Asian J. Med. Biol. Res. 2016, 2 (1), 121-130; doi: 10.3329/ajmbr.v2i1.27577

Asian Journal of Medical and Biological Research
ISSN 2411-4472 (Print) 2412-5571 (Online)
www.ebupress.com/journal/ajmbr

Article

Biochemical and phytochemical evaluation of *Stevia rebaudiana*

Md. Moniruzzaman Sohag Howlader[1], Sheikh Rashel Ahmed[1*], Khadizatul Kubra[2] and Md. Khairul Hassan Bhuiyan[3]

[1]Department of Biotechnology, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[2]Department of Biotechnology and Genetic Engineering, Noakhali Science and Technology University, Noakhali, Bangladesh
[3]Department of Physics, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

[*]Corresponding Author: Sheikh Rashel Ahmed, Department of Biotechnology, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh. Mobile: +8801925652984; E-mail: rashel_gebcu@yahoo.com

Received: 10 March 2016/Accepted: 21 March 2016/Published: 31 March 2016

Abstract: The present study was aimed to evaluate biochemical and phytotochemical of dry leaves of *Stevia rebaudiana*. Biochemical analysis indicated that Stevia leaves are a good source of carbohydrate and reducing sugar. Different extraction methods were used to prepare four different dry extracts (Extract A, B, C, D). Total soluble sugars and reducing sugars were analyzed for these four dry extracts and commercial Stevia powder. The highest amount of total soluble sugar (477 mg sugar g^{-1} dry extract) was obtained from extract C and higher amount of reducing sugar (82 mg g^{-1} dry extract) was obtained from extract D among the extracts. But commercial Stevia powder showed higher total soluble sugar content (754 mg g^{-1} dry powder) and highest amount of reducing sugar (98 mg g^{-1} dry extract) than all the extracted dry samples. The extraction process of dry extract C was feasible for the extraction of total soluble sugar. For the phytochemical screening, crude extract was tested for the presence of different chemical groups and presence of alkaloids, phenolic compounds, steroids, tannins, flavonoids, cardiac glycosides and saponins that were identified. The highest amount of total phenolic compounds (92 mg) was recorded from methanolic extract of extraction B. The lowest amount of total phenolic compounds (36 mg) was recorded in ethanolic extract of extraction A. So, Methanol proved as best solvent to extract increased quantity of total phenolic compounds than other solvents.

Keywords: biochemical, phytochemical, evaluation, *Stevia rebaudiana*

1. Introduction

Stevia is wonderful alternative to sources and artificial sweetener for those who are diabetic. It nourishes the pancreas and thereby helps to restore normal pancreatic function (Adesh *et al*., 2012). The latest estimation of WHO for the number of people with diabetes, worldwide in 2000 is 171 million. This is likely to increase in diabetes will occurs at least 366 million by 2030. On the other hand, the current WHO estimate for the number of people with diabetes, Bangladesh, in 2011 is 7 million (Hossain *et al.*, 2013).

Stevia rebaudiana is a herbaceous perennial shrub belonging to the sunflower family Asteraceae which is indigenous to Brazil and Paraguay (Uddin *et al.*, 2006; Alhady, 2011). It is commonly known as honey leaf which is estimated to be 300 times sweeter than sugar cane (Chalapathi and Thimmegowda, 1997). The sweet herb Stevia is rapidly replacing the chemical sweetener like Splenda, Saccharine and Aspartame. Savita *et al*. (2004) analysed the leaves of *S. rebaudiana* on dry weight basis and calculated the energy value of 2.7 kcal g^{-1}. The leaves of Stevia contain a natural complex mixture of eight zero caloreic sweet diterpene glycosides, including isosteviol, stevioside, rebaudiosides (A, B, C, D, E, F), steviolbio-side and dulcoside A (Rajasekaran

et al., 2008; Goyal *et al*., 2010). Structurally, stevioside is a glycoside with a glucosyl and a sophorosyl residue attached to the aglycone steviol, which has a cyclopentanon hydrophenanthrene skeleton (Gupta *et al*., 2013). Stevioside can be degraded to its major metabolite, steviol, by intestinal bacterial micro flora of human being. It does not have the neurological or renal side effects associated with some of the artificial sweeteners (Goyal *et al.*, 2010). Toxicological studies have shown that stevioside does not have mutagenic or carcinogenic effects and no allergic reactions have been observed when it is used as sweetener (Aminha *et al.*, 2014). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Siddique *et al*., 2014). The documented properties of Stevia are anti-bacterial, anti-fungal, anti-inflammatory, anti-microbial, anti-viral, anti-yeast, cardio-tonic, diuretic, hypoglycaemic, hypotensive and as a vasodilator (Goyal, 2010). It also has a healing effect on blemishes, wounds, cuts and scratches. In 1999 the JECFA clearly stated that there was no indication of carcinogenic potential of stevioside (WHO, 1999).

2. Materials and Methods

The research work was conducted at laboratory of the department of biotechnology and physics with a common help of central laboratory, Bangladesh Agricultural University, Mymensingh during the period from July to November, 2015.

