OPEN ACCESS

International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

ISSN 2091-2609

Indexing and Abstracting

CrossRef, Google Scholar, Global Impact Factor, Genamics, Index Copernicus, Directory of Open Access Journals, WorldCat, Electronic Journals Library (EZB), Universitätsbibliothek Leipzig, Hamburg University, UTS (University of Technology, Sydney): Library, International Society of Universal Research in Sciences (EyeSource), Journal Seeker, WZB, Socolar, BioRes, Indian Science, Jadoun Science, Jour-Informatics, Journal Directory, JournalTOCs, Academic Journals Database, Journal Quality Evaluation Report, PDOAJ, Science Central, Journal Impact Factor, NewJour, Open Science Directory, Directory of Research Journals Indexing, Open Access Library, International Impact Factor Services, SciSeek, Cabell's Directories, Scientific Indexing Services, CiteFactor, UniSA Library, InfoBase Index, Infomine, Getinfo, Open Academic Journals Index, HINARI, etc.

CODEN (Chemical Abstract Services, USA): IJASKD

Vol-3(4) December, 2015

Available online at:

http://www.ijasbt.org

&

http://www.nepjol.info/index.php/IJASBT/index

SEM-Biotech Publishing

Impact factor*: **1.422**
Scientific Journal Impact factor#: **3.419**
Index Copernicus Value: **6.02**
IBI Factor 2015**: **4.19**

*Impact factor is issued by Universal Impact Factor. Kindly note that this is not the IF of Journal Citation Report (JCR).
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M.M.A. Shuvo et al. (2015) Int J Appl Sci Biotechnol, Vol 3(4): 721-726
DOI: 10.3126/ijasbt.v3i4.13972

ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

AN ASSESSMENT OF MAJOR NUTRITIONAL COMPONENTS AND SOME SECONDARY METABOLITES OF *IN VITRO* PROPAGATED *STEVIA REBAUDIANA* (CULTURED IN BANGLADESH) PLANT LEAVES DRY POWDER

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Abstract

Stevia rebaudiana, belongs to the family of Asteraceae, is a perennial and medicinal shrub. It's leaves dry powder widely used as a natural sweetener which has no calories but reported to be 50– 300 times sweetener as sweet as sugar, that are said to be having insulin balancing properties without any side effects. This research was first time conducted in Bangladesh where a quantitative analysis were performed for determining the major constituents of *in vitro* propagated *Stevia rebaudiana* plant leaves dry powder that was prepared through oven dry after sun dry. The analysis revealed the powder as mild acidic (P^H 5.345). Total chlorophyll contents were found to be 0.845mg% where chlorophyll–a and -b were 0.088 and 0.761 mg% respectively. The major macro nutrients such as ash, crude fiber, total carbohydrate, total protein and fat were determined at the amount of 7.05, 10.5, 53.52, 13.13 and 3.55 gm% respectively where the micro nutrients like Iron (Fe), Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg), Phosphorus (P) and Chloride (Cl) were found at the amount of 34.2, 184.3, 2500, 534.43, 465.34, 304.7 and 49.6 mg% accordingly. Screening for secondary metabolites showed the presence of alkaloids, flavonoids, saponins and tannins as the amount of 2.83, 17.20, 6.69 and 5.84 mg% respectively in dry powder. These findings also have been compared with the other reported values on *Stevia* from different countries of the world.

Keywords: *Stevia rebaudiana*; Nutrition; Minerals; Secondary Metabolites.

Introduction

Stevia rebaudiana plant leaves dry powder is a non-caloric alternative to artificially produced sugar substitute which traverse to the chemical degradation process without breaking down, and is safe for those who need to control their blood sugar level, especially for the diabetic patient. In recent studies depicted, the locally found various types of synthetic or pharmaceutical sweetener like sucralose, aspartame, saccharine etc. which are used as a substitute of sugar, is associated with various types of potential risk like migraines, memory loss, slurred speech, dizziness, stomach pain, seizures, obesity, diabetes and heart diseases, including bladder cancer (Jaroslav *et al.*, 2006). Whereas this endemic shrub, originated in the culture of native Guarani tribes of Paraguay, becoming high demanding uses as a natural sweetener and also considered as a medicinal plant against various types of complicated diseases.

Stevia plants, discovered by Antonio Bertoni (a South American Natural Scientist) in 1887, are widely situated along the 25th Degree line in South Latitude. It is a subtropical plant which prefers the climate of 75^0 F of mean temperature and semi - humid. Studies showed that its leaves contain diterpene glycosides, commonly known as Stevioside, Rebaudioside A – F, Ducloside A and Steviolbioside (Kinghorn *et al*, 1985), which are responsible for the typical sweet taste (GRAS Assessment) and its extract contain chlorophylls, xanthophylls, hydroxyl cynnamic acids (caffeic, chlorogenic etc), oligosaccharides, amino acids, lipids and trace elements, alkaloids, flavonoids, saponins and tannins (Gasmalla *et al.*, 2014).

