Technology, Faculty of Food Nutrition and Home Sciences, University of Agriculture-Faisalabad - E-mail: m.farhan.chughtai@hotmail.com; [2] Department of Biochemistry, University of Agriculture-Faisalabad

Summary. Nutritional and health augmenting facets of *Stevia rebaudiana* as intense natural sweetener have been studied in different parts of world. Biochemical characterization of well adapted and well cultivated Pakistani Stevia have been carried out which is the need of hour to establish a better framework from nutritional viewpoint. Semi-arid conditions of central Punjab city, Faisalabad, have impacted the chemical composition that have been depicted in carbohydrates, crude protein, crude fat, crude fiber ash and moisture content as 63.59%, 10.64%, 5.47%, 7.60%, 8.75 and 3.95% respectively. Functional attributes have been well observed with slightly acidic to neutral pH 6.14, exhibits good swelling power, WHC, OHC, Bulk density. K, P, Mg, Na, Fe are found in maximum amount coinciding their ADI. Saturated, mono and polyunsaturated fatty acids like Palmitic, Palmitoleic, Stearic, Linoleic, Linolenic and Oleic have been identified in appreciable quantities like 28.31%, 2.17%, 2.39%, 13.65%, 25.48 and 4.95% respectively. FTIR mapping have concluded alcohols, alkanes, ketones, amines, esters, carboxylic acids, alkenes, hydroxyl groups as the major functional groups in raw powder and water extracts of Stevia. The study opens new horizons for further research covering safety aspects and steviosides characterization.

Key words: Pakistani Stevia, biochemical composition, mineral profile, fatty acids, AAS, GC-FID, FTIR mapping

1. Introduction

The ever growing food manufacturers are interested in substituting synthetic sweeteners with natural sources to provide segment of consumers that are calorie conscious, diabetics and unable to consume calorie rich/artificial sweetener in order to satisfy their requirements (1). Stevia *(Stevia rebaudiana)* is perennial herb, endemic to semitropical area of Paraguay, where it is well adapted to small niche environments between wetter marshlands and drier vegetated areas. Nowadays Stevia powder and extracts are consumed globally (2). The Stevia powder and extracts are sweeter than sucrose and have gained much more attention for being naturally zero caloric and not causing spikes in blood sugar levels. The sweetness bestowed to Stevia is due to diterpenes glycosides (Steviol glycosides): Stevioside (4-13%), rebaudioside A (2-4%), Dulcoside A (0.4-0.7%), rebaudioside C (1-2%) along with other less abundant types like rebaudioside B, rebaudioside F, steviolbioside, steviolmonoside and rubusoside (3). Food and drug administration (FDA) have given the GRAS (generally recognized as safe status) to Stevia and established the ADI (acceptable daily intake) for Stevia which is 4 mg/kg bw/day (4, 5).

In recent years, *Stevia rebaudiana* has earned global fame by successfully growing over diverse climatic domains. The biochemical composition of stevia is dependent on environmental conditions and plant cultivation techniques which have become the subject of several research topics (6). Stevia is getting the market place and emerged as synthetic/artificial sweeten-

ers replacer that do not provide the pragmatic taste of sugar with bitter after taste as well. Artificial sweetener disease (ASD) is affecting masses across USA, Europe and several other developed as well as underdeveloped countries. Artificial sweeteners have linked diseases which have the symptoms like anxiety, recurring headaches, depression, arthritis flare ups, unbearable migraines, muscle pain, chronic fatigue, buzzing or ringing in the ears, potential risk of bladder cancer, phenylketonuria, etc (7). Therefore, people are looking towards new alternates in which Stevia came out as alternate to be used in beverages, confectionaries, desserts and various delicacies that are popular in masses (8). Stevia is native to South American countries like Paraguay and Brazil, but now it is cultivated in Asian countries as well (9). Pakistan is blessed with different seasons and have excellent growing conditions including soil, water, fertilizer, etc. Stevia is grown in some parts of Pakistan specially Punjab province and acclimatized successfully to environmental conditions with 1.6-1.8 tons per acre yield. There is no research work done so far on biochemical characterization of Pakistani grown Stevia for its nutritional as well compositional parameters. Current research has been carried out to augment and provide sufficient information about Stevia acclimatized to Pakistani environment.

2. Methodology

2.1 Chemicals and reagent

The research work was carried out at National Institute of Food Science and Technology (NIFSAT), University of Agriculture, Faisalabad, Pakistan. *Stevia rebaudiana* (Stevia) leaves were procured from Ayyub Agricultural Research Institute (AARI), Faisalabad. All chemicals used in research were analytical grade and purchased from Sigma Aldrich, RCI Labscan, Merck, etc. Fatty acid methyl esters standards were obtained from Sigma Aldrich, St. Louis, MO, USA.

2.2 Preparation of raw material

The leaves were separated from the stalk, dust or any foreign material was removed and selected to provide a homogeneous quality of leaves based on the color and freshness according to visual analysis. Leaves were washed with water followed by drying in hot air oven at 30±5°C for 6 hours and then converted into powder by grinding dried leaves using high speed grinder. The resultant powder was stored in air tight containers prior to analysis. Stevia water extract obtained by mixing the stevia powder in water with subsequent heating for 30 minutes. After that centrifuged the solution for 15 minutes at 6000 rpm. The amber colored supernatant was collected and used for further analysis (10).

2.3 Chemical composition

The proximate composition of Stevia was done by following the method (11): moisture content (Method 925.09), crude fat (Method 920.39), ash (Method 923.03), crude fiber (Method 962.09) and crude protein (Method 954.01). The carbohydrate content was calculated by subtracting the rest of the components from the total dry weight and estimated as the nitrogen-free extract (NFE). All analyses were performed in triplicate and expressed in g/100 g dry matter (d.m.).

2.4 Functional properties

2.4.1 Bulk density

Bulk density of stevia powder was determined by using the method of (12). By using this method, 50 g stevia powder was put in 100 mL measuring cylinder. The cylinder was tapped several times on a laboratory bench to a constant volume. The volume of sample was recorded.

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Volume of sample after tapping}}{\text{Wt of sample}}$$

2.4.2. pH

The pH of stevia powder was determined by using pH meter (Inolab). KCl solution of 7 pH was used to standardize the equipment.

