

QUANTITATIVE ESTIMATION OF BIOCHEMICAL CONTENT OF VARIOUS EXTRACTS OF STEVIA REBAUDIANA LEAVES

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ABSTRACT

The purpose of current research work is quantitative investigation of total carbohydrates, reducing sugars, protein and amino acids in various leaf extracts of *Stevia rebaudiana*. Leaf extracts of *Stevia* were evaluated for their biochemical composition. In fresh leaves extract the percentage of carbohydrates is higher than reducing sugars and amino acids and least protein content. In dry leaves extract reducing sugars are present in highest percentage followed by carbohydrates, proteins and amino acids respectively. In the present work the concentration of sugars, proteins, amino acids were found to be higher in dry leaves than that of fresh leaves due to reduction in moisture content and net increase in dry mass. Protein and carbohydrate content was found to be highest in dry leaf extract of *Stevia* as compared to fresh leaf extract. The demand of *Stevia* is increasing widely due to its non caloric nature and usages as natural supplement for sugar. This study will help to set up certain protocols for purification of *Stevia* extract.

Keywords: *Stevia rebaudiana*, Biochemical, protein, carbohydrate

INTRODUCTION

Stevia rebaudiana (Bertoni) is a new and versatile herb with incredible sweetness that is gaining very high popularity amongst all type of sweetener users as most ideal substitute for sugar. It produces sweet steviol glycosides (Soejarta *et al.*, 1982 and Savita *et al.*, 2004). It is high demanding antidiabetic medicinal plant belonging to Astaraceae family. It is a perennial and endemic, medicinal shrub (Sivaram and Mukundan, 2003). It is also called as honey plant due to its sweetness. The fresh leaves have a nice liquorice taste. It is recommended for diabetes and has been extensively tested on animals and has been used by humans with no side effects (Megaji *et al.*, 2005). The components obtained from *Stevia* are the best alternative natural sweetener for diabetes. Leaves of *Stevia* contain sweetening compounds viz., Stevioside, Rebaudioside-A, Rebaudioside-B,C and six other compounds which are said to be having insulin balancing properties (Farooqi and Sreeramu, 2001). Diabetic persons with hyperglycemia can use *Stevia* as alternative natural sweetener (Din *et al.*, 2006). *Stevia* have versatile medicinal uses without any side effects that focus the interest towards *Stevia* in World wide. It is used for the treatment of various conditions such as cancer (K. Yasukawa *et al.*, 2002) diabetes (N. Lailerd *et al.*, 2004), obesity, cavities, hypertension (Dyrskog *et al.*, 2004) fatigue, depression, and in cosmetic and dental preparations. It possesses hypoglycemic, hypotensive, vasodilating, taste improving, sweetening, anti-fungal, anti viral, anti inflammatory, anti bacterial (Ghosh, 2008) properties and increases urination function of the body. For Patients of diabetes, hypoglycemia, high blood pressure, obesity and chronic yeast infections, *Stevia* is the ideal sweetener. It can be safely used in herbal medicines, tonics for diabetic patients and also in the daily usage products.

The purpose of this research work is quantitative investigation of biochemicals present in various leaf extracts of *S. rebaudiana*. The demand of *Stevia* is increasing widely due to its non caloric nature and usages as natural supplement for sugar. The leaves are having commercial importance due to presence of di-terpene sweet glycosides which are 300-400 times sweeter than sugar without any side effects. The plant was domesticated in India in last 20th century from the wide source. So there is a need to set up certain protocols for purification of *Stevia* extract by various techniques. This investigation was carried to study and compare presence of biochemical components in various leaf extracts of *Stevia*.

MATERIALS AND METHODS

S. rebaudiana plants were obtained from Jamna Biotech, Pune. Leaves were collected and dried under shade at room temperature. Dry leaves were packed in polyethylene bags and stored in deep

freezer until used. The fresh leaves and dry leaves were used for were subjected for extract preparation.