Stevia has versatile medicinal uses for the treatment of obesity, diabetes, cavities, hypertension (Dyrskog *et al.*, 2005; Jeppesen *et al.*, 2000, 2002, 2003) and dental carries (Das S *et al.*, 1992). It is also characterized for having anti – hyperglycemic (Jeppesen *et al.*, 2002), anti-tumor and antibacterial (Satishkumar *et al.*, 2008), anti-viral (Kedik *et al.*, 2009) and anti-fungal (Silva *et al.*, 2008) properties. Stevia is an ideal sweetener and recommended for diabetes, tested on animals and used by humans with no side effects (Megeji *et al.*, 2005). Glycosides of Stevia leaves which are responsible for sweet taste do not participate in the metabolic process and are eliminated from the body with no caloric absorption (Mantovaneli *et al.*, 2004). In view of this, the objective of this study was to evaluate its major

nutrients, minerals and phytochemical compositions to concern people of our country for using Stevia leaf extract or dry powder as a substitute of sugar rather than synthetic sugar substitute which have partial toxicity and wide range of damage inside the body.

Materials and Methods

Sample Collection and Taxonomy

In Bangladesh, tissue culture methods are followed for the rapid mass propagation of Stevia Plant in some nurseries in the month of June to August, 2014 due to its low germination percentage. For this analysis Stevia plants were collected from the Green Nursery at October, 2014 (Mirsorai, Dhaka - Chittagong highway) and brought to the ethno - botany lab for taxonomy, where most of the plants were found 30 cm in height with 3-4 cm long, sessile, elongated – lanceolate or spatulate shape with blunt – tipped laminal leaves. The upper surface of the leaf was slightly glandular pubescent. The stem was weak at bottom and woody. Flowers were white, tubular and bisexual (Classified & identified by the Department of Botany, Chittagong University, Chittagong 4331, Bangladesh).

Scientific Name : *Stevia rebaudiana* (Bertoni) Bertoni

Synonyms : *Eupatorium rebaudianum* Bertoni

Stevia rebaudiana (Bertoni) Hemsl.

Sample Preparation

The mature fresh green leaves were collected before flowering from the plants. The leaves were removed from the plants and washed in clean running water and spread on trays. Then the leaves were allowed for drying under sunlight for five days. Again, they were dried for 24 hrs at 37^0 C in an incubator (Brand: Binder, Model: E 28, Country: Germany). Then fine powders from dry leaves were prepared by using high speed blending machine (Brand: Miyako, Model: BL – 152 PF - AP, Speed: 25000 RPM) for 3 times until powder form and then again ground with mortar and pestle for getting fine powders. Fine powders from dry leaves were used as sample for experimental purpose.

Proximate Chemical analysis of Stevia dry leaves powder

Determination of P^H

2 gm dry powder were homogenized well with 30 ml distilled water and then filtered through Whatman's No.1 filer paper. Then the filtrate was centrifuged for 10 min at 5000 rpm and the clear supernatant was collected. The P^H of the extracted solution was determined by a Corning 215 – P^H meter using standard buffer solution.

Determination of Chlorophyll

The extraction of five gram dry powder was carried out with 80% acetone and after filtration; the filtrate was pooled and made up to 100 ml in a volumetric flask with 80% acetone. The absorbance was measured at 645nm and 663nm for the determination of chlorophyll. The chlorophyll content of Stevia dry powder were calculated employing the formula using the specific absorption coefficient for Chlorophyll –a and Chlorophyll – b at 645nm and 663nm in 80% acetone, respectively as described in Methods of Physiological Plant Pathology (Mahadavan *et al.*, 1982).

Determination of Moisture

Moisture was determined from fresh leaves as well as from dry powder also. Five gram of water clean fresh leaves were allowed to kept at 20^0C at room temperature for evaporating the water. After that the fresh leaves and five gram of dry powder were kept separately in crucible at 100^0C in incubator for six hours and the conventional procedure of moisture determination that is described in ICOMR, 1971; was followed for measuring the moisture content from Stevia leaves and dry powder.

Determination of Ash, Crude Fiber, Total Carbohydrate, Total Protein and Lipid Content

Ash and Crude fiber content were determined through the established procedure of A.O.A.C, 2000. Phenol Sulphuric Acid Method of Dubois *et al.*1951; was used for the determination of the total carbohydrate, whereas the total sugar by anthrone method by Jayaraman, 1981; total reducing sugar by Di – Nitrosalicylic acid (DNS) Method by Miller, 1972; total protein by Micro – Kejeldahl Method of AOAC, USA, 2000; and total lipid content by Bligh, E.G and Dyer Method, 1959.

