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Research Paper On Stevia Rebaudiana Leaf Preliminary Studies: Intermediate Composition, Minerals Examination, And Phytochemical Evaluation

1.Jay Prakash Singh, . 2.Prof. Dr. Subhashish Tripathy 3. Devendra Kr. Rawat 4. Saloni Srivastava 5. Shivam Tiwari 6. Neeraj Bharti 7. Swati Pandey

1. Ph.D. Research Scholar Lords University, Alwar Rajasthan-301001
2. Director at BMS College of Pharmacy Amethi,UP- 229309
3.Assistant Professor at BMS College of Pharmacy Amethi,UP- 229309
4.Assistant Professor at BMS College of Pharmacy Amethi,UP- 229309
5.Assistant Professor at BMS College of Pharmacy Amethi,UP- 229309
6.Assistant Professor at BMS College of Pharmacy Amethi,UP- 229309
7.Assistant Professor at BMS College of Pharmacy Amethi,UP- 229309

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Abstract

The study examined the proximate composition, mineral content, and phytochemical screening of leaves of Stevia rebaudiana. Additionally, leaf oil's fatty acid composition was examined using gas chromatography. On a dry weight basis, the contents of The percentages of protein, fat, carbs, and ash were found to be 20.41, 4.33 out of 5, 35.21, and 13.13 mg%, in that order. Substantial concentrations of K, as well as magnesium, calcium, phosphorus, Na, and Si were found in the leaves. according to mineral analysis, but only negligible amounts of cobalt(Co), copper (Cu), iron (Fe), manganese(Mn), zinc(Zn), selenium(Se), and molybdenum(Mo) were discovered. Tannins were found to be present in large concentrations, followed by anthraquinones, reducing chemicals, alkaloid substances, heart glycosides, saponins, sterols, and triterpenes. Glycosides cyanogenetic were discovered to be missing from the leaf. The presence in leaves of palmitic acids, plamitoleic, a combination of , oleic, linoleic, and linolenic acids was shown by GC analysis of leaf oil. For this reason, it may be concluded that Stevia rebaudiana leaves, as demonstrated here, do contain significant levels of K, as well as magnesium, calcium, phosphorus, carbohydrates, proteins, and some active chemical substances which includes tannins, alkaloid substances, and saponins that, and the fatty and linoleic acids.

Keywords

Stevia rebaudiana, rebaudioside-A, stevioside, column chromatography, thin layer chromatography

Introduction-The disparity between population growth and food availability is widening, especially in emerging nations. All organisations interested in supplying enough food and enhancing the nutritional state of the populace have always had the hunt for innovative, high-quality, yet affordable food sources as a top priority (Sankhala et al., 2005). The health of both people and societies is greatly impacted by medicinal plants (Hill, 1952). Numerous

(A)Stevia Leaf

(B) Dry Stevia Leaf

(C) Stevia Leaf Powder

Native culinary and medicinal herbs are utilised as spices (Okwu, 2001). These kinds of foods are abundant in India, despite the fact that not much research has been done to establish the nutritional worth of uncommon foods (Mohan and Janardhanan, the year 1995; Rajalakshmi and Geeravani, 1990). Consequently, The present the research's objective is to find Stevia rebaudiana leaves that may improve food and nutritional security of the general public in an effort to investigate some of these underutilised sources of good nutrition. Native to both Brazil and Argentina, The flowering plant Ste (The research team of Bert) is a shrub of the Asteraceae family. Additionally, it is grown throughout Europe, the United States, and Asia (Crammer and Ikan, 1987). Lately, a plant called Stevia rebaudiana has been cultivated in India. Stevioside is the primary sugar found in Stevia rebaudiana leaves (Geuns, 2000). According to Soejarto et al. (1983), it is three hundred times sweet than glucose and has grown in popularity as a calorie-free, natural sugar substitute While Extracts of leaves from stevia are utilised as nutritional supplements in the US, they are sweetened Yoghurt, beverages, soju, which is soybean sauce, along with other cuisines in Japan, Korean, and other Chinese American nations. According to several studies Stevia sweetener extractives, such as those that are hypertensive medications, antihyperglycemic, noncariogenic, & anti-human rota virus, may benefit human health (Chan in et al., 2000; the work of Lee et al., the year 2001). properties (Jeppesen et al., 2000, 2002). Additionally, it is believed that these sweeteners affect renal function (Jutabha et al., 2000) and The utilisation of hyperglycemia (Suanarunsawat & Chaiyabutr, 1997; Toskulkao et al., 1995). Up till now, no one has looked at the possible nutritional value or screening of the phytochemical

components of the plant's stems in India. As a result, this study looked at the fatty acid composition, mineral and phytochemical analysis, and approximate compositions of leaf oil.