2.1. Preparation of leaves powder of *Stevia rebaudiana*

Leaves were removed from the plants then, washed in clean water and spread on trays. Leaves were dried under shade at room temperature ranged from to 25 - 30°C for 24-48 h. Dry leaves were packed in polyethylene bags and stored until used. By using mortar and pestle, fine powder of leaves was prepared. The dry powders of leaves were used for extract preparation.

2.2. Preparation of dry extracts

a) Preparation of Extract A: 20 g dried ground leaves powder of *stevia* plant was mixed with 1 litre water at a ratio of 1:50. Water was heated using Bunsen burner at 65^0C temperature for 1hr with uninterrupted stirring. The crude extract containing stevioside was filtered through hand-made cloth and again filtered through Whatman No. 1 filter paper. The filtrate was purified with two times addition of 100 gm dried local soil of Mymensingh and 5% Ca $(OH)_2$ .The filtrate, collected through filtering with Whatman No. 3 filter paper, was passed through a separating funnel packed with amberlite-4B resin to remove undesirable pigment and colour (Plate 1). The clear and colourless elute was used to get concentrated liquid which was stored at -4^0C in refrigerator to get crystalline Stevia powder (Abou-Arab *et al.*, 2010).

b) Preparation of Extract B: Water was added to ground leaves powder at ratio (50:1 v/w) and extracted by heating water at 65^0C for 1hr on hot water bath with continuous stirring, then filtered through Whatman No. 3 filter paper. On the basis of weight of dried leaves) laboratory grade CaO was added two times to the deep brownish filtrate which was again filtered with Whatman No. 3 filter paper. Small quantity of $FeSO_4$ and activated charcoal was added to the filtrate which was again filtered. This step was repeated until the elute was cleared and then concentrated by using rotary evaporator at 45^0C to the maximum concentrated value (Slightly modified Abou-Arab *et al*., 2010).

c) Preparation of Extract C: The dried leaves powder was extracted by water according to slightly modified Nishiyama *et al*. (1992). The dried powder were mixed at ratio 1:50 with water and heated at 65^0C for 1hr with non-stop stirring .It was incubated at room temperature for 8 hrs at 150 rpm in an orbital shaker. This was filtered through Whatman No. 3 filter paper and the filtrate was mixed wih equal amount of ferrus sulphate and activated charcoal. Ultrasonication was applied for 30 min to the solution to settle down brown precipitate. This was filtered to get clear pale yellowish solution. The addition of small quantity of laboratory grade calcium oxide and further filtration through Whatman No. 3 filter paper improve purification process (Figure 1).

d) Preparation of Extract D: Green leaf extract: Methanolic extract of fresh *Stevia* leaves (5g) was prepared by vigorous grinding in 80% alcohol and distilled water respectively by using mortar and pestle. The mixture was centrifuged at 6000 rpm for 30 min. Supernatant was collected and dried for analysis in hot air oven at 45^0C (Figure 1).

e) Preparation of commercial stevia powder: Commercially available stevioside powder was taken in 1mg/ml concentration that was further diluted to make 0.01% solution.

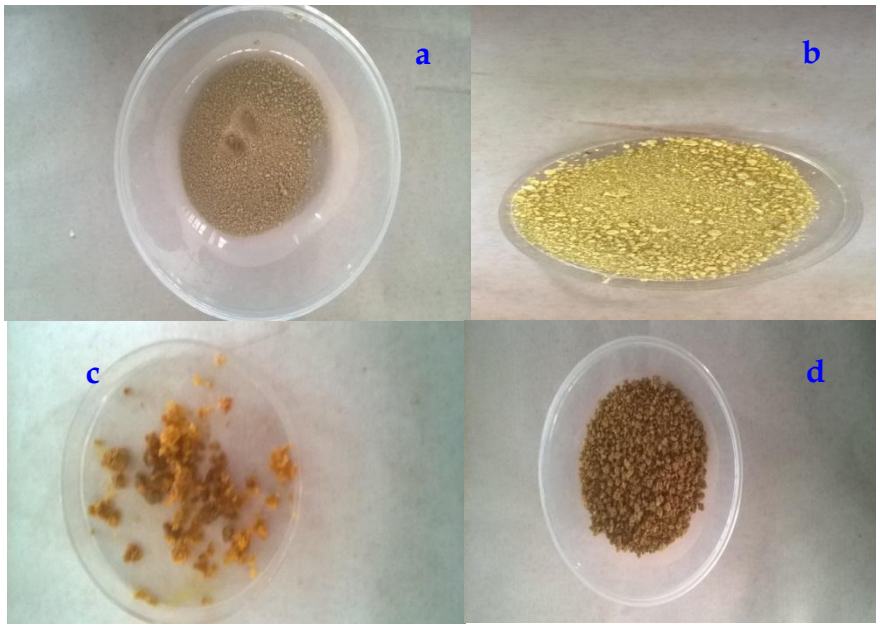


Figure1. Preparation of extract of Stevia leaves (a) Extract A; b) Extract B; c) Extract C and d) Extract D

2.2.1. Biochemical analysis of dry extracts

Total soluble carbohydrates were estimated quantitatively by using Anthrone reagent according to the method of Yem and Willis (1954). Green to dark green color was read at 630 nm using spectrophotometer. The reducing sugar content was estimated as per the method of Lindsay (1973). For the estimation of reducing sugar, DNSA method was used. The intensity of dark red color was read at 510 using spectrophotometer. Total soluble carbohydrate and total reducing sugar was calculated with the help of a reference curve using D- glucose as standard.