2.4.3. Swelling power

Swelling power of stevia powder was determined by using the method (12). According to this method, 1 g of the stevia powder was placed in a conical flask. It was hydrated with 15 mL distilled water, shook for 5 min with mechanical shaker at low speed. Heating was done for 40 min at 80-85°C with constant stir-

ring in a water bath. The content was transferred into a clean, dried and pre weighed centrifuge tube. 7.5 mL of distill water was added and centrifuged at 2200 rpm for 20 min. The supernatant was decanted into a pre-weighed can and dried at 100°C to a constant weight.

2.4.4. Emulsification capacity

Emulsification capacity of stevia powder was found by using the method (12). By using this method 2 g of stevia sample was blended with 25 mL distilled water for 30 sec in a blender at 1600 rpm. After complete dispersion, refine corn oil was added from a burette and blended until there was a separation into two layers of water and fat. Emulsifying capacity was expressed as mL of oil emulsified by 1 g of stevia powder.

2.4.5. Water absorption capacity

According to the method of (12), 5 mL of distilled water was added to 1g of stevia powder in a weighed 25 mL centrifuge tube. The tube was agitated on a vortex mixer for 2 min. It was centrifuged at 4000 rpm for 20 min. The clear supernatant was decanted and discarded. The adhering drops of water were removed and then reweighed. Water absorption capacity is expressed as the weight of water bound by 100 g dried stevia powder.

2.5 Mineral profiling of Stevia

Mineral profiling of Stevia was determined by using Flame Photometer (Sherwood Scientific Ltd., Cambridge, and Model 410) and Atomic Absorption Spectrophotometer (AA240, Varian). Samples were prepared by wet digestion method according to (11) method No. 965.17-968.08 in which HNO_3 and $HClO_4$ were used with 10:3 concentration. 2 g sample was ignited in order to remove the organic portion after that wet digestion was done on hot plate till the color of the fume becomes light green or clear. After that sample was diluted with 25 ml of deionized water and filtered for further analysis at flame photometer or AAS. The results are expressed in mg/100 g d.m.

2.6 Fatty acid profile

Fatty acid profile of stevia oil was estimated in such a way that oil was extracted by using Soxhlet apparatus using hexane as solvent and afterwards solvent is removed by rotary evaporator in order to get the pure oil. The fatty acids were converted to their respective methyl esters prior to analysis by following the method of (13, 14). By using Pasteur pipettes transferred 100 µL±5 µL of oil sample to the Pyrex test tube with a tight sealing cap. 5 mL of hexane was added in the test tube and vortex briefly to dissolve lipid. Then added 250 µL sodium methoxide reagent, cap the test tube tightly and vortex for one minute, pausing every 10 seconds to allow the vortex to collapse. Added 5 mL of saturated NaCl from Rci Labscanto the test tube capped the test tube and shacked vigorously for 15 sec and stand it for 10 minutes. After that removed the hexane layer and transferred to a vial containing a small volume of sodium sulphate from Sigma Aldrich. Now the hexane phase containing the methyl esters to be in contact with sodium sulphate for at least 15 min prior to analysis. Hexane layer was transferred to a vial for subsequent GC analysis. Fatty acid methyl esters were analyzed by using GC (Agilent 6890) equipped with Flame Ionization Detector (FID) as described by. GC analysis was performed on an Agilent 6890N Network GC system, under the following conditions: DB wax capillary column; 60.0m×0.25mm×0.25m; oven temperature programmed: the column held initially at 60°C for 3 min after injection, then increased to 185°C with 10°C/m in heating ramp for 1 min and increased to 200°C with 5°C/min heating ramp for 10 min. Then the final temperature was increased to 220°C with 5°C/m in heating ramp for 20min; injector temperature, 250°C; detector (FID) temperature, 275°C; carrier gas, nitrogen; inlet pressure, 40.65 psi; linear gas velocity, 39 cm/s; column flow rate, 2.7 mL/m in; split ratio, 40:1; injected volume, 1 µL.

2.7 Mapping with FT-IR

The Fourier transform infrared spectra of Stevia leaves powder and water extract were recorded in order to characterize various functional groups present, gather sufficient information about the dried powder and water extract of stevia. A total scan of 16 scans per sample with a 4-cm^{-1} interval of spectral resolution were obtained over operating in the mid-infrared region of 400 to 4000 cm-1 using Tensor 27 by Bruker Optics GmbH, Ettlingen, Germany with OPUS Data Collection Program. The instrument was fitted with a

sealed and desiccated interferometer, with a deuterated triglycine sulfate (DTGS) detector. The standard sample cell in the FT-IR was a Pike Miracle single bounce attenuated total reflectance (ATR) cell equipped with a ZnSe single crystal comprising transfer optics within the chamber through which the infrared radiation was directed to ATR crystal and a KBr beam splitter. The zinc selenide crystal, a 45° parallelogram with mirrored angled faces with 10 internal reflections, was mounted in a plate with a shallow trough for sample containment. The depth of penetration, which gives a measure of the intensity of the resulting spectrum, was 60 µm. All spectral measurements were made at 32-cm^{-1} resolutions, with 256 interferograms co-added before Fourier transformation (15, 16).

3. Results and Discussion

3.1 Chemical composition

Proximate composition is a key factor for assessing the quality of raw material. It helps to formulate a quality stable product. Chemical composition of stevia leaves powder have been found by different scientists previously. Findings of current research are in accordance with some variations to previous researches. Variation in results obtained from Pakistani grown stevia may be due to environmental factors like soil and water quality, genetic makeup, growing conditions, temperature variations and genetic makeup. Stevia is not indigenous to Pakistan as it is native to South American countries like Paraguay, Brazil, Canada, etc. Stevia acclimatized to Pakistani environment but the environmental parameters are quite different as compared to South American countries.