Sample preparation (A, B and C)

Green leaf extract: Methanolic extract of fresh *Stevia* leaves (5g) was prepared by vigorous grinding in 80% alcohol and distilled water respectively by using pestle and mortar. The mixture was centrifuged at 8000 rpm for 20 min. Supernatant was collected and used for analysis. B) Dry leaf extract: Same procedure was carried by using dry leaf powder. C) Stevioside powder: Commercially available stevioside powder was taken in 1mg/ml concentration. Alcoholic (methanol) extract of pure stevioside powder were prepared and used for analysis. Sample preparation (D and E): 250 g of finely powdered dry leaves of *Stevia rebaudiana* were extracted with hot water (1 litre) for two hours at 70°C. The extract was filtered through Whatman #1 filter paper and the clear green solution (800ml) was concentrated to 400ml by heating. Two methods were used for partial purification of extracts. D) pH of partially purified extract was brought down to pH 3.5 with fumaric acid. It was refiltered and the pH of the filtrate adjusted to 10.0 with dilute sodium hydroxide. A pasty mass separated out. It was filtered and the pH of the filtrate readjusted to 8.5 with addition of potassium aluminum sulphate (alum). The solution was clear and clarified. Thus the solution was partially purified. E) pH of the extract was adjusted to 11.5 with calcium oxide filtered and the pH of the filtrate adjusted to 6.5 with glucono-delta-lactone. The solution was clear and clarified. Both the extracts D and E were stand for 4-5 hours and supernatant was taken. The solution was further diluted 10 times with distilled water and used for analysis (1ml extract+9ml distilled water).

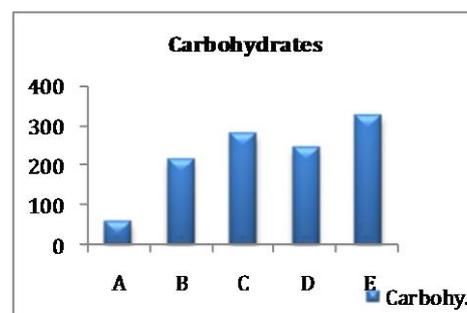


Fig 1: Concentration of carbohydrates (µg/ml) in different samples.

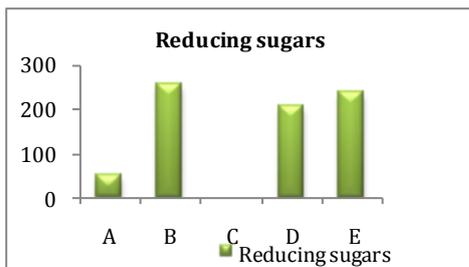


Fig 2: Concentration of reducing sugars (µg/ml) in different samples.

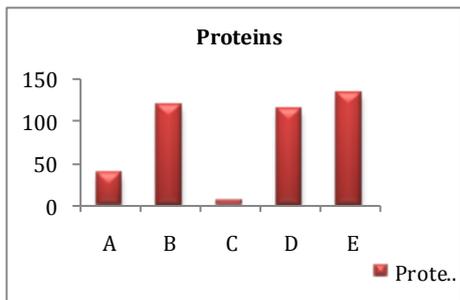


Fig 3: Concentration of proteins (µg/ml) in different samples

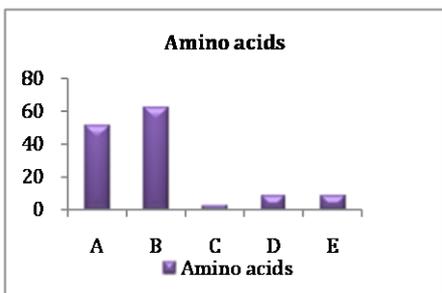


Fig 4: Concentration of amino acids (µg/ml) in different samples.

BIOCHEMICAL ANALYSIS

Total soluble carbohydrates were estimated quantitatively by using Anthrone's method. Total soluble carbohydrate was calculated with the help of a reference curve using D- glucose as standard. The reducing sugar content was estimated following the method of Lindsay (1973). For the estimation of reducing sugar DNSA method was used. The quantitative estimation of proteins was done by using Lowry et al., (1951) method. Folin-Lowry's method is the most commonly used method for determination of proteins in cell free extract because of its high sensitivity. The method was outlined by Lee and Takahashi (1966).The method used for the estimation of amino acids using ninhydrin reagent.

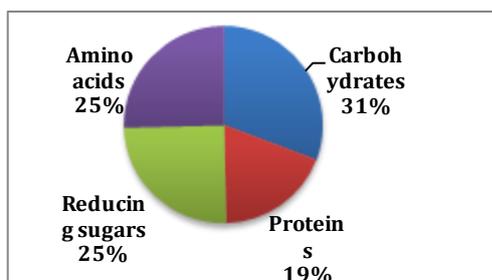


Figure 5: Comparison of biochemical content in fresh leaf extract of Stevia.