Determination of Minerals

The dry materials (Stevia dry leaves powder) were kept in an incubator at 105°C for overnight due to presence of moisture and digested by HNO_3 and Perchloric Acid ($HClO_4$). Then the important minerals content of Stevia were determined by Analytical Method of Peterson, 2002; where Ca, Mg and Fe were analyzed by Atomic Absorption Spectrophotometry, P & Cl by Spectrophotometry and Na & K by Flame photometry.

Screening for Secondary Metabolites

Prepared Stevia dry leaves powder were used for the screening of phytochemicals. 100 gm dry powder was soaked in 400 ml of 96% ethanol in a glass container for sixteen days with additional regular shaking and stirring. The extract was then separated from the debris by filtration using by a piece of clean, white cotton material and it was done for two times. The filtrate was taken in a beaker and was wrapped a sheet of aluminium foil to which perforation was done for evaporation of ethanol. The concentrate was designated as a crude extract of ethanol. The different chemical groups – Alkaloids, Glycosides, Cardiac Glycosides, Anthraquinone Glycosides, Flavonoids, Tannins, Steroids and Saponins were performed for screening of phytochemicals (Myers, 1982; Boham and Kocipai, 1974).

Quantitative analysis of some secondary Metabolites

After screening, quantitative analyses were performed for Alkaloids, Flavonoids, Saponins and Tannins by following the extraction method (dry weight basis) that was described by Boham and Kocipai, 1974; Obdoni and Ochuko, 2001; and Pearson, 1976 consequently.

Statistical Analysis

All experiments were executed in triplicate while the minerals were analyzed by duplicate. The optical density of each sample was measured with the help of spectrophotometer and was plotted on a graph of respective standard used particularly for each biochemical's. From the graph, concentration of biomolecules in one ml was calculated and then converted into 100 gm and the results were the means of triplicate or duplicate ± Standard Deviation (SD).

Results and Discussions

Studies are going on upon Stevia plants due to its advantages as a dietary supplement and wide range of medicinal properties based on its chemical compositions which may be varied for different climatic conditions from region to region and its cultivation processes. In this analysis, the findings have been compared with the published reports of Ena gupta *et al.* 2015; Mishra *et al.* 2010; Savita *et al.* 2004; Gasmalla *et al.* 2014; Kaushik *et al.* 2010; Tadhani *et al.* 2006; and Abou–Arab and Abu-Salem, 2010; on major constituents of Stevia leaves or powder which were from different climatic regions and cultivation processes. The proximate chemical analysis of Stevia dry leaves powder revealed that the powder is mild acidic (pH 5.3– 5.4). Its chlorophyll content was 0.845±0.05 mg% where chlorophyll – a & b were found 0.088±0.024 and 0.761±0.028 mg% respectively (Table.1) that are comparatively found similar with the findings of Ena gupta *et al.*, 2015. Fresh leaves of Stevia Plant contained high amount of moisture that was up to 82% where the dry leaves powder contained 6.32±0.10 gm% which is very similar to the findings of Gupta *et al.*2015; Mishra *et al.* 2010; Savita *et al.* 2004; but less than the reported value of Gasmalla *et al.* 2014 and Kaushik *et al.* 2010.

Table.2 represents the nutritional compositions of Stevia dry leaves powder which contains total carbohydrate 53.52±0.45 gm per 100 gm that is highest among the major nutrients where the total protein and fat were determined 15.13±0.1 and 3.55±0.45 gm% respectively. Total carbohydrate and fat contents in present analysis were found to be very similar while the protein content was estimated to be much higher than most of the reported values but found less than the value of Tadhani & Subhash, 2006. So, it might be concluded that Stevia is a good sources of carbohydrate and protein. The amount of crude fiber and ash are found less as reported by Mishra *et al.*, 2010 and Savita *et al.*, 2004 but similar to the finding of Abou–Arab and Abu-Salem, 2010 for ash. Total sugar, total reducing sugar and total non-reducing sugar contents were also analyzed in this study and calculated as 10.73±0.23, 3.62±0.29 and 7.11±0.49 gm% respectively.

Table 1: pH, Chlorophyll and Moisture contents of Stevia leave dry powder.

Parameters	Amounts
pH	5.345±0.02
Total Chlorophyll	0.845±0.05 mg/100gm
Chlorophyll – a	0.088±0.024 mg/100gm
Chlorophyll – b	0.761±0.028 mg/100gm
Moisture (Fresh Leaves)	82.26±0.42 gm/100gm
Moisture (Dry leaves powder)	6.32±0.10 gm/100gm

Table 2: Nutritional composition of Stevia leaves dry powder.