2.2.1.1. Estimation of total soluble sugar

Anthrone reagent was prepared by dissolving 0.2 g of anthrone in 100 ml conc. H_2SO_4. This reagent was freshly prepared whenever needed. 100 mg oven dried pure glucose was dissolved in a volumetric flask of 100 mL. The volume was made up to 100 mL. 10 mL of this solution was further diluted to 100 mL by adding 90 mL distilled water for the preparation of 0.01% glucose solution.

Procedure:

1 ml extracted solution was taken in the test tubes and 4-5 mL freshly prepared anthrone reagent was added slowly into the tube. The tube was placed into an ice-bath to prevent the loss of water by evaporation. After cooling, the tube was heated in a boiling water-bath for 10 min and was cooled again in running tap water. The test tubes were incubated for 20 min., at room temperature (25º C). Blank was maintained with distilled water and reagents. The absorbance of the green solution was measured at 625 nm on a spectrophotometer. Total sugars were estimated by comparing the absorbance of sample with the absorbance of gallic acid standard.

2.2.1.2. Estimation of reducing sugar

100 mg anhydrous glucose was dissolved in distilled water and then raised the volume to 100 ml with distilled water.

Preparation of reagent:

- DNS reagent: This reagent was prepared by dissolving 1 g 3,5-dinitrosalicylic acid (DNS), 1 g sodium hydroxide, 0.2 g crystalline phenol, 0.05 g sodium sulphite in 100 ml distilled water.
- 40% (w/v) sodium potassium tartrate (Rochelle salt)

Procedure:

A 1 ml liquid sample was mixed with 3 ml of DNS reagent.1 ml of 40% (w/v) sodium potassium tartrate was also added to reagent. All the tubes were heated in water bath at 99°C for 5 min to develop red-brown colour. After cooling to room temperature, 5 ml of distilled water was added to mixture. The absorbance was recorded with a spectrophotometer at 575 nm and total reducing sugar was calculated with the help of a reference curve using D- glucose as standard.

2.2.1.3. Estimation of non reducing sugar
Non-reducing sugar was estimated by following equation-
Non reducing sugar =Total sugar -Reducing sugar

2.3. Evaluation of phytochemicals of *Stevia rebaudiana*
2.3.1. Preparation of extracts for phytochemical screening of *Stevia rebaudiana*
A total of three types of extracts using three different solvents of ethanol, methanol and water were collected from the samples of *Stevia rebaudiana*.

2.3.1.1. Aqueous extract
Collection of aqueous extract was done in a different way than the other two. 40 g of Stevia powder was measured and mixed with 480 ml of distilled water. This was left for 2 days in sterile environment. The liquid extract was then filtered through Whatman Filter paper no.1. The filtrate was kept in water bath at 80-90 ºC till the extract was dried out. The dried extracts were then stored at -4 ºC in refrigerator.

2.3.1.2. Ethanoic extracts
For collection of ethanoic extracts 32 g of Stevia powder was packed in thimble and extracted in Soxhlet apparatus using 320 ml of ethanol. The temperature was kept between 60-80ºC. The samples in the thimble of the Soxhlet apparatus was kept boiling for approximately 6 hours till the solution becomes clear and the dark colored extract was collected at the bottom of the apparatus. This was then collected in petri dishes and left to dry for 24 hours. The dried extract having a sticky appearance was stored in 25 ml at temperatures at 4 ºC in the refrigerator for further use. The whole process was repeated 3 times for the collection of a substantial amount of extracts for the study.

2.3.1.3. Methanolic extract
The methanolic extracts were collected in the same way as the ethanolic extracts. 32 g of powdered sample was weighted in the weighing machine and packed in the soxhlet apparatus with 320 ml methanol. As the methanol boiled the extract was slowly collected in the flask and extract collection through soxhlet apparatus below. The temperature here too was adjusted between 60-80 ºC. After leaving to dry overnight in Petri dishes, the extracts were collected with spatula.

2.3.1.4. Preparation of stock solution for phytochemical assays
For working the extracts were dissolved and a stock solution of 10µg/µl was made. This was done by mixing 1 g of crude extract with 100 ml of solvent. All three stock solutions were made this way.

2.3.2. Tests for preliminary phytochemical screening
2.3.2.1. Test for alkaloids
a. **Dragendroff's test:** To 2 ml of each of the extract , few drops of Dragendorff's reagent (potassium bismuth iodide solution) was added. A turbid orange/orange red precipitate was observed for detecting the presence of alkaloids.
b. **Wagner's test:** To indicate the presence of alkaloids, a few drops of Wagner's reagent was added in 2-3 ml extracts. A brown flocculant/ reddish- brown precipitate confirms the test as positive.
c. **Hager's test :** Presence of alkaloids was confirmed by the formation of yellow coloured precipitate from Hager's reagent treated with extract.