Stevia leaves powder (dry weight basis) was subjected to chemical composition analysis expressed in Table 1 and Figure 1. Moisture content was found to be 3.95±0.21%. Previous studies, (8, 12, 17-21), have found the moisture content varying from 7%, 4.65%,

Table 1. Proximate analysis (%) of Stevia leaves powder

Parameter	Stevia Leaves Powder
Moisture	3.95±0.21
Ash	8.75±.035
Crude Protein	10.64±0.03
Crude Fat	5.47±0.06
Crude Fiber	7.60±0.05
NFE/ Carbohydrate	63.59±.62

Values expressed are means ± standard error

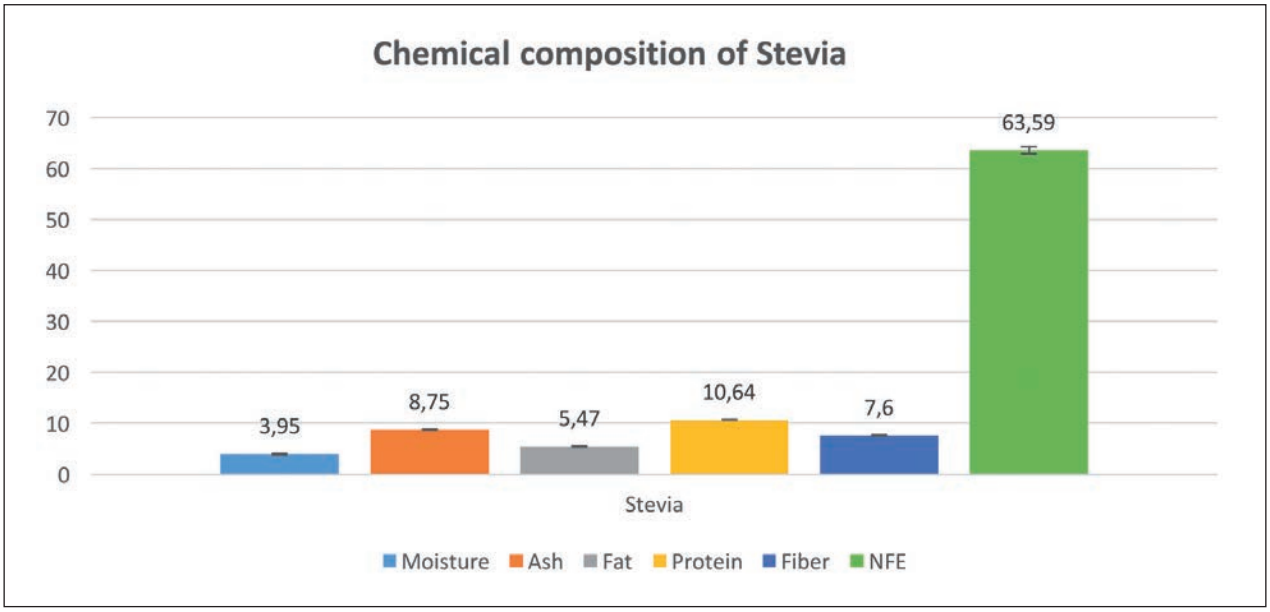

Figure 1. Chemical composition of Stevia leaves powder

5.37%, 7%, 7.45-7.80%, 4.45-10.73% and 6.97-8.06% respectively. The ash content found in this study was 8.75±.035%. However, in previous studies ash content have been reported to be 7.73-9.25%, 7.82-11.93%, 7.41%, 11%, 13.1% and 10.5% according to the studies of (12, 17, 19-22) respectively.

Stevia has been found to be very impressive in protein content representing as a good source for the development of body structure and various physiological functions. Previously various scientists have conducted research on chemical composition of Stevia and found it to be out of the box if we consider any other plant material as a protein source. In this study, crude protein is found to be (10.64±0.03%). However (17), declared 9.8% of protein content in their study (22), found the maximum protein content which is 20.4% (18, 23), found it to be 11.2%. On the other hand (8), in Chinese Stevia (12), in Mexican grown Stevia and (21) declared in their studies to be 12.44-13.68, 12.11-15.05, and 10.51-11.75%.

Lipids are biologically active substances essential for the human organism, storing energy, forming elements of cell membrane structures and regulating physiological functions. In this study, crude fat was find out to be 5.47±0.06%. The results are in accordance with studies of various scientists like (8, 12, 18, 19, 22, 23), which reported that fat content is in appreciable amount varying to be 1.9%, 3.73%, 4.34%, 5.6%, 4.18-6.13% and 3.04-3.23% respectively.

Crude fiber finds out to be 7.60±0.05% which is in considerable amount as compared to previous studies. In this context, stevia crude fiber varies according to (3, 8, 19, 21, 23, 24), which declared that 9.52-10.65%, 12.09-13.17%, 4.34-5.26%, 2.6%, 3.73% and 5.6% respectively. Carbohydrates or Nitrogen free extract (NFE) are the main sources of energy and they are found as structural components of cellular elements and in current study NFE is find out to be 63.59±2.62%. However, previously it has been reported to be 63.73-66.43%, 63.10-73.99%, 72.42-79.77%, 61.9%, 53% and 35.2% according to (3, 8, 19, 21-23) respectively.

3.2 Functional Properties of Stevia leaves powder

The functional properties of any food helps in assessing appropriateness of that particular ingredient of cooking and in different aspects of handling. These determine the application and use of different components for food products. Different functional properties of stevia powder have been determined by using their respective protocols including water absorption capacity, bulk density, pH, swelling power and emulsification value. The results are expressed in table 2 and graphically presented in figure 2.

pH is a measure of hydrogen ion concentrations of particular solution which determines the alkalinity as well acidity. Aqueous solutions at 25°C with a pH <7 is acidic in nature while pH >7 refers to alkaline solution, however pH 7.0 is neutral. pH of stevia powder dissolved in deionized water was found to be 6.1 which is slightly acidic and close to neutral showing that hydrogen ions concentration is less than hydronium ion (H_3O^+) concentration. The results of Pakistani grown stevia is found to be in range of previously done studies on functional properties of Stevia grown in different climatic conditions (17) as well as (20) have declared 5.95 pH of stevia. However (8), have found Stevia pH in the range varying from 5.95-6.24.