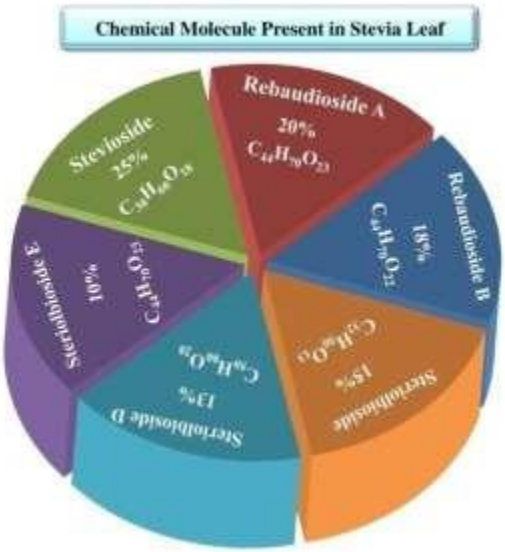
Components and Procedures

The Hosur, India Tamil Nadu, India-based Growmore Biosciences Ltd. was the provider of dried Stevia rebaudiana leaves. Prior to them becoming utilised, leaves were kept at -18°C in

polyethylene bags. The chemicals used were bought from Hi-media and Qualigens firms and were of research grade purity. Sigma Company provided the standard methyl esters of fatty acids that were acquired.

Proximal composition: The proximal composition of leaves of Stevia rebaudiana was assessed. The protein content was estimated using Oser's macro Kjeldahl technique (1976) for nitrogen. The amount of nitrogen was doubled by 6.25. The Soxhlet technique was used to assess the total fat content. The AOAC (1984) technique was used to determine the amount of ash. Using anthrone reagent, the carbohydrate content was calculated using Dreywood's technique (1946). Using an atomic absorption spectrophotometer and a conventional procedure, the mineral

content, such as sodium, The elements iron, manganese, zinc, copper, selenium, cobalt, calcium, magnesium, the mineral phosphorus sodium, are a sulphur, an, was measured.



Phytochemical screening: The method described by Harbone (1973), a and Trease and Owens (1987) was used to the qualitative phytochemical examination of Stevia rebaudiana leaves in order to identify many classes, including active component elements.

Check for polyphenols. **:** In a test tube, and 0.5 g of the powdered material was brought to a boil in 20 mL of water, and the mixture was then filtered. After applying adding a few sprays of ferrous chloride (0.1%), resulting hue turned blue-black or yellowish emerald.

Test for alkaloids: On a steam bath, one millilitre of The aqueous extract was combined with a single percent aqueous hydrochloric acid, and it's this acid and then immersed. Next, Dragedorff's reagent was applied to 1 millilitre of the filtrate. When using this reagent, turbidity or precipitation was thought to be indicative of an abundance of cannabinoids.

Test for cardiac glycosides: 10 mL of 70% alcohol were combined with one gramme of leaf powder, cooked for two to three minutes, and then filtered. Next, 5 millilitres of filtrate were obtained, and 10 millilitres ofAfter adding, thoroughly mixing, and filtering 0.5 millilitres of containing an aggressive lead acetate solution, distilled water was added. Two layers were allowed to separate after After giving the filter mixture a good tremble, five millilitres of aldehyde was added. In a porcelain dish, the chloroform layer pipetted out and evaporated. Three millilitres of glacial acetic acid were used to dissolve the residue, along with two drops of a five per cent solution of ferric chloride. This mixture was carefully put to the outside of two millilitres of acid sulfuric that had been focused on, and the development of a brownish-reddish coating at the interface between the highest stratum and two separate fluids darkened and became blue green as it stood.