2.3.2.2. Tests for phenolic compounds
Equal amounts of 1% ferric chloride solution and 1% potassium ferrocyanide was mixed.3 drops of this freshly prepared mixture was added to 2 ml extract. A positive result shows the formation of a bluish-green color.

2.3.2.3. Test for tannins
a. **Ferric chloride reagent test:** Each of the Extracts was allowed to react with 3-4 drops of 5% ferric chloride solution. Formation of intense green/ greenish black/ brownish green colour indicates the presence of tannin.
b. **Potassium dichromate test:** Each extract solution was allowed to treat with 1 ml of 10% of aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggested the presence of tannins.
c. **Gelatin test:** Formation of white precipitate from the addition of 1% gelatin solution containing sodium chloride to the extract indicates presence of tannin.

2.3.2.4. Tests for flavonoids

a. Alkaline reagent test: 1 ml of 10 % sodium hydroxide solution was added to test sample to produce intense yeollow color which which becomes colorless on the addition of dilute acid, indicates the presence of falvonoids.

b. Lead acetate test: Extracts were treated with a few drops of 10% lead acetate solution for the formation of a yellow color precipitate suggested the presence of flavonoids.

2.3.2.5. Test for steroids

a. Salkowki's test: 1ml of chloroform was added to 2ml of each extract followed by the careful addition of concentrated sulphuric acid on the wall of test tube to form a lower layer. A reddish brown ring produced immediately at the interface indicated the presence of steroid.

2.3.2.6. Test for cardiac glycoside

a. Keller-kilani test: 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3 was added to the 2ml extract, followed by the addition of 1 ml concentrated sulphuric acid. A brown ring was formed at the junction of two layer indicated the presence of cardiac glycosides.

2.3.2.7. Test for saponins

a. **Froth test:** 5ml of extract was shaken vigorously for 30 second and left to stand for 10 min, then observed for the formation of a stable persistent froth that confirms the presence of saponins.

2.3.3. Extraction for the estimation of total phenolic compounds

Two extraction methods were performed by different solvent to estimate total phenolic compounds.

a. Extraction A: Methanol, ethanol and acetone of 10 ml of 80% were used to extract 1 gm of sample. The homogenate was centrifuged at 6000 rpm for 30 minutes. The supernatant was collected and evaporated to dryness. Methanolic solution of the extract in the concentration of 0.1 mg/ml was used in the analysis**.**

b. Extraction B: A touch type vortex mixer was used to dissolve 10 mg of the sample in 2 ml 50% methanol followed by adding 8 ml of 50% methanol to it. Sonication technique was applied to make a sample of concentration 0.1 mg/ml**.**

2.3.4. Estimation of total phenolic compounds

The concentration of phenolics in plant extracts was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content.

a) Preparation of standard gallic acid

100mg pure standard gallic acid was dissolved in 100ml distilled water to prepare a concentration of 1mg/ml. From this, 10ml solution was taken in 100 ml volumetric flask and 90 ml distilled water was added to make a volume of 100 ml.

b) Materials

I. Follin-ciocalteau reagent (FCR): 2N commercially available FCR reagent was diluted to make 1N FCR reagent.

II. 35% sodium carbonate solution (Na_2CO_3)

c) Procedure

1 ml extract was taken in a test tube. Then 1ml FCR reagent and 1 ml Na_2CO_3 was added. It was mixed well and left for 15 minutes. After 15 minutes, 7 ml distilled water was added. The absorbance was recorded at 620 nm in the UV spectrophotometer against the reagent blank. Finally, the content of total phenolic compounds was determined using a standard curve prepared with gallic acid.

3. Results and Discussion

3.1. Biochemical evaluation

The quantitative estimation of biochemical contents of different dry extract was carried out to investigate carbohydrates, reducing sugars and non-reducing sugar. The optical density of sample of each extract was measured with the help of spectrophotometer and was plotted on graph of respective standard used particularly for each biochemical. The concentration of total soluble sugar and reducing sugar present in different samples of extracts was compared with the help of standard glucose graph (Figures 2 and 3). Commercial stevia powder showed higher total soluble sugar content 754 mg per 1 gm dry powder than all the extracted dry sample (Table 1, Figure 4). Among the extracts, extract C contained the highest amount of total soluble sugar (477 mg sugar

per 1 gm extract powder). The highest amount of reducing sugar (98 mg) was estimated in commercial stevia powder. However, among the extracted samples, extract D contained higher amount of reducing sugar (82mg) (Table 1, Figure 4). The dry leaves were showing greater total soluble sugar content than that of fresh leaves. 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 was responsible to enhance the content of total sugar. The highest amount of non-reducing sugar was also found in high amount in commercial powder 656 mg g^{-1} dry powder. The lowest amount of non-reducing sugar (153 mg) was estimated in Extract A powder. So, the extraction process of extract C extracted higher amount of total soluble sugar than the other extraction process. The comparison of biochemical contents showed that in overall samples the amount of total soluble sugar was maximum followed by reducing sugars. According to Rafiq *et al. (*2007), the soluble total sugar contents were 306 mg g^{-1} in sample 1 with reducing sugars 27.28 of dry weight. Deshmukh *et al.* (2014) reported that the amount of total carbohydrates was found to be 20 mg g^{-1} and the amount of total reducing sugar was found to be 0.877 mg g^{-1} dry extract. They commented that the percentage of carbohydrates was higher than reducing sugars in fresh leaves extract.

