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Research Article

Evaluation of Dried *Stevia* (*Stevia rebaudiana bertoni*) Leaf and its Infusion Nutritional Profile

Abdela Befa[1*], Abadi Gebre[2], Tesfu Bekele[2]

[1]Department of Food Science and Nutrition, Ethiopia Institute of Agriculture Research, Wondo Genet, Ethiopia; [2]Department of Food Science and Technology, Hawassa University, Ethiopia

ABSTRACT

Stevia is a recently cultivated kitchen garden as vegetables and on a large scale by entrepreneurs in Ethiopia for herbal production due to its potential uses as the sweetener (250-330 times sweeter than table sugar). *The demand for Stevia currently increased due to its low calories and medicinal values. However, there is no such information about their Nutritional profile evaluation in dried form and its infusion in Ethiopia. The purpose of this study was to evaluate of dried Stevia (Stevia rebaudiana bertoni) Leaf and its infusion Nutritional profile.* The dried *Stevia* leaf had 5.5 g/100 g moisture, 8.2 g/100 g ash,16.2 g/100 g crude protein, 3.8 g/100 g crude fat, 7.9 g/100 g crude fiber, 58.8 g/100 g carbohydrate,359.6 mg/100 g Ca, 102.9 mg/100 g Na,347.4 mg/100 g K, 324.1 mg/100 g Mg, 297.9 mg/100 g Fe, 3.7 mg/100 g Zn, 1.1 mg/100 g Cu and 9.4 mg/100 g Mn, 5.3 mg GAE*/g Total Phenolic Content (TPC), 28.6 mg CE/g Total Flavonoid Content (TFC) , 4.6 mg CE/g Condensed Tannin Content (CTC), 0.04 (IC-50, g/mL) DPPH scavenging activity, 6.67 mg AAE/g Ferric Reducing Power (FRAP) and 1.92 mg BHTE/g Total Antioxidant (TAA). *This study provides evidence on the nutritional profile of dried Stevia leaf and its infusion.*

Keywords: Antioxidant activity; *Stevia*; Phenolic content; Infusion

INTRODUCTION

Stevia is a perennial, calorie-free natural sweet herb that has cultivated in many areas of Indian, subtropical, and tropical South and Central America, Japan, Brazil, and Paraguay [1]. *Stevia* is also grown like other vegetables in the kitchen garden. The fresh leaves and leaf extracts of *Stevia* are 15-30 and 250-330 times sweeter than common sugar and table sugar, respectively [2]. The chemical composition of *Stevia* is very dependent on factors like environmental and plant age [2]. *Stevia* leaves are used in food products as low calories intense natural sweeteners [3]. *Stevia* is used in soft drinks, coffee, pastry, pickles, candies, jam, and yogurt, like sugar substitutes [4]. *Stevia* is used treat diabetics due to it not affect blood sugar levels and renal side effects as other artificial sweeteners, exhibit anti-fungal and anti-bacterial properties, can be safely used in herbal medicines and tonics and also in daily usage products such as mouthwashes and toothpaste and mild *Stevia* leaf tea offers excellent relief for an upset stomach [1,4]. *Stevia* is recently cultivated on a large and small scale by entrepreneurs in Ethiopia and its demand currently increased due to its low calories and health benefits [1,2]. However, there is no such information about their Nutritional profile in dried *Stevia* leaf and its infusion in Ethiopia. Therefore, exploring and compile the potentials of the dried *Stevia* (*Stevia rebaudiana bertoni*) Leaf and its infusion Nutritional profile.

MATERIALS AND METHODS

Sample collection and preparation

About 5 kg of fresh *Stevia* leaves were obtained from Wondo Genet Agriculture research centers (Ethiopia) and transported to Wondo Genet Food Science and Nutritional laboratory using polyethylene (plastic) bags. The fresh leaves were subjected to shade drying by spread thinly on paper-lined wooden trays and protected from direct sunlight to prevent the loss of volatile aroma compounds and also photo oxidation. The dried samples were milled using an electric Blender (Model BLG401, Zhejiang YiLi Tool Co., Ltd., China) and sieved using 2 mm sieve size to

***Correspondence to:** Abdela Befa, Researcher, Department of Food Science and Nutrition, Ethiopia Institute of Agriculture Research, Wondo Genet, Ethiopia, Tel: 916002205; E-mail: befabdela@gmail.com

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separate the milled leaves. The sieved samples were prepared and kept in an air-tight container and stored at room temperature until further analysis (Figure 1).

