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Phytochemical and Pharmacognostic study of *Stevia rebaudiana* Bertoni

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Abstract

Stevia rebaudiana Bertoni sweetening plant is used for curing various diseases. The aim of this study is conferring the comparative abundances of phytochemicals and physiochemical analysis of *Stevia rebaudiana* Bertoni obtained from two different places. Amount of total soluble sugar, reducing sugar and non-reducing sugar contents were analysed. The moisture percentage, ash percentage and organoleptic evaluation were obtained by employing standard methods of analysis. Extraction of *Stevia rebaudiana* Bertoni gave positive result.

Keywords: *Stevia rebaudiana*, total soluble sugar, reducing sugar, non-reducing sugar

Introduction

In recent years use of medicinal plants is increasing rapidly as plant products are more preferred for treatment of different diseases over synthetic chemical due to less side effects on human life. In daily life our feeding habits have been dependent on major portions of carbohydrates and sweeteners have become integral part of our daily diet. The use of sucrose are not suitable for human health in food items as it leads to the development of diabetes which in long terms affect many internal organs of human. The *Stevia* have high potency of alternative sweetener^[1,2] for the growing natural food market in whole world including India. *Stevia rebaudiana* Bertoni called as *Eupatorium rebaudi-anum* Bertoni, draws its name from the botanist PJ Esteve. *Stevia rebaudiana* Bertoni is commonly called by many attractive name viz. *Stevia*, sweet leaf of Paraguay, Candy leaf, Sweet herb, Honey yerba and Honey leaf. *Stevia* belongs to family compositae (Asteraceae), the largest family of vascular plants. The genus *Stevia* contains steviosides which were found to be 150-300 times sweeter than sucrose which were in 1931 when eight novel plant chemical called glycosides were discovered^[3]. Three non-sweet labdane diterpens, two triterpenes, two sterols, a flavonoid and unidentified tannins as well as certain vitamins A, B and C were present in *Stevia* plant^[3]. There are many therapeutic application of *Stevia*^[4, 5]. It possesses hypoglycaemic^[6-8], hypotensive^[9], vasodilating^[10], taste improving, sweetening, anti-fungal, antiviral, anti-inflammatory, antibacterial and anti-tumour^[11] properties. It is also used for curing gastrointestinal and skin related problems^[12].

There are various varieties of *Stevia rebaudiana* developed all around the world^[13]. Basically all these varieties have been developed for different climatic requirements. Many times these varieties perform strangely in different climatic conditions. Like sugar cane, its Stevioside and rebaudioside content in found in leaves determine the price and market-ability of *Stevia* leaves. Therefore, it becomes imperative that grower may select proper varieties to earn high price.

Materials and Methods

Investigated Plant: *Stevia rebaudiana* Bertoni was selected and research work was focused only on this plant.

Botanical Characters: *Stevia rebaudiana* Bertoni is an herbaceous perennial plant which produces small white hermaphrodite flowers at the terminal end of the stems. *Stevia* possess fine and thick roots. Fine roots spread out near soil surface but thicker roots grow deeper into the soil. The plant shows opposite leaf arrangement. These leaves are sessile, lanceolate to oblanceolate. The seeds are small and leaves are mainly used as sweetening agent.

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Taxonomy – Scientific classification ^[14, 15]

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Genus	<i>Stevia</i>
Species	<i>S. rebaudiana</i>

Collection of plants

Healthy plants of *Stevia rebaudiana* Bertoni were obtained from Indira Krishi Gram Vikash Kendra Vishwavidyalay, Raipur and were named as L1, and those which were obtained from Krishi Gram Vikash Kendra, Rukka were named as L2. The plant parts like roots, stem, leaves and the flower were used to prepare dried sample and fresh sample. The powdered samples were preserved in airtight polythene bags separately. In present research work an attempt has been made to investigate phytochemical and physiochemical of *Stevia*.

1. Phytochemical Analysis

The standard procedure methods of phytochemical analysis were carried out for collected samples.