Bulk density is a property of granules, divided solids and powders which are particularly used in foodstuffs as ingredients or any other masses of particulate matter. It is the ratio of weight particles and the volume occupied by these particles. These particles have different volumes which contribute to the total volume of the matter. This includes internal pore, inter-particle and particle volumes. Foods with higher bulk densities are considered desirable by reducing paste thickness especially in child feeding where bulk is of concern. Stevia leaf powder appears to lack this property. Bulk density of Stevia powder was reported to be lower than

Table 2. Functional properties of Stevia leaves powder

Functional property	**Stevia**
pH	6.143±0.077
Swelling power gm/gm	5.003±0.029
Water Absorption/Holding Capacity ml/gm	3.933±0.049
Emulsification value/Oil-Holding Capacity ml/gm	5.963±0.069
Bulk Density gm/ ml	0.547±0.061

Values expressed are means ± standard error

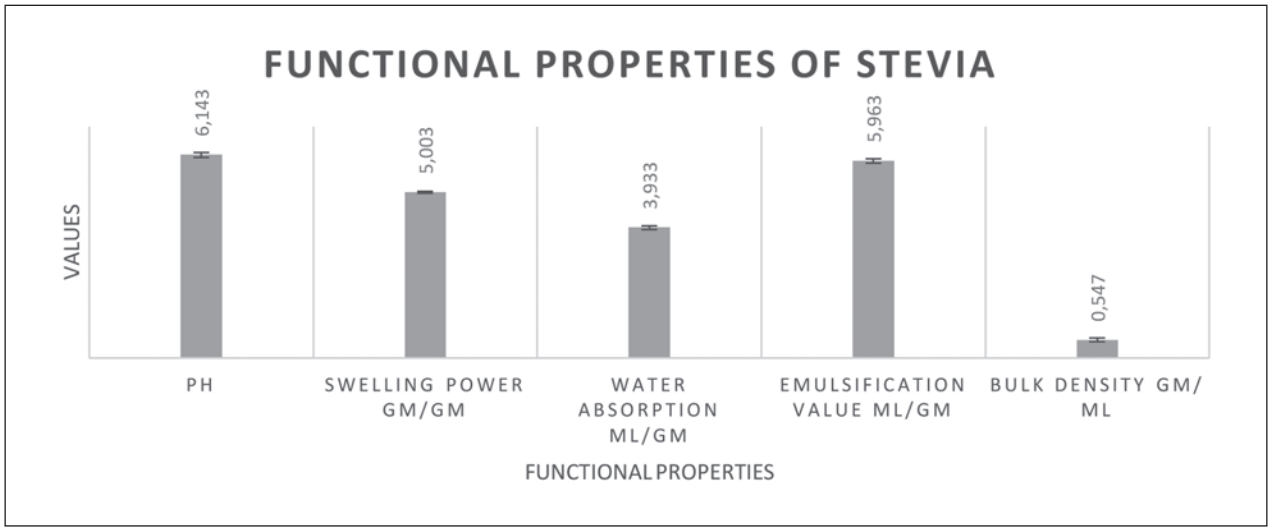

Figure 2. Functional properties of Stevia leaves powder

pulses and find out to be 0.547±0.061gm/ml in this study (20) found 0.443 gm/ml bulk density of Stevia powder.

In food product development, protein or fiber enriched foodstuffs are important for their various properties to control how much water they can hold. Water holding capacity (WHC) relates to many sensory, health, nutritional and convenience properties. Thermal processing of protein-rich food is accompanied by a loss of water (containing various nutritional components), so better control of WHC means reducing loss of the nutritional value. WHC of indigenous Stevia is found to be 3.933±0.049ml/gm. WHC of Stevia is beneficial in recipe management and considered due to high protein content. Proteins play their role to increase water holding capacity and stevia have considerable good protein content (12) showed in their studies that stevia water holding capacity ranges from 2.87-4.07 ml/gm while (17) have found it as 4.7 ml/gm.

Swelling power or ability is an important protein function for preparation of soups, gravies, confectionaries and baked products. Swelling power is composition and stress depended that the product gets during processing. Therefore, the swelling ability or power of indigenous Stevia which is 5.003±0.029 gm/gm and found to be in accordance with the results of (20, 17) which declared it as 5.01 gm/gm.

Fat absorption capacity determines the entrapment of oil in food preparation. Stevia powder have been reported to have adequate fat absorption capacity which helps in food processing by retaining flavors and increasing mouthfeel. Indigenous Stevia shows good fat absorption capacity which appears to be 5.963±0.069 ml/gm making it desirable for usage as main stream sweetener in bakery as well as beverage industry. Similar results were found by (9, 20) which is 5.0 ml/gm. However (12), reported it to be in range of 6.49-6.79 ml/gm.

3.3 Mineral profiling of Stevia

Minerals/metals elements are the diet components inevitable for the upkeep of life and good health. Metabolic processes need them for proper functioning; some are required in major quantity while other in minor or trace amount for proper metabolic processes. Majority of mineral elements are part of earth crust. Major elements that are required include magnesium, potassium, chlorine, sodium, phosphorous, Sulphur, calcium are classified as macronutrients. However micronutrients include iron, cobalt, zinc, copper, selenium, iodine, molybdenum, manganese, etc (25, 26). From nutritional point of view, the recommended average daily intake (ADI) of minerals like Na, K, P, Mg, Fe, Zn, Mn, Cu, Ni and Co are 2400 mg, 3500 mg, 1000 mg, 350 mg, 15 mg, 15 mg, 5 mg, 2mg, <1 mg and 5 µg respectively per day have been reported by (27).

Stevia leaves have good mineral profile with nutritionally important essential minerals in reasonable

amount in fresh as well as dried leaves. In this study macro, micro, trace metals have been investigated both qualitatively as well as quantitatively which are exhibited in Table 3. Potassium (K) is found to be in appreciable quantity have the highest value 2195.3 mg/100 g which can be due to the soil fertility as in Pakistan NPK fertilizers are used in quite an amount for the proper growth and soil fertility management that ultimately affect the amount of potassium in Stevia. The results found are quite near to the findings of (28) which have reported it to be ranging from 1585-1915 mg/100 g. However, previously (20, 22) have reported it as 1800 and 2510 mg/100 g respectively. The results of Phosphorous (372.1 mg/100 g) and magnesium (286.2 mg/100 g) are coincidences with the outcomes of (9, 28) who reported them to be in range of 318 mg/100 g and 349 mg/100 g. Sodium (29.4 mg/100 g), iron (24.9 mg/100 g) and manganese (10.24 mg/100 g) being important in maintaining the blood pressure, brain and nervous functions, play role in fat and carbohydrate metabolism, blood cells production, sugar regulation and oxygen transport in the body as part of hemoglobin and myoglobin. The similar results for sodium, iron and magnesium have been reported by (17, 19) with some difference due to water and soil as they affect the amount of these minerals. Zinc (1.423 mg/100 g), nickel (1.26 mg/100 g), copper (0.85 mg/100 g) and cobalt (0.35 mg/100 g) have an important impact *i-e* zinc is required for growth, tissue repair, immune system, construct and maintain DNA, helps in tissue repair and wound healing. Nickel provide optimal skin growth, bone strengthening and structuring and enhances zinc absorption, its deficiency leads to dermatitis and retarded growth rate (3, 19, 24), have reported the similar results for these minerals.