Parameters	Amounts (gm/100gm)
Ash	7.05±0.13
Crude Fiber	10.5±0.7
Total Carbohydrate	53.52±0.45
Total Sugar	10.73±0.23
Total Reducing Sugar	3.62±0.29
Total Non-Reducing Sugar	7.11±0.49
Total Protein	15.13±0.1
Total Fat	3.55±0.45

K was found as highest in amount in mineral analysis as presented in Table.3 that was 2500±6.1 mg% and found to be very similar with the findings of Tadhani & Subhash, 2006 but greater than the reported value of Savita *et al.* 2004 and Kaushik *et al.* 2010. Ca was determined as second largest mineral that was 534.43±4 mg% in amount and found almost different from the reported values. Other major minerals like Mg, P, Na, Cl and Fe were also determined at the amount of 465.34±4, 304.70±2.5, 184.30±3.5, 49.6±3.5 and 34.2±0.6 mg% respectively.

Table 3: Mineral composition of Stevia leaves dry powder.

Parameters	Amounts (mg/100gm)
Iron (Fe)	34.2±0.6
Sodium (Na)	184.3±3.5
Potassium (K)	2500±6.1
Calcium (Ca)	534.43±4
Magnesium (Mg)	465.34±4
Phosphorus (P)	304.7±2.5
Chloride (Cl)	49.6 ±3.5

The present data in Table.4 clearly indicate that Stevia leaves are good sources of alkaloids, flavonoids, Saponins and tannins where they have found at the amount of 2.83±0.03, 17.20±0.2, 6.69±0.52 and 5.84±0.01 mg per 100gm respectively (Table.5) while glycosides and steroids were found to be present in a very less amount.

Table 4: Screening for secondary metabolites.

Parameters	Result
Test of Alkaloids	+++
Test of Glycosides	+
Test of Cardiac Glycosides	++
Test of Anthraquinone Glycosides	++
Test of Flavonoids	+++
Test of Tannins	+++
Test of Steroids	+
Test of Saponins	+++

Here, +++ indicate high, ++ indicate medium and + indicate low.

Table 5: Quantitative analyses of some secondary metabolites.

Parameters	Amounts (mg/100gm)
Alkaloids	2.83 ±0.03
Flavonoids	17.20 ±0.21
Saponins	6.69 ±0.52
Tannins	5.84±0.01

Flavonoids, a secondary metabolite with much medical importance are found to be present in highest amount in the experimental leaves dry powder. It regulates plant growth by inhibition of the exocytosis of the auxin indolyl acetic acid, as well as by induction of gene expression (Havsteen, 2002). It also inhibits bacterial strains; viral enzymes, i.e reverse transcriptase and protease, and destroy some pathogenic protozoans (Havsteen, 2002).

Further, Saponin contents are found to be second higher amounts in present study which possess significant anti – cancer properties and also display anti-tumorigenic effects (Man *et al.*, 2010). Tannins were also present in sufficient amount which are useful in various applications in the chemical and pharmaceutical industry and also considered as anti – oxidants and prevent the onset of degenerative diseases such as cancer and cardiovascular diseases.

longing for sweetness led to man to ascertain several forms of unconventional intense sweeteners, which have made possible to offer patrons the sweet taste without calories like Stevia, which is several hundred times sweeter than sucrose but do not contain calories and used in very small amount for their resolute sweetening property. Energy value analyzed were 2.7 Kcal per gram which is a low calorific sweetener in evaluation with other low calorie sweeteners (Savita *et al.*, 2004). Due to its low calorie and nutritional properties, it can be used in food processing industries and has immense potential benefits for diabetic patients and also has remarkable therapeutic value. Stevia prevents the growth of certain bacteria and use for treating wounds, sores and gum diseases (Saurabb *et al.*, 2013). Leaves of *Stevia rebaudiana* have been recommended for the treatment of chronic and non – chronic diseases like obesity, inflammatory bowel diseases, dental caries, cardiovascular and renal diseases (Gupta *et al.*, 2013).

According to the above mentioned upshot, it can be accomplished that *Stevia rebaudiana* Plant leaves dry powder are a good source of crude fiber, carbohydrates, protein, fat, macro and micro elements that have abundant health benefits and remarkable healing properties of different types of diseases for their organolyptic distinctiveness. Further study is needed to determine the physico - chemical properties and biological activities of Stevia, which will be helpful for food industries and pharmaceutical companies to prepare different food stuffs and medicines for diseases diagnosis and recovery.

Acknowledgement

The Authors gratefully acknowledge The Department of Biochemistry and Biotechnology, University of Science and Technology Chittagong (USTC), Foy's Lake, Chittagong, Bangladesh and Chittagong Veterinary and Animal Sciences University (CVASU), Khulshi, Chittagong, Bangladesh, for their kind consideration for using their research laboratory and equipments to complete this research and also thankful to Ethno Botany Laboratory of Chittagong University, Bangladesh for Taxonomy.

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