Test for saponins: For two minutes, a 5-milliliter aqueous extract was agitated violently using 10 millilitres of distilled water. The presence of saponins was verified by the formation of an emulsion with the addition of olive oil or by the emergence of foam that lasts for at least fifteen minutes.

Check for triterpenes and sterols : Ten millilitres of watery extract were dried out by evaporating it in a tiny beaker. The remaining substance had been dispersed in 0.5 millilitres of chloroform and acetic anhydride, respectively. strong sulfuric acid that was concentrated was placed in a dry tube for testing that was filled with the solution. Sterols and triterpenes were indicated by reddish red or violet bands at the supernatant contact zone and a green to violet colouring.

Test for reducing compounds: Five millilitres of Fehling's solution until it reaches the A and B were combined with two millilitres of the aqueous extract, subsequently the combination was cooked for just under five minutes in a vessel of water. Brick colour precipitate was looked for in the examination tube.

Test for anthraquinones: Afterwards filtering, ether was used to macerate the leaf powdery substances, and then aqueous ammonia was added. Following shaking, the aqueous layer's hue changed. Anthraquinones were identified by colours in the aqueous layer that were pink, red, or lavender.

Test for cyanogenetic glycosides: A little amount of toluene was added to a tiny cylindrical vessel containing dried leaf powder and enough water to wet it. A cork was used to hold an appropriately embedded filtering paper piece that underwent ammonium picrate treatment within the flask neck, and the surface of the strip's colour was monitored for modifications.

Fatty acid analysis: Gas Chromatography (GC) was employed to ascertain the essential oil's fatty acid content. Christie's (1982) approach was followed in the preparation of methyl esters. Five millilitres of 0.5 N methanolic sodium methoxide were put to a clean screw-capped tube containing one or two droplets of oil extracted from the leaf at 40–60 degrees Fahrenheit using ether from petroleum. The sealed tube was submerged in a bath of hot water and left there for ten minutes. After cooling, 0.5 mL of trifluoride of boron (fourteen percent BF3) was added, and the instrument was heated for a further five minutes to complete oxygenation. Following that, it was let to cool to room temp.The tube was filled with 2 mL of spectroscopic grade hexane, mixed on a cyclomixer, and then The GC (Sigma Chromatographic Equipment Co., Vadodara) connected with a Flame The ionisation Detector (It) has been loaded with 1-5 μL of acetone. obtained methyl ester compounds. A constant 220°C column temperature and 250°C injector temperature were maintained. By contrasting the duration of retention with benchmarks, the storage time was determined and saturated fats have been determined.

Result

Proximal composition: The stevia leaf's dietary fibre, protein, and ash contents have been determined to be greater, whereas its dry weight fat content was predicted to be lower (Table 1).

Mineral Examination: Mineral examination revealed that On a scale of dry weight, concentrations of manganese,iron, It was discovered that the amounts of zinc, copper, the element selenium and copper were at percent, along with notable quantities of potassium, phosphorus, calcium, magnesium, and sodium. sulphate (Table 2).

Phytochemical analysis: The amount of feedback present is reflected in the strength of responses, as shown by the number of positive indications. The most prevalent chemicals in the Alkaloids are substances and tannins were found in extracts from leaves, followed by anthraquinones, saponins that sterols, is composed of triter, reducing chemicals, and glycosides from heart tissue. A thorough plant chemicals assessment of the powdered leaf was conducted using an analytical tool approach. Nevertheless, the cyanogenetic glycoside that's test yielded an undetectable conclusion (Table 3).

Examination of fatty acids : By comparing both fatty acid and methyl ester standards, six lipids have been identified. The leaf oil was found to include acids such as the stearic, oleic, fatty acid l, linoleic, palmitic acids, and palmitoleic. Among the fatty acids found, palmitic acid included determined to have the greatest amount and stearic acid was determined to have the lowest (Table 4).

Proximal composition of stevia leaves

Component	Value g%
Protein	20.41±0.56
Fat	432±0.01
Carbohydrates	35.20±1.25
Ash	12.11±0.30

Table 1- The values are three assessments' means±SEM.