Table 3. Mineral profile of Stevia leaves powder

Minerals	Value (mg/100g)
Na	29.4±0.12
K	2195.3±0.88
P	372.1±16.74
Mg	286.2±0.06
Fe	24.29±0.04
Zn	1.423±0.04
Mn	10.24±0.06
Cu	0.85±0.02
Ni	1.26±0.02
Co	0.035±0.02

Values expressed are means ± standard error

3.4 Fatty acids profiling of Stevia

Fats are the most concentrated form of energy for body (37 kJ/g) helping in absorption of fat soluble vitamins (A, D, E and K). Chemically composed of triglycerides, with a unit of glycerol attached with three same or different fatty acids which ultimately change the chemical nature of fatty acids as well. Fatty acids being saturated or unsaturated present in different proportion in food components and have nutritional as well medicinal importance (29). Omega-3 and omega-6 fatty acids are two major classes of polyunsaturated fatty acids (PUFAs). Omega-3 fatty acids (omega-3s) have a carbon-carbon double bond located three carbons from the methyl end of the chain including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (30). However, Omega-6 fatty acids (omega-6s) have a carbon-carbon double bond that is six carbons away from the methyl end of the fatty acid chain. The human body can only form carbon-carbon double bonds after the 9th carbon from the methyl end of a fatty acid. Therefore, ALA and linoleic acid are considered essential fatty acids, meaning that they must be obtained from the diet. ALA can be converted into EPA and then to DHA, but the conversion (which occurs primarily in the liver) is very limited, with reported rates of less than 15% (Harris, 2010). Therefore, consuming EPA and DHA directly from foods and/or dietary supplements is the only practical way to increase levels of these fatty acids in the body (31).

In this research work, fatty acids profile of stevia leaves dried by using hot air oven for saturated, monounsaturated and polyunsaturated fatty acids was investigated and identified by comparing with standards of 21 fatty acids methyl ester kit using GC-FID. The results are expressed in table 4, figure 3 and 4 are chromatograms for fatty acid methyl esters standards and Stevia fatty acids, while figure 5 is the graph de-

Table 4. Fatty acid profile of Stevia leaves powder

Serial No.	Fatty acid	Carbon No.	Cons. of Fatty acids (g/100g)
1	Caprylic acid	C:8	ND
2	Capric acid	C:10	ND
3	Lauric acid	C:12	ND
4	Tri-decanoic acid	C:13	ND
5	Myristic acid	C:14	ND
6	Myristoleic acid	C:14:1	ND
7	Pentadecanoic acid	C:15:0	ND
8	Palmitic acid	C:16:0	28.31
9	Palmitoleic acid	C:16:1	2.17
10	Heptadecanoic acid	C:17:0	ND
11	Stearic acid	C:18:0	2.39
12	Oleic acid	C:18:1	4.95
13	Linoleic acid	C:18:2	13.65
14	Arachidic acid	C:20:0	ND
15	Eicosaenoic acid	C:20:1	ND
16	Linolenic acid	C:18:3	25.48
17	Behenic acid	C:22:0	ND
18	Erucic acid	C:22:1	ND

picting the stevia fatty acids. Saturated fatty acids like Caprylic acid (C:8:0), Capric acids (C:10:0), Lauric acid (C:12:0), Tri-decanoic acid (C:13:0), Myristic acid (C:14:0), Pentadecanoic acid (C:15:0), Heptadecanoic acid (C:17:0), Arachidic acid (C:20:0) and Behenic acid (C:22:0) have not been detected due to negligible amount or unavailability. However, Palmitic acid (C:16:0) and Stearic acid (C:18:0), the only two saturated fatty acids that have been found in appreciable quantities 28.31 g/100 g and 2.39 g/100 g respectively.

On the other hand, monounsaturated fatty acids identified in stevia leaves include Palmitoleic acid (C:16:1), Oleic acid (C:18:1) have been detected and ranges as 2.17 g/100 g and 4.95 g/100 g respectively. Myristoleic acid (C: 14:1), Eicosaenoic acid (C: 20:1), Erucic acid (C: 22:1) are the monounsaturated fatty acids that have shown no amount in stevia extracted oil.

Linoleic acid (C: 18:2) and linolenic acid (C: 18:3) are the polyunsaturated fatty acids also known as omega-3 fatty acid alpha linolenic acid (ALA). Both are the essential fatty acids, therefore these are needed

