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Preliminary Studies on *Stevia rebaudiana* Leaves: Proximal Composition, Mineral Analysis and Phytochemical Screening

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Stevia rebaudiana leaf was investigated for it's proximal composition, mineral content and phytochemical screening. Further, fatty acid analysis of leaf oil was also carried out using gas chromatography. Protein, fat, carbohydrate and ash content were found to be 20.42, 4.34, 35.20 and 13.12 g% on dry weight basis, respectively. Mineral analysis showed that the high content of K, Ca, Mg, P, Na and S present in the leaf whereas cobalt, copper, iron, manganese, zinc, selenium and molybdenum found as trace amount. The phytochemical analysis revealed the presence of tannins in high concentration followed by alkaloids, cardiac glycosides, saponins, sterols and triter penes, reducing compounds and anthraquinones. Cyanogenetic glycosides were found to be absent in the leaf. GC analysis of leaf oil indicated the presence of palmitic, plamitoleic, stearic, oleic, linoleic and linolenic acids. In conclusion therefore, leaves of *Stevia rebaudiana* as shown here do posses high content of protein, carbohydrate, K, Ca, Mg, P and some active chemical constituents like tannins, alkaloids, saponins as well as palmitic and linolenic acids.

Key words: *Stevia rebaudiana*, proximate composition, minerals, phytochemicals, fatty acids

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INTRODUCTION

There is an ever increasing gap between food supplies and population growth, particularly in the developing countries. The search for novel high quality but inexpensive sources of food has always remained a major concern of all agencies involved in providing adequate food and improving nutritional status of the population (Sankhala *et al*., 2005). Medicinal plants are of great importance to the health of individuals and communities (Hill, 1952). Many indigenous medicinal plants are used as spices and food plants (Okwu, 2001). India owns a rich biodiversity of such foods although few studies have been conducted to document the nutritive value of less familiar foods (Rajalakshmi and Geeravani, 1990; Mohan and Janardhanan, 1995) but information available is quite meager. Hence, in an attempt to explore some such untapped sources of good nutrition, the present investigation has been undertaken to identify *Stevia rebaudiana* leaves with a potential to contribute towards food and nutrition security of the masses.

Stevia rebaudiana (Bertoni) is a shrub of the family Asteraceae that is indigenous to Paraguay and Brazil and it is also cultivated in some regions of Asia, Europe and Canada (Crammer and Ikan, 1987). Recently, cultivation of *Stevia rebaudiana* has started in India. The main sweet component in the leaves of *Stevia rebaudiana* is stevioside (Geuns, 2000). It is 300 times sweeter than sucrose (Soejarto *et al*., 1983) and has recently gained importance as natural non caloric sweetener. Stevia leaf extracts are used in Japan, Korea and certain countries of South America to sweeten soft drinks, soju, soy sauce, yogurt and other foods, whereas in the United States they are used as dietary supplements. Stevia sweetener extractives have been suggested to exert beneficial effects on human health, including antihypertensive (Chan *et al*., 2000; Lee *et al*., 2001), antihyperglycemic (Jeppesen *et al*., 2000, 2002), noncariogenic (Das *et al*., 1992) and anti-human rotavirus (Takahashi *et al*., 2001) activities. These sweeteners are also thought to influence glucose metabolism (Suanarunsawat and Chaiyabutr, 1997; Toskulkao *et al*., 1995) and renal function (Jutabha *et al*., 2000).

Nobody has investigated the potential nutritive and screening of phytochemical constituents of *Stevia rebaudiana* leaves in India until now. Therefore, in this study the proximate compositions, mineral and phytochemical analysis as well as fatty acid composition of leaf oil were investigated.

MATERIALS AND METHODS

Dried leaves of *Stevia rebaudiana* were supplied by Growmore Biotech Ltd., Hosur, Tamil Nadu, India. Leaves were packed in Polyethylene bags and stored at -18°C until used. Chemicals used were research grade purity and purchased from Hi-media and Qualigens companies. Standard methyl esters of fatty acids were purchased from Sigma Company.

Proximal composition: *Stevia rebaudiana* leaves were evaluated for their proximal composition. For protein content, nitrogen estimation was done by the macro Kjeldahl method of Oser (1976). Nitrogen content was multiplied by a factor of 6.25. Total fat content was estimated by Soxhlet method. Ash content was determined by the method described in AOAC (1984). Carbohydrate content was estimated using anthrone reagent according to method given by Dreywood (1946). Mineral content e.g. potassium, calcium, magnesium, phosphorus, sodium, sulphur, iron, manganese, zinc, copper, molybdenum, selenium and cobalt were determined using atomic absorption spectrophotometer using standard method.