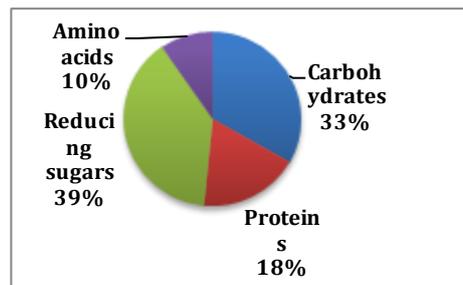


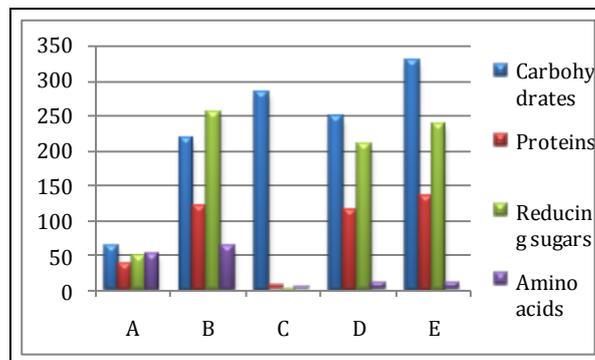
Figure 6: Comparison of biochemical content in dry leaf extract of Stevia.

Table 1: Biochemical content in five extracts of *S.rebaudiana*.

Contents (µg/ml)	A	B	C	D	E
Carbohydrates	61.66	217.50	282.50	246.66	326.66
Reducing sugars	50	254.16	*ND	208.33	237.5
Proteins	37.91	120	5	115	133.75
Amino acids	50.83	62.5	2.91	9.165	9.165

*ND=Not detected (Present in very low concentration)

Fig 7: Comparison of biochemical contents (µg/ml) in all the samples.



RESULT AND DISCUSSION

Biochemical analysis

The quantitative estimation of biochemical contents of major biomolecule such as carbohydrates, reducing sugars, proteins, amino acids was done. The optical density of each sample was measured with the help of colorimeter and was plotted on graph of respective standard used particularly for each biochemical. From the graph, concentration of biochemical content present in one ml of sample was calculated and is presented below in tabulated form. The concentration of carbohydrates present in different samples is compared with the help of graph below. The amount of carbohydrates content is higher in dry leaves than that of fresh leaves. Pure stevioside powder is showing higher carbohydrate content followed by solution A and solution B. The concentration of reducing sugars present in different samples is compared with the help of graph below. The dry leaves are showing greater reducing sugar content than that of fresh leaves. In case of pure stevioside powder the reducing sugars content was not observed in considerable amount. Solution A and solution B also contain reducing sugars in higher amount. The concentration of proteins present in different samples is compared with the help of graph below. The fresh leaves sample showed less protein content than that of dry leaves sample, whereas least amount of protein content was seen in pure stevioside sample. Solution A and solution B also showed higher amount of protein content. The concentration of amino acids present in different samples is compared with the help of graph below. The dry leaves sample is showing slight higher free amino acids content than that of fresh leaves sample. Least amount of amino acid content was seen in pure stevioside. While equal amount of amino acids content was seen in solution A and solution

B. Comparison of carbohydrates, proteins, reducing sugars, amino acids present in fresh and dry leaves of *Stevia rebaudiana* was done using the results obtained from the quantitatively estimated biochemical contents. In fresh leaves extract the percentage of carbohydrates is higher reducing sugars and amino acids and least protein content. In dry leaves extract reducing sugars are present in highest percentage followed by carbohydrates, proteins and amino acids respectively. The comparison of biochemical contents shows that in overall samples the amount of carbohydrates is maximum followed by reducing sugars. Proteins content is also higher than that of amino acids but it is less than reducing sugars. In the present work the concentration of sugars, proteins, amino acids were found to be higher in dry leaves than that of fresh leaves due to reduction in moisture content and net increase in dry mass. Partial purification of sample using calcium oxide and acid-alkali treatment is responsible to enhance the content of carbohydrate but amount of protein remains unaffected by partial purification. In the case of pure stevioside sample purification results in decrease in protein, amino acids, and reducing sugars content in response to increase in carbohydrate content. The amount of total carbohydrate and reducing sugars estimated are found to be in similar amounts. Negligible amount of protein observed in pure stevioside sample corresponds to least protein content.

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