A. Total Soluble Sugars: The amount of total soluble sugar was estimated using Anthrone method colorimetrically ^[16].

B. Reducing Sugars: Reducing sugar was estimated by Dinitro Salicylic Acid (DNS) method ^[17].

C. Non-Reducing Sugars: Non-reducing sugar content was calculated by difference of total soluble sugar and reducing sugars.

D. Extractive Percentage
2. Physiochemical Studies

The ash value generally considered as inorganic residue present in the medicinal plant which illustrate the quality as well as purity of herbal medicine. Physiochemical constants such as organoleptic, microscopic evaluation, moisture percentage and ash percentage were calculated based on standard procedures.

Results and Discussion

Phytochemical analysis: Phytochemical analysis of medicinal plants is very essential for manufacturing new drugs. The station of different types of sugar from root, stem, leaf and flower both from before and after flowering the plant were presented in Table 1. Carbohydrate contents including reducing and non-reducing sugars, starch etc. play a vital role in plant metabolism. These governs all most all the metabolic processes of the plant. Most of the literatures available regarding biochemical analysis were related to its Stevioside content ^[18].

Table 1: Estimation of total soluble sugars (%), reducing sugars (%) and non-reducing sugars (%) in *Stevia rebaudiana* Bertoni

Locality	Plant parts	Total soluble sugars		Reducing sugars		Non-reducing sugars	
		Before Flowering	After Flowering	Before Flowering	After Flowering	Before Flowering	After Flowering
L1	Root	4.43±0.44	3.85±0.14	1.77±0.13	1.74±0.03	2.66±0.50	2.10±0.12
	Stem	5.07±0.33	4.81±0.11	2.50±0.28	1.54±0.39	2.56±0.31	3.26±0.28
	Leaf	15.88±0.88	15.23±0.47	6.14±0.14	5.79±0.13	9.74±0.73	9.44±0.57
	Flower	–	2.56±0.31	–	3.39±0.04	–	2.17±0.26
L2	Root	4.62±0.11	4.38±0.38	1.85±0.01	1.84±0.02	2.90±0.13	2.54±0.36
	Stem	5.13±0.08	4.85±0.03	2.83±0.12	1.88±0.49	2.30±0.14	2.97±0.50
	Leaf	16.01±0.42	15.38±0.20	6.15±0.08	6.14±0.52	9.86±0.48	9.24±0.62
	Flower	–	2.94±0.48	–	0.41±0.04	–	2.53±0.45

The results obtained by the chemical analysis of *Stevia rebaudiana* showed that in L1 the total soluble sugar ranged from 4.43% - 15.88%, while in L2 it ranged from 2.94% - 16.01%. Anthrone was used for the detection of total soluble sugar as it is a rapid and convenient method for determination of sugars. The approximate composition of reducing sugars contents of leaf was found to be higher in leaf of both the varieties. The reducing sugar ranged from 1.54% to 6.14% in L1 and in L2 it ranged from 0.41% to 6.15%. The DNS was used for analysis as it was simple, sensitive and adaptable during handling of a large number of samples at a time.

The non-reducing sugars are difference of total soluble sugar and reducing sugar. It ranged from 2.10% - 9.74% in L1 and 2.30% - 9.86% in L2. The results obtained are in line with earlier findings ^[19]. The sugars were found to be declined in after flowering samples. This might be due to the breakdown

of carbohydrates during plant development. From the present study it was observed that *Stevia* obtained from two different places are good source of sugars (carbohydrates) which are the essential factors for maintenance of health. The consumers are also benefited when the leaves of *Stevia rebaudiana* was used as substitutes of sugar in place of pure stevioside in different food preparations. Results of quantitative analysis for extractive percentage in different solvents are presented in Table 2. Extractive percentage indicated that the extractive percentage is high in methanol in both L1 and L2. This indicates that the methanol is the best solvent for *Stevia rebaudiana* extraction. The extractive values were also reasonably high in butanol and water, which indicate the solubility of the active constituents in these three extractive solvents. This could be seen when compared with certain results obtained earlier ^[20].