Phytochemical screening: Leaves of *Stevia rebaudiana* were subjected to qualitative phytochemical screening for the identification of various classes of active chemical constituents using the method described by Trease and Evans (1987) and by Harbone (1973).

Test for tannins: About 0.5 g of the dried powder was boiled in 20 mL of water in a test tube and then filtered. A few drops 0.1% ferric chloride was added and was observed for brownish green or a blue black coloration.

Test for alkaloids: One milliliter of aqueous extract was stirred and placed in 1% aqueous hydrochloric acid on a steam bath. Then, 1 mL of the filtrate was treated with Dragedorff's reagent. Turbidity or precipitation with this reagent was considered as evidence for the presence of alkaloids.

Test for cardiac glycosides: One gram of leaf powder was added to 10 mL of 70% alcohol, boiled for 2-3 min and was filtered. Then, 5 mL filtrate was taken, to this 10 mL distilled water and 0.5 mL of strong solution of lead acetate were added, mixed thoroughly and filtered. To the filtrate, 5 mL chloroform was added, shook vigorously

and allowed to separate two layers. Chloroform layer pipette out and was evaporated in porcelain dish. The residue was dissolved in 3 mL of glacial acetic acid containing two drops of 5% ferric chloride solution. This solution was transferred to the surface of 2 mL concentrated sulphuric acid carefully and was observed for formation of reddish brown layer at the junction of the two liquids and the upper layer was becomes bluish green and was darkening with standing.

Test for saponins: Five milliliter aqueous extract was vigorously shaken with 10 mL distilled water for 2 min. The appearance of foam that persists for at least 15 min or the forming of an emulsion when olive oil was added confirmed the presence of saponins.

Test for sterols and triter penes: Ten milliliter aqueous extract was placed in a small beaker and evaporated to dryness. The residue was dissolved in 0.5 mL each of acetic anhydride and chloroform. The solution was transferred into a dry test tube and concentrated sulphuric acid was added. Brownish red or violet rings at the zone of the contact with the supernatant and green or violet coloration denoted the presence of sterols and triter penes.

Test for reducing compounds: Two milliliter of the aqueous extract were placed in test tube and 5 mL mixture of equal volumes of Fehling's solution A and B were added and boiled in a water bath for 5 min. The test tube was observed for brick red precipitate.

Test for anthraquinones: Leaf powder was macerated with ether and after filtration aqueous ammonia was added. After shaking, change in colour of aqueous layer was observed. Pink, red or violet colour in aqueous layer indicated the presence of anthraquinones.

Test for cyanogenetic glycosides: Dried leaf powder was placed in small conical flask with sufficient water to moisten with a drop of toluene. In the neck of the flask a suitably impregnated strip of filter paper treated with sodium picrate was suspended by means of a cork and observed for changes in colour of paper strip.

Fatty acid analysis: To determine the fatty acid composition of the essential oil, Gas Chromatography (GC) was used. Methyl esters were prepared according to the method given by Christie (1982). About 1-2 drops of oil extracted from leaf using petroleum ether (40-60° C) was taken in a clean screw capped tube and 5 mL of 0.5 N methanolic sodium methoxide was added. Tube was capped and heated in a boiling water bath for 10 min. After cooling, 0.5 mL of Boron trifluoride (14% BF_3) was added, again heated in a similar manner for 5 min for complete methylation and that tube was allowed to cool at room temperature. Spectroscopic grade hexane (2 mL) was added to the tube, mixed on cyclomixer and 1-4 μL of hexane containing the extracted methyl esters was injected in to the GC (Sigma Chromatography Instruments Co., Baroda) fitted with a Flame Ionization Detector (FID). The column temperature was maintained at 220°C and injector temperature at 250°C. The retention time was measured and fatty acids were identified by comparing with the retention time of standards.

RESULTS

Proximal composition: Protein, carbohydrate and ash content of stevia leaf were found to be higher whereas fat was estimated to be less in the leaf on dry weight basis (Table 1).

Mineral analysis: Mineral analysis showed that the presence of high amount of potassium, calcium, magnesium, phosphorus, sodium and sulphur content and iron, manganese, zinc, copper, molybdenum, selenium and cobalt content were found to be at ppm levels on dry weight basis (Table 2).

Phytochemical screening: The number of positive signs indicated the intensity of reactions that reflects the quantity present. The powdered leaf subjected to preliminary phytochemical screening using chemical method showed the most abundant compounds in the leaf extract were of tannins and alkaloids, followed by cardiac glycosides, saponins, sterols and triter penes, reducing compounds and anthraquinones. Test for cyanogenetic glycosides, however, showed negative result (Table 3).

Fatty acid analysis: There were six fatty acids identified by comparison with the fatty acid methyl ester standards. Palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were identified in the leaf oil. Palmitic acid content was found to be highest whereas stearic acid was found to be least among the identified fatty acids (Table 4).