Table 1. Contents of biochemicals in extracts.

Extract	Content(mg g^{-1} dry extract)		
	Total soluble sugar	**Total reducing sugar**	**Non-reducing sugar**
Extract A	188	35	153
Extract B	264	59	205
Extract C	477	39	438
Extract D	279	82	197
Commercial stevia powder	754	98	656

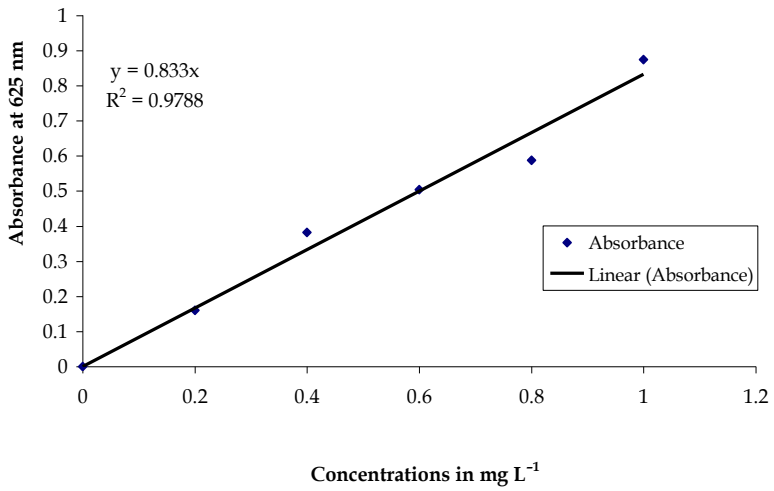


Figure 2. Standard glucose curve for estimation of Total soluble sugar.

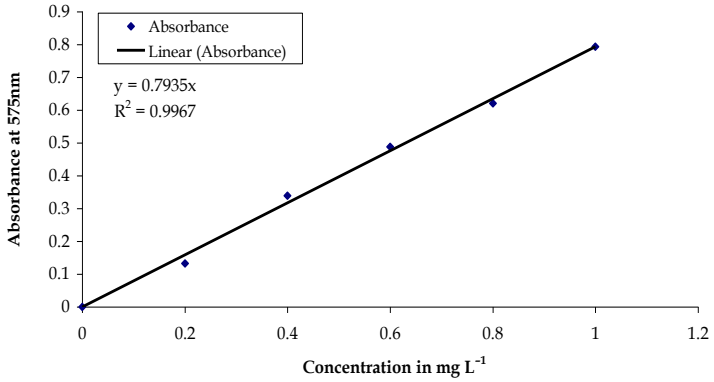


Figure 3. Standard glucose curve for estimation of reducing sugar.

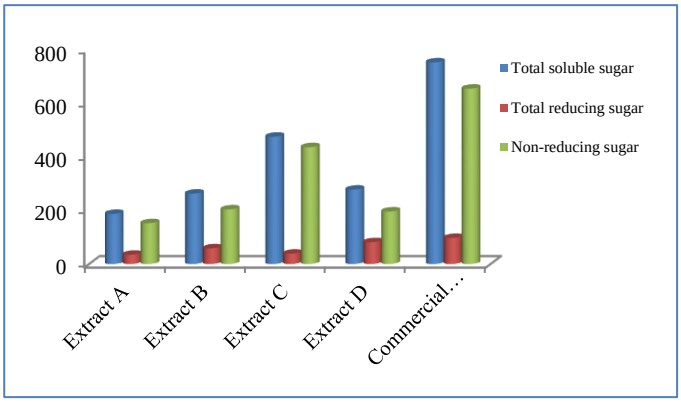


Figure 4. Contents of total soluble sugar, reducing sugar and non-reducing sugar (mg g^{-1} dry extract) in different samples.

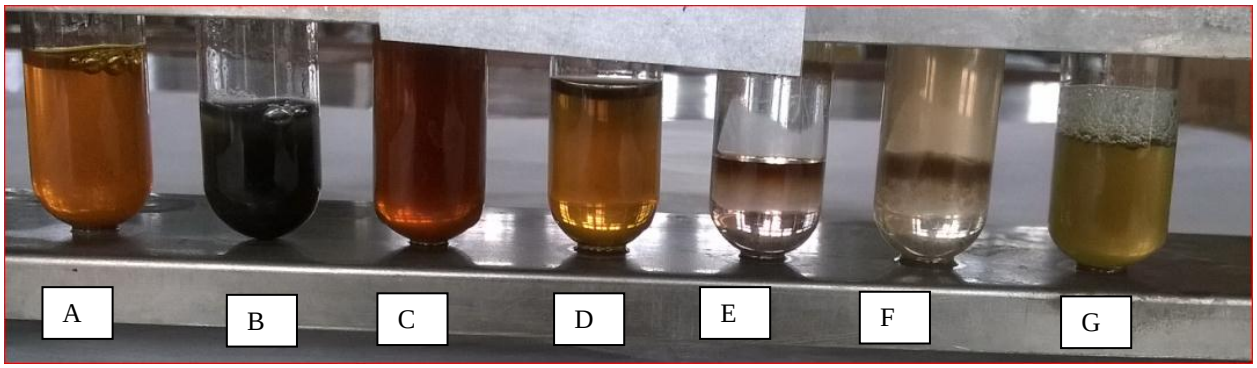
3.2. Evaluation of phytochemicals of *Stevia rebaudiana*