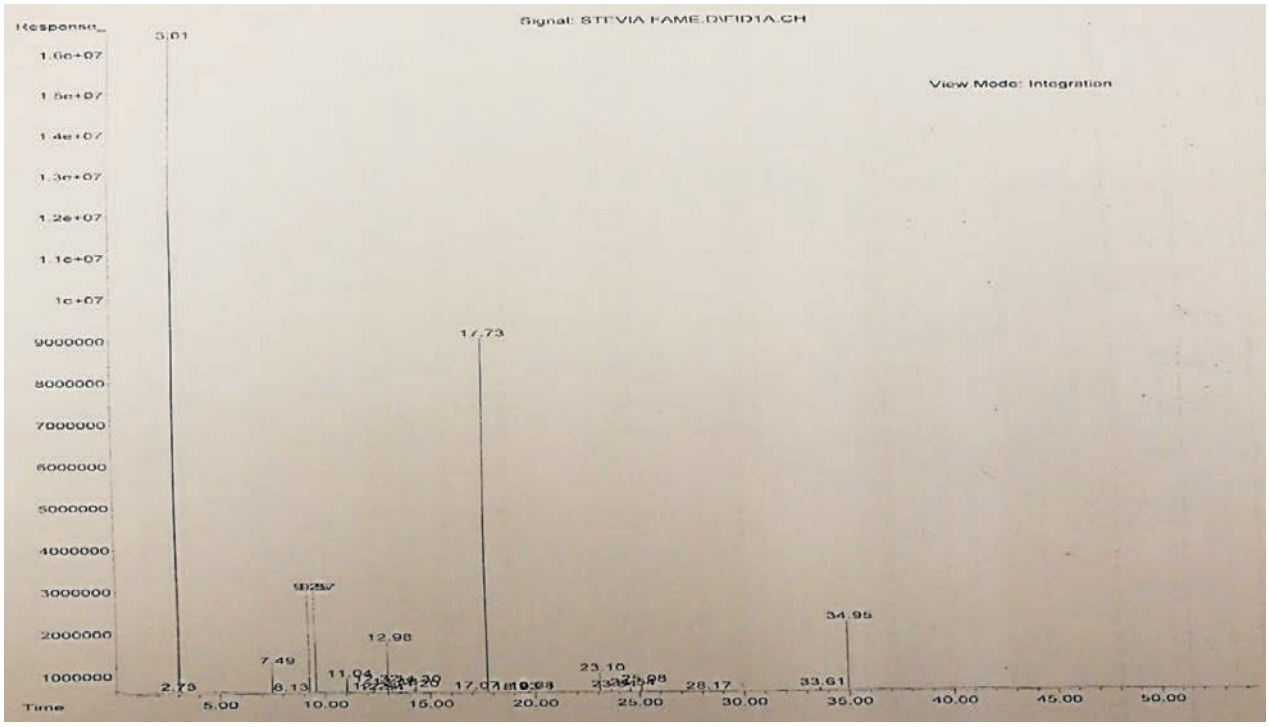

Figure 3. Fatty acids methyl esters standard chromatogram

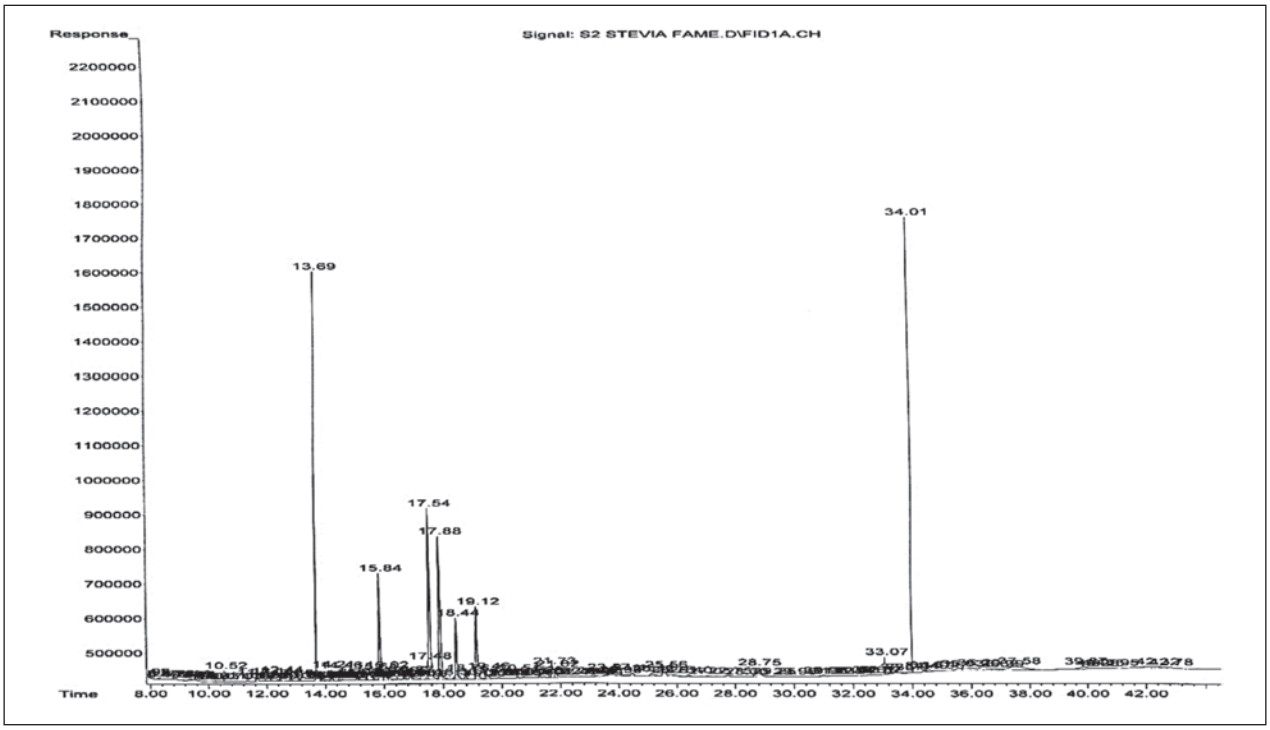

Figure 4. Stevia Fatty acids chromatogram

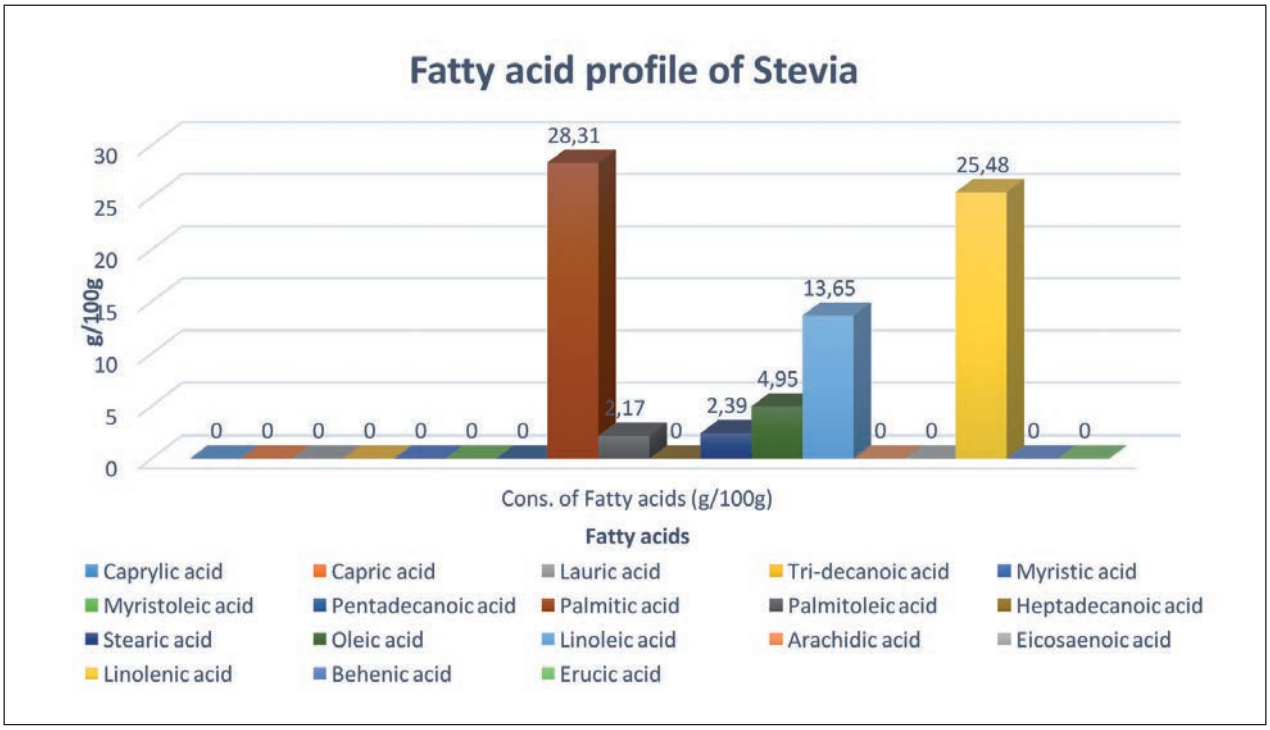

Figure 5. Fatty acid profile of Stevia leaves powder

from diet for proper metabolic functioning and energy generation. Linoleic and linolenic fatty acids are found in good amount in stevia oil with 13.6 g/100g and 25.48 g/100 g respectively. Though stevia is not a very good source of lipids or oils and gives only a small amount of it, however its fatty acid profiling make it peculiar and a plant of interest for some healthy as well as handsome amount of essential and non-essential fatty acids (32). Essential fatty acids have crucial impact both in life and death of heart cells (33). The results obtained in this study are found to be similar with few previous investigations performed on Stevia grown in diverse environmental conditions. According to the research outcomes of (22), Palmitic acid, Linoleic and linoleic acids have been found to be in high amount varying from 27.51 g/100 g, 12.40 g/100 g and 21.59 g/100 g respectively. However, Palmitoleic, Stearic and Oleic acids are in minute quantities 1.27 g/100 g, 1.18 g/100 g and 4.36 g/100 g respectively.

In a research work conducted in Bangladesh by (34) for determining the chemical composition of stevia leaves oil extracted by hydro-distillation method using gas chromatography mass spectroscopy (GC-MS) in which they declared that Palmitic acid found in high relative percentage reported 86.50% while stearic and linoleic acids being the least abundant with share of 2.20% and 3.26% respectively.