Table 1: Proximal composition of stevia leaves

Component	Value (g%)
Protein	20.42±0.57
Fat	4.34±0.02
Carbohydrates	35.20±1.26
Ash	13.12±0.31

Values are mean of three reading±SEM

Table 2: Mineral content of leaves of *Stevia rebaudiana*

Minerals	Concentrations
Potassium	2.51 g%
Calcium	1.55 g%
Magnesium	0.50 g%
Phosphorus	0.35 g%
Sodium	0.16 g%
Sulphur	0.12 g%
Iron	363.00 ppm
Manganese	98.30 ppm
Zinc	63.90 ppm
Copper	10.40 ppm
Molybdenum	1.14 ppm
Selenium	0.57 ppm
Cobalt	0.27 ppm

Table 3: Phytochemical constituents of leaves of *Stevia rebaudiana*

Phytochemical constituents	Results
Tannins	++++
Alkaloids	+++
Cardiac glycosides	++
Saponins	++
Sterols and triter penes	++
Reducing compounds	++
Anthraquinones	+
Cyanogenetic glycosides	-

- = Negative result, + = Low concentration, ++ = medium concentration, - +++ = High concentration, ++++ = Very high concentration

Table 4: Fatty acid composition (g/100 g) of stevia leaf oil

Fatty acids	g/100 g
Palmitic acid (C16)	27.51
Palmitoleic acid (C16-1)	1.27
Stearic acid (C18)	1.18
Oleic acid (C18-1)	4.36
Linoleic acid (C18-2)	12.40
Linolenic acid (C18-3)	21.59

DISCUSSION

The proximate analysis shows that *Stevia rebaudiana* leaves are good source of protein and carbohydrate. Due to this, consumers are more benefited when they use leaves as a substitute of sugar in place of pure stevioside in various food preparations. High ash content indicates that the stevia leaves are good source of inorganic minerals. A low fat value obtained in this study confirms that the leaf is not a good source of oil. The low oil yield, will not enhance the use of the oil for domestic purposes.

Potassium, calcium, magnesium, phosphorus, sodium and sulphur which are nutritionally important, were found in reasonable amount in the leaf. The high concentration of these minerals could be an advantage. It is known that certain inorganic elements (potassium, zinc, calcium, etc.) play important roles in the maintenance of normal glucose-tolerance and in the release of insulin from beta cells of islets of Langerhans (Choudhary and Bandyopadhyay, 1999). Selenium, zinc and manganese are considered as antioxidant micro nutrients (Talwar *et al*., 1989) and their presence could therefore boost the immune system (Jimoh and Oladiji, 2005) and in prevention of free radical mediated diseases. Iron is an essential element for synthesis of hemoglobin. Higher amount of iron in stevia leaves is again useful in maintenance of normal hemoglobin level in the body. Moreover, stevia leaves could also be use to prepare various sweet preparations for combating iron deficiency anemia which is a major nutritional disorder of developing countries.

The presence of secondary plant products in the leaf that are biologically important e.g. tannins, alkaloids, cardiac glycosides, saponins, sterols and triter penes, reducing compounds and anthraquinones contribute to its medicinal value as well as exhibiting physiological activity (Sofowara, 1993). Tannins have been reported to have several pharmacological activities such as spasmolytic activity in smooth muscle cells (Tona *et al*., 1999). It has been also reported that they have free radical scavenger properties (Bharani *et al*., 1995) and antioxidant action (Yoshizawa *et al*., 1987). Saponins inhibit Na^+ efflux, by the blockage of the entrance of the Na^+ out of the cell. This leads to higher Na^+ concentration in cells, activating a Na^+-Ca_2^+ antiporter producing elevated cytosolic Ca_2^+ which strengthens the contractions of heart muscle and thereby reducing congestive heart failure (Schneider and Wolfling, 2004). The hypoglycemic property of *Stevia rebaudiana* leaves may be attributed to the presence of bioactive compound like glycosides, mineral salts etc. These compounds have been shown to be responsible for hypoglycemic activity in *Momordica charantia* (Akhtar *et al*., 1981). Though, the stevia leaves contain very low amount of oil, it could not be use for oil extraction, but looking to the fatty acid composition, stevia leaf oil is a rich source of linolenic acid. This high value of linolenic acid may be helpful to maintain ideal fatty acid ratio in human diet.

It is worthwhile to indicate that there are no data in the literature of investigated the potential nutritive and phytochemical constituents of *Stevia rebaudiana* leaves for comparison. The results of the present study could be showing the additional therapeutic and functional properties of *Stevia rebaudiana* leaves. Due to the limitation of the present study, author could not evaluate the quality of protein, bioavailability of various elements and clinical trials of stevia leaves, these aspects of stevia leaves are suggested for future research.

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