The results on preliminary phytochemical screening of dry leaves extract of Stevia revealed the presence of alkaloids, tannins, flavonoids, cardiac glycosides, steroids, saponins. Presence of alkaloids was confirmed through Hagers, Wagners and Dragendroffs test. The dragendroffs test gave a strong positive result for the aqueous extract. Hagers and Wagners test gave a strong positive result for both methanolic and ethanolic extract. The presence of phenol, flavonoids and tannins was comparatively high except for aqueous extract which was negative for phenols. Steroids and cardiac glycosides were found to be higher amount in all the extracts (Table 2, Figure 5). The highest amount of saponin was observed in aqueous and methanolic extract where traces of saponin were found in the ethanolic extract. According to Shukla *et al.* (2013) the alcoholic and aqueous extracts of *S. rebuadiana* contained steroids, glycosides, tannis, alkaloids and saponnis like phytochemicals.

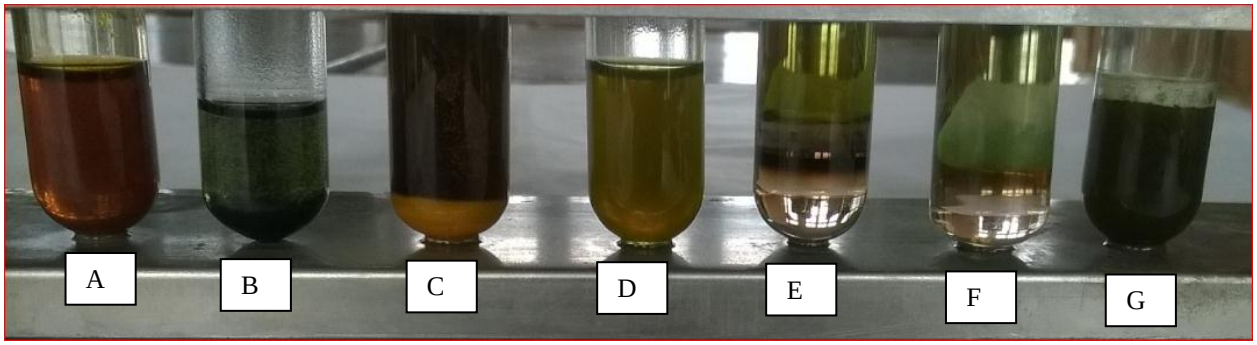
Table 2. Results of various phychemical tests for the detection and identification of chemical constituents.

Name of the test	Reagents	Aqueous extract	Methanolic extract	Ethanolic extract
Test for alkaloids	Dragendorff's	+++	++	+
	Wagners	+	+++	+++
	Hagers	+	+++	+++
phenolic compounds	% ferric chloride solution +1% potassium ferrocyanide	-	+++	+++
Test for tannins	Ferric Chloride Reagent	+++	+++	+++
	Potassium dichromate	-	+++	+++
	Gelatin	+	+++	+++
Tests for flavonoids	Alkaline reagent	+++	+++	+++
	10% Lead acetate	+++	+++	+++
Test for steroids	Salkowki's test	+++	+++	+++
Test for cardiac glycoside	Keller-kilani test:	+++	+++	+++
Test for saponins	Froth test	+++	+++	+

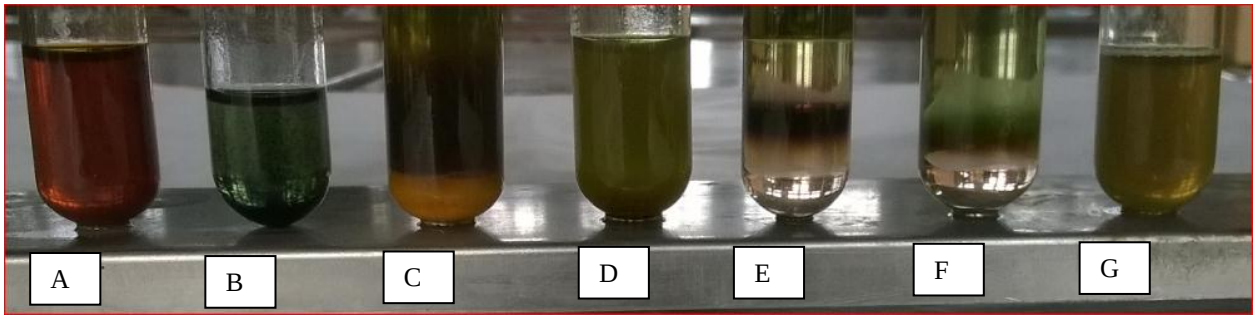
+++= Highly present,++ = Moderately present, + =Trace, - =Absent