Mineral composition of The leaves of Stevia leaves

Minerals	Concentrations
Potassium	2050g%
Calcium	1.54g%
Magnesium	0.51g%
Phosphorus	0.36g%
Sodium	0.15g%
Sulphur	0.13g%
Iron	364.00ppm
Manganese	98.25ppm
Zinc	36.85ppm
Copper	10.35ppm
Molybdenum	1.15ppm
Selenium	0.56ppm
Cobalt	0.26ppm

Table – 2

Phytochemical Constituents The herb Stevia rebaudiana stems

Phytochemical Constituents	Results
Tannins	++++
Alkaloids	+++
Cardiac glycosides	++
Saponins	++
Sterols and triterpenes	++
Reducing Compounds	++
Anthraquinones	+
Cyanogenetic glycosides	-

Table-3 The results are as follows: -= Negative, += Low, ++= Medium, +++= High, and ++++= Very High Concentration.

The fatty acid composition of stevia leaf oil (g/100g)

Table -4

Fatty acids	g/100g
Palmitic Acid (C16)	27.50
Palmitoleic acid (C16-1)	1.26
Steamic acid (C18)	1.19
Oleic acid (C18-1)	4.35
Linoleic acid (C18-2)	12.39
Linolenic acid (C18-3)	21.60

Discussion

The rebaudiana plant's leaves are a rich supply of protein and carbohydrates, according to the proximate analysis. As a result, when leaves are used in various culinary preparations instead of pure stevioside as a sugar replacement, customers profit more. The stevia leaves' high ash

content suggests that they are an excellent source of elemental minerals. This study's low fat value attests to the leaf's poor suitability as an oil source. The poor oil yield won't increase the amount of oil used domestically.

Nutritionally significant elements such as potassium, a mineral calcium, magnesium, phosphorus, sodium, and sulphur were present in the leaf in acceptable amounts. These minerals' high concentration may work to their benefit. Potassium, zinc, calcium, and other inorganic elements are known to have significant effects on the preservation of normal glucose tolerance as well as the islets of Langerhans beta cells' ability to secrete insulin (Choudhary and Bandyopadhyay, 1999). According to Talwar et al. (2009), manganese, zinc, and selenium are antioxidant minerals 1989), and as a result, their being there may strengthen the body's immunity and aid in the prevention of illnesses caused by free radicals (Jimoh and Oladiji, 2005). The production of haemoglobin requires iron as a necessary component. The increased iron content of stevia leaves aids in the preservation of the body's normal haemoglobin levels. Furthermore, stevia leaves may be used to make a variety of sweet concoctions to treat low iron levels anaemia, a common dietary problem in impoverished nations. The previously leaf's biological functions and therapeutic usefulness are enhanced by the presence of physiologically significant secondary plant products such as anthraquinones, reducing chemicals, tannins, and alkaloids, cardiac glycosides, saponins, sterols, and triterpenes (Sofowara, 1993). Numerous pharmacological actions of tannins have been documented, including the ability to cause spasmolysis in smooth muscle cells (Tona et al., 1999). Additionally, they have been shown to have antioxidant and free radical scavenger qualities (Bharani et al., 1995; Yoshizawa et al., 1987). Saponins prevent Na+ from leaving the cell by blocking its entry, which inhibits Na+ efflux. Higher Na+ concentrations as a result cause cells to activate a Na+- Ca2+ antiporter that generates increased cytosolic Ca2+, strengthening the cardiac muscle spasms and lowering the risk of Heart failure is a condition that is congestive (Schneider and Wolfling, 2004). Mineral salts and other bioactive substances may be the cause of the hypoglycemic property of Stevia rebaudiana stems. and glycosides. Momordica charantia has demonstrated hypoglycemic action due to these chemicals (Akhtar et al., 1981). Despite having relatively little oil, stevia leaves

cannot be used for oil extraction. However, based on the amount of fatty acids; linolenic acid, one kind of fatty acid, is abundant in stevia leaf oil. The ideal ratio of fat to protein in those who are diet may be maintained by this high naturally occurring content. It is important to note that no information on the possible nutritional and phytochemical components of the rebaudiana plant's leaves has been analysed or published in the literature. The current study's findings may indicate further medicinal and practical attributes of the leafy parts of Stevia rebaudiana. The author had no opportunity to evaluate the stevia's clinical studies, availability of various elements, or the quality of the proteins. plants due to the limitations of the current study; more research on these aspects of stevia leaves is recommended.

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