Recently (35), have worked on the stevia dried from different techniques and investigated the fatty acid profile of stevia. Palmitic, Stearic and Oleic acids found in low amount ranging from 0.46-1.47%, 0.23-0.47% and 0.45-1.39% respectively. However they have discovered Linoleic and Linolenic acids in appreciable amount ranging from 1.37-2.22% and 1.36-5.96% respectively.

3.5 FTIR mapping of Stevia

The Fourier transform infrared spectroscopy (FTIR) is used in order to characterize and identification of different functional groups present in Pakistani grown stevia. FTIR analysis of chemical constituents and Steviol/diterpene glycosides in stevia leaves powder and water extract was carried out. The functional groups corresponding to their peaks in infrared spectra (Fig. 6) of Stevia leaves powder is shown in Table 5. The IR spectra for raw stevia powder gives different bands indicating the particular functional groups at distinct IR wavelength. At 3301.05cm^{-1}, a broad band showed the presence of alcohols -OH groups stretching as well as secondary amides groups. This indicates protein availability as amides serve as defining molecular character of proteins. Hydrogen bonding capabilities represents secondary amide structures. However, sp2 and sp3 hybridization of carbon is indicated by

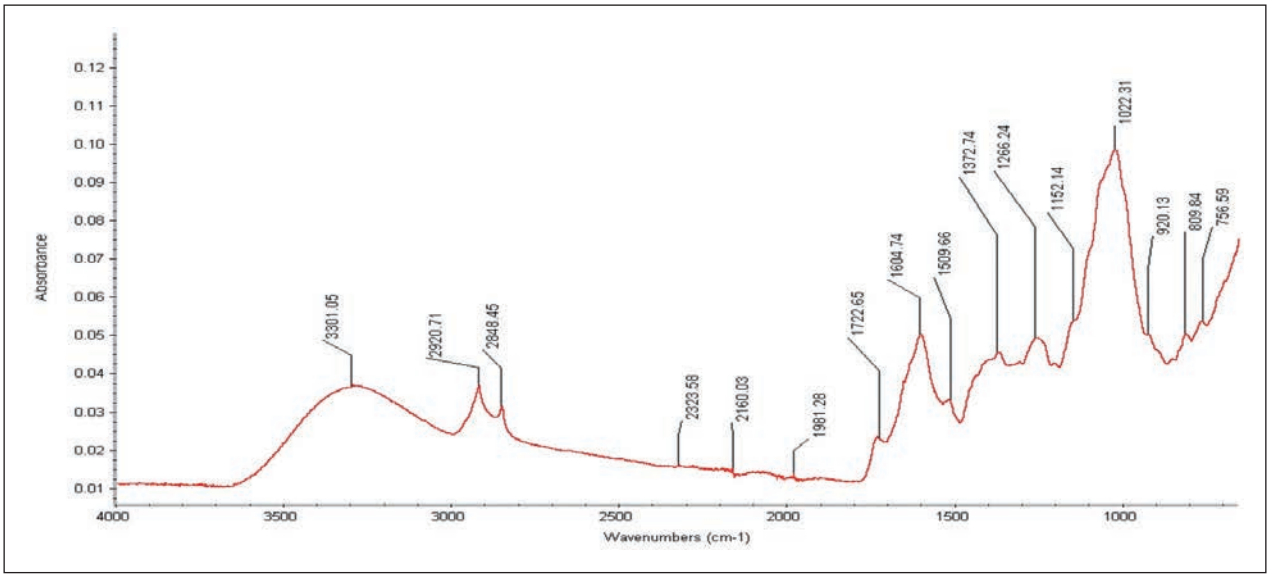

Figure 6. FTIR spectrum of Stevia leaves powder

Table 5. FTIR spectrum values of Stevia leaves powder

Sr. No.	Wave Number	Functional group	Vibration type
1	3301.05	Alcohols, secondary amides	O-H stretching, N-H
2	2920.71	Alkane	$=CH_2$ stretching
3	2848.45	Alkane	C-H stretching
4	1604.74	Ketones	C=O stretching
5	1509.66	Alkene, Primary amines	C=C stretching, N-H
6	1372.74	OH Bending	-OH stretching
7	1022.31	Esters	(RCOOR`)
8	809.84	Alkanes, Carboxylic acids	C-C, O-H stretching