Phytochemical screening of aqueous extract



Phytochemical screening of methanolic extract



Phytochemical screening of ethanolic extract

Figure 5. Phytochemical screening of aqueous, methanolic and ethanolic extract of dry leaves of Stevia.
A-Test for alkaloid with dragendroffs reagent
B-Test for phenolic compounds
C- Test for tannins with potassium dichromate
D- Tests for Flavonoids with alkaline reagent
E- Test for steroids (Salkowki's test)
F- Test for cardiac glycoside (Keller-kilani test)
G- Test for Saponins (Froth test)

The difference in the above results in the respective solvents may be due to the interaction of the phytoconstituents with the solvent system or the process employed for extraction. Alkaloids act in inhibiting microbial growth. The anti-inflammatory, anti-apoptotic anti-ageing properties of the plant may be due to the the presence of phenols (Archana *et al.*, 2012). Tannins hasten the healing of wounds and have antioxidant activities. Saponins have traditionally used in detergents, pesticides and molluscicides in addition to their industrial applications such as foaming and surface active agents. Flavonoids are a group of polyphenolic compounds that have potent antimicrobial, anti-inflammatory activity. Flavonoids are free radical scavengers which prevent oxidative cell damage and have strong anti-cancer activity (Archana *et al.*, 2012).

3.3. Estimation of total phenolic compounds

The total phenolic content in plant extracts depends on the type of extract, i.e. the polarity of solvent used in extraction. The high solubility of phenols in polar solvents provides high concentration of these compounds in the

extracts obtained using polar solvents for the extraction (Mohsen and Ammar, 2008; Zhou and Yu, 2004). Different solvents were used to estimate total phenolic compounds. Total phenolic compounds were estimated by comparing the absorbance of samples with the absorbance of gallic acid standard (Table 3, Figure 6). Total phenols were the major bioactive components found in extracts expressed as mg g^{-1} extract which ranged from 36-92 mg per gram extract. The highest amount of total phenolic compounds (92 mg) was recorded from methanolic extract of extraction B. The lowest amount of total phenolic compounds (36 mg) was recorded in ethanolic extract of extraction A. So, the extraction method B was most feasible to extract phenolic compounds. In both the extraction process, methanolic extract gave the highest amount of phenolic compounds. The contents of total phenolic compounds are given (Table 3). According to Tadhani *et al*. (2007) total phenolic compounds were found to be 25.18 and 35.86 mg per gram of stevia leaves and callus on dry weight basis, respectively.

Table 3. Total phenolic compounds of *S. rebaudiana* extracts.

Extraction	Solvent used for extraction	Absorbance of Extract	Total Phenolic Content in mg g^{-1} extract
Extraction A	Methanol	0.045	64
	Ethanol	0.026	36
	Acetone	0.032	46
Extraction B	Methanol	0.065	92

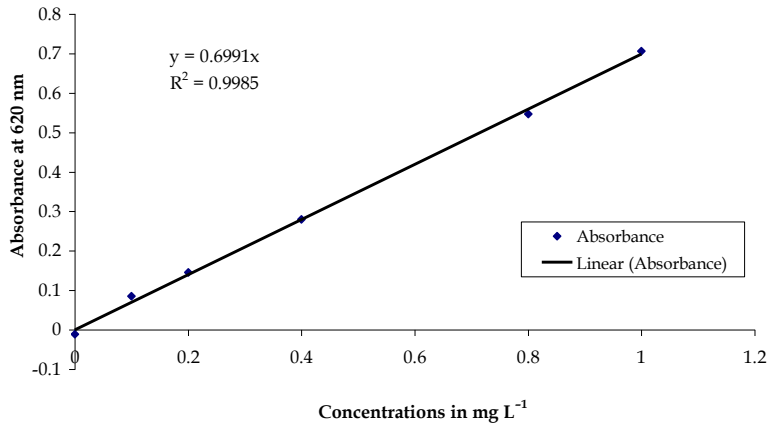


Figure 6. Standard gallic acid curve for the estimation of total phenolic compounds.

4. Conclusions

Stevia plants are a good source of carbohydrates and reducing sugar which are vital for human nutrition and maintains a good health. Total soluble sugars and reducing sugars were analyzed for four different extracts (Extract A, B, C, D) and commercial Stevia powder which were extracted through different extraction process. The extraction process which extracted dry extract C was feasible to get increased amount of total soluble sugar in extract. The demand of Stevia is increasing widely due to its non caloric nature and usages as natural supplement for sugar. Because, it might help in preventing diabetic complications and may serve as a good alternative in the present armamentarium of antidiabetic drugs. The presence of phytoconstituents in *Stevia rebaudiana* may serves as an agent to treat many infectious diseases. The primary phytochemical analysis revealed the presence of alkaloids, flavonoids, tannin, saponins, phenols, steroids and cardiac glycosides. Since the plant contains high amounts of these bioactive compounds, it is reliable to possess large number of medicinal values like anticancerous, antimutagenic, antioxidant, antifungal, laxative and antibacterial activities. The highest amounts of total phenolic compounds (92 mg) were recorded from methanolic extract of extraction B. In considering both cases of extraction method (extract A and extract B), total amount of phenolic compounds were maximum in methanolic extract. So, Methanol solvent proved to be the most effective among the other solvents used.