Table 2: Determination of extractive percentage in *Stevia rebaudiana* Bertoni in different solvents

Locality	Plant Parts	Water	Methanol	Benzene	Butanol	Acetone
L1	Root	1.73±0.06	4.53±0.02	0.33±0.13	3.40±0.11	1.00±0.30
	Stem	25.80±0.41	26.60±1.17	2.06±1.03	24.26±0.26	6.66±0.17
	Leaf	28.66±0.35	34.66±0.61	4.13±0.13	30.46±0.29	8.13±0.13
	Flower	1.20±0.23	4.86±0.29	0.53±0.13	3.20±0.11	0.80±0.30
L2	Root	1.40±0.11	4.86±0.06	0.46±0.06	3.46±0.24	1.20±0.11
	Stem	25.86±0.48	27.33±0.48	3.20±0.23	24.33±0.17	6.93±0.29
	Leaf	29.00±0.34	35.26±0.46	4.46±0.24	31.00±0.30	8.66±0.17
	Flower	1.80±0.11	5.06±0.35	0.73±0.24	3.40±0.30	1.06±0.17

Physiochemical Analysis

The organoleptic and microscopic evaluation presented in

Table 3 and moisture percentage and ash percentage in Table 4.

Table 3: Organoleptic and microscopic evaluation in *Stevia rebaudiana* Bertoni

Plant Parts	Organoleptic evaluation					Microscopic Evaluation
	Appearance/ Texture	Colour	Taste	Odour		
				Strength	Sensation	
Root	Moderately coarse powder	Dark Brown	None	Weak	Aromatic	Pieces of epidermis, cells and colleen-chymatous cells and oil droplets
Stem	Moderately coarse powder	Brown	Sweet	Weak	Fruity	Pieces of epidermis, parenchyma with xylem, broken varcular bundles
Leaf	Fine powder	Green	Sweet	Distinct	Fruity	Pieces of epidermal cells, anomocytic stoma, palisade mesophyll, trichome, pieces of vessels-reticulate, spiral and starch grain, calcium oxalate
Flower	Moderately fine powder	Light Green	Sweet	Distinct	Aromatic	Broken cells, oil droplets

Table 4: Determination of Moisture and Ash percentage in *Stevia rebaudiana* Bertoni

Locality	Parameter	Root	Stem	Leaf	Flower
L1	Moisture %	71.59±0.59	65.77±1.97	35.74±0.52	27.84±1.49
	Ash %	17.68±0.34	11.04±0.17	7.08±0.20	6.76±0.39
L2	Moisture %	71.97±0.53	66.90±1.05	36.04±0.54	27.94±1.04
	Ash %	17.72±0.37	10.84±0.22	7.00±0.14	6.44±0.30

Both the L1 and L2 showed the similar characteristics like fine powder to moderately fine powder texture, brown and green colour of stem and leaf respectively exhibiting sweet taste and distinct odour. The result of microscopic evaluation revealed the presence of anomocytic stomata, trichome, oil droplets, calcium oxalate, starch grain, vessels etc. These microscopical characters are useful in the identification of plant species. Two types of trichomes were also reported earlier in *Stevia rebaudiana* [21]. Trichomes are taxonomical importance as these help in attracting the pollinators, defence against herbivorous and pathogen attacks [22]. The presence of calcium in form of oxalate testified the literature that, the plant has small or low amount of soluble calcium. On this basis it was suggested that the plant families like Umbelliferae, Compositae (Asteraceae) and others are regarded as potassium type because of their high content of potassium when compared with the presence of calcium [23]. The presence of both major and minor elements in the plant (varieties) as well as oxalate confirms the plant to be of nutritional importance [24]. Less Moisture content in leaves indicated that there is less chances of microbial degradation of drug during storage. On the other hand high ash content in both L1 and L2 indicates that *Stevia rebaudiana* are good source of inorganic minerals [25].

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