the peaks at 2848.45 cm^{-1} and 2920.71 cm^{-1} respectively. These indicates the presence of compounds with alkane functional groups and configurations. Amide linkages in a biochemical context are called peptide bonds when they occur in the main chain of a protein and isopeptide bonds of protein. The 1604.74 cm^{-1} band in IR spectra of stevia leaves powder indicates the ketone C=O stretching group components which is attributed flavor along with different aldehyde groups. Alkenes and primary amines have been observed according to the C=C stretching at 1509.66 cm^{-1} which are important components of all steviosides ranging from steviosides to Steviol, etc. At 1372.74 cm^{-1} bending of -OH groups have been seen which is an important constituent of different chemical groups including glucose attached to the Steviol which is considered as the basic building block to all steviosides. Bands at 1022 cm^{-1} and 809.84 cm^{-1} in IR spectrum are attributed to RCOOR` stretching of ester groups, alkanes (C-C) and carboxylic groups (ROOH) stretching respectively.

The FTIR spectrum of stevia water extract was analyzed (Fig. 7) and different bands were obtained which coincides with stevia leaves powder spectrum which are presented in table 6. However, number of stretching bands from different functional groups are less as compared to stevia powder. These bands help in identification of Steviol glycosides present in stevia water extract. At 3330.0 $cm^{-1,}$ distinct and broad band was observed which indicate the -OH alcoholic groups stretching, however -COOH carboxylic acids also shows their presence at this IR wavelength. Concentration of steviosides, nature solvent extract which is water and temperature for extraction process plays an important role in maximum absorption of IR which gives a broad and prominent band. Compounds having carboxylic groups attached have been identified from

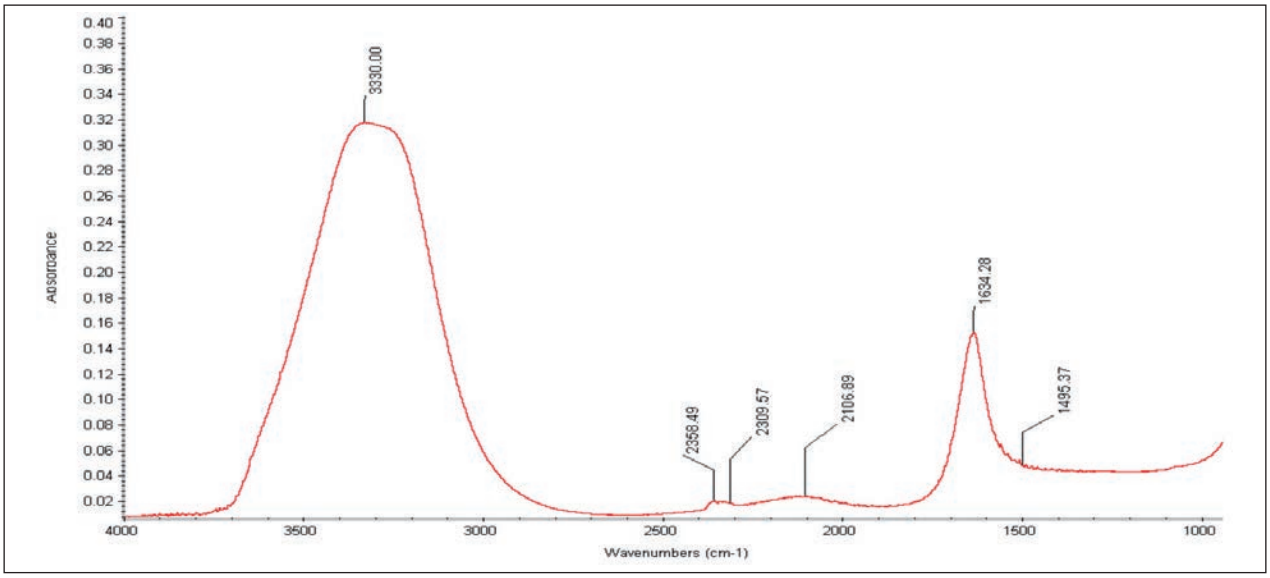

Figure 7. FTIR spectrum of Stevia water extract

Table 6. FTIR spectrum values of Stevia water extract

Sr. No.	Wave Number	Functional group	Vibration type
1	3330.00	Alcohols, Carboxylic acids, Amines	-OH, -COOH, N-H stretching
2	2358.49	Thiol, CO_2	S-H, O=C=O stretching
3	1634.28	Alkene, Inorganic phosphates	C-H, P, C=C stretching
4	1495.37	Alkanes, Aromatic groups	C-H, -NO_2 stretching

band position. Primary amines have been observed which indicate high protein content in stevia powder and water extract as well. At 2358.49 cm^{-1}, stretching of thiol as well as carbon dioxide (S-H and O=C=O) group have been observed which ultimately indicates the presence of Sulphur compounds. Absorption shifts are directly dependent on concentration of components that can clearly observed in intermolecular bonds of different molecules. The presence of a band at 1634 cm^{-1} are assigned to alkenes, inorganic phosphates, C=C groups stretching. The band at 1495.37 cm^{-1} indicates the presence of -CH_2 stretching of alkane group, C=C stretching and -NO_2 stretching representing aromatic groups which are attached to Steviol base which is benzene ring having different functional groups attached to it indicating the presence of diterpene glycoside in water extract of Stevia (15) have published the functional groups identification of stevia leaves. However, in current study elaborated results on functional groups mapping of stevia leaves powder and stevia water extracts have been discussed.

Conclusion

Stevia rebaudiana has progressively sweeping range of global appliance not only as a sweetener, but also as a nutrient rich additive minimizing the calorie intake for diabetics and diet conscious masses. Current research concluded that Pakistani stevia is well adapted and endorse health boosting equities. The chemical composition illustrated that stevia is rich source of proteins and carbohydrates which declares it not only as sweetener but food additive as well. The results of functional properties from indigenous Stevia affirmed its application in product development and stabilities. The mineral and fatty acid profile is of worth importance, affirming appositeness as collateral in health subsistence and chronic ailments cure. FTIR studies showed variety of functional groups and moieties availability that can be useful in future research management.

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Correspondence:
Muhammad Farhan Jahangir Chughtai
National Institute of Food Science and Technology,
Faculty of Food Nutrition and Home Sciences,
University of Agriculture-Faisalabad
E-mail: m.farhan.chughtai@hotmail.com