Conflict of interest

None to declare.

References

Abd-Alhady MRA, 2011. Micropropagation of *Stevia rebaudiana* Bertoni. A New Sweetening Crop in Egypt. Global Journal of Biotechnology and Biochemistry, 6: 178-182.

Abou-Arab A, A Azza, M Ferial, 2010. Physicochemical assessment of natural sweeteners steviosides produced from *Stevia rebaudiana* bertoni plant. African Journal of Food Science, 4: 269- 281.

Adesh AB, B Gopalakrishna, SA Kusum, OP Tiwari, 2012. An Overview on Stevia: A Natural Calorie Free Sweetener. International Journal of Advances in Pharmacy, Biology and Chemistry**,** 1**:** 362-368.

Aminha S, AN Soumya, VG Raju, BM Goud, M Irfath, SAP Quadri, 2014. Isolation and extraction of artificial sweetner(Stevia). World Journal of Pharmaceutical Research**,** 3**:** 481-486.

Archana P, T Samatha, B Mahitha, Chamundeswari, N Ramaswamy, 2012. Preliminary phytochemical screening from leaf and seed extracts of *Senna alata* L. Roxb-an Ethno medicinal plant. International Journal of Pharmaceutical and Biological Research, 3: 82-89.

Chalapathi MV and S Thimmegowda, 1997. Natural non-calorie sweetener Stevia (*Stevia rebaudiana* Bertoni), A future crop of India. Crop Research Hisar**,** 14: 347-350.

Deshmukh SR and VR Kedari, 2014. Isolation, purification and characterization of sweetners from *Stevia rebaudiana* (bertoni) for their anticancerous activity against colon cancer. World Journal of Pharmacy and Pharmaceutical Sciences, 3: 1394-1410.

Goyal S, Samsher, R Goyal, 2010. Stevia (*Stevia rebaudiana*) a bio-sweetener: a review. International Journal of Food Sciences and Nutrition, 61: 1-10.

Gupta E, S Purwar, S Sundaram, GK Rai, 2013. Nutritional and therapeutic values of *Stevia rebaudiana*: A review. Journal of Medicinal Plants Research, **7:** 3343-3353.

Hossain MM, SM Shoaib, R Mallick, R Hore, TT Tanmy, 2013. An assessment of present status and related complications of Diabetes Mellitus (DM) at port city Chittagong in Bangladesh. Standard Research Journal of Medicine and Medical Sciences**,** 1: 6-11.

Koyama E, K Kitazawa, Y Ohori, O Izawa, K Kakegawa, A Fujino, 2003. In vitro metabolism of the glycosidic sweeteners, Stevia mixture and enzymatically modified Stevia in human intestinal microflora. Food and Chemical Toxicology**,** 41: 359–374.

Lindsay HA, 1973. Colorometric estimation of reducing sugar in potatoes with 3,5-dinitrosalicylic acid. Potatoes Res., 16: 176-179.

Mohsen MS and SMA Ammar, 2008. Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chem., 112: 595-598.

Nishiyama P, 1991. Correlation between total carbohydrate content and stevioside content in *Stevia rebaudiana* leaves. Arquivos de Biologia e Technologia, 34: 3-4

Rafiq M, MU Dahot, SM Mangrio, HA Naqvi, IA Qarshi, 2007. *In vitro* clonal propagation and biochemical analysis of field established *Stevia rebaudiana* bertoni. Pakistan Journal of Botany, 39: 2467-2474.

Rajasekaran T, A Ramakrishna, SK Udaya, P Giridhar, G Ravishankar, 2008. Analysis of predominant steviosides in *Stevia rebaudiana* bertoni by liquid chromatography/electrospray ionization-mass spectrometry. Food Biotechnology**,** 22: 179-188.

Savita SM, K Sheela and S Sunanda, 2004. *Stevia Rebaudiana*- A functional Component for Food Industry. Journal of Human Ecology, 15: 261-264.

Shukla S, A Mehta and VK Bajpai, 2013. Phytochemical screening and anthelmintic and antifungal activities of leaf extracts of *Stevia rebaudiana*. Journal of Biologically Active Products from Nature, 3: 56-63.

Siddique AB, SMM Rahman, MA Hossain, MA Rashid, 2014. Phytochemical screening and comparative antimicrobial potential of different extracts of *Stevia rebaudiana* Bertoni leaves. Asian Pacific Journal of Tropical Disease**,** 4: 275-280.

Tadhani MB, VH Patel, R Subhash, 2007. *In vitro* antioxidant activities of *Stevia rebaudiana* leaves and callus. Journal of Food Composition and Analysis, 20: 323–329.

Uddin MS, MSH Chowdhury, MMMH Khan, M Belal, R Ahmed, MA Baten, 2006. In vitro propagation of *Stevia rebaudiana* Bert in Bangladesh. African Journal of Biotechnology, 5: 1238-1240.

WHO Food Additives, 1999. Series 42: Safety evaluation of certain food additives ''Stevioside'', Genf, pp.119–143.

Yem EW and AJ Willis, 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochemistry Journal, **57:** 769-773.

Zhou K and L Yu, 2004. Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT, 37: 717-721.