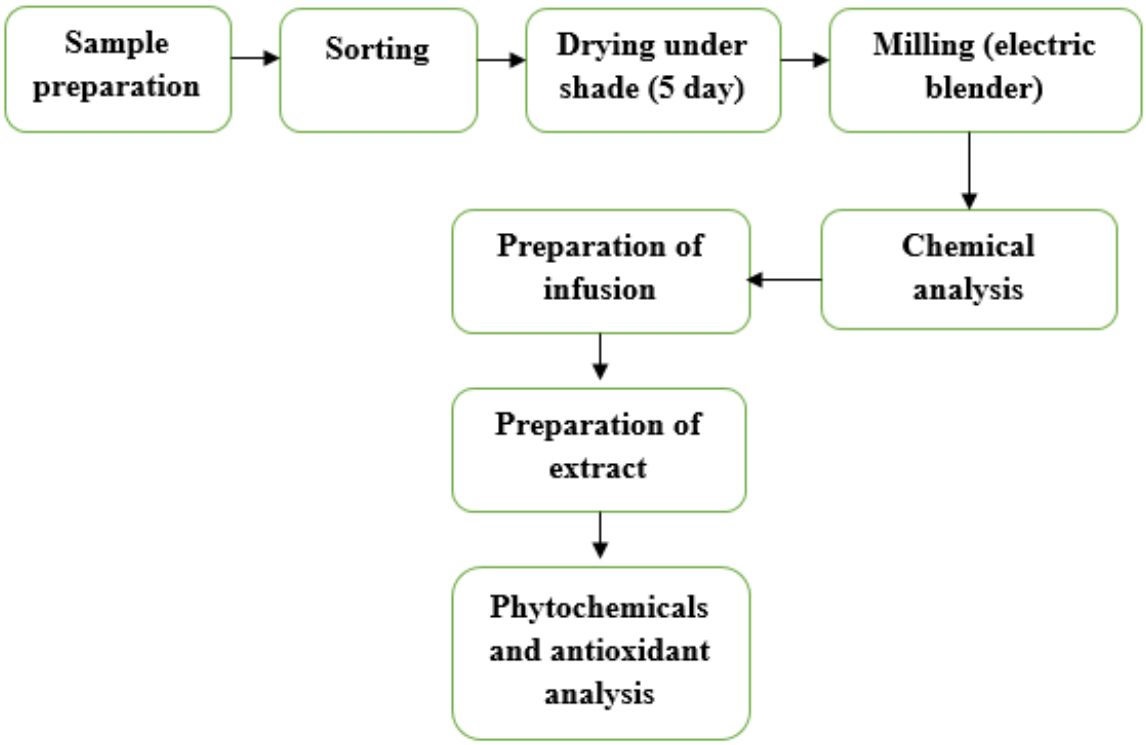

Figure 1: Flow diagram of sample preparation and analysizing.

Proximate analyses

Moisture: Moisture content was determined according to Sabir et al. [5]. A sample of 2 g was placed in the oven at 105°C for 3 hrs and cooled in desiccators. The percentage of moisture was calculated using equation 1.

Moisture (%) $=W_2\text{-}W_3/W_1 \times 100$..... 1

Where W_1= Weight of fresh samples, W_2=Weight of fresh samples and crucibles and W_3= Weight of dried samples and crucible.

Ash: Ash content was determined according to Sabir et al. [5]. A sample 2 g was measured into crucibles of a known weight and incinerated in a muffle furnace at 550°C for 3 hrs. The samples were then cooled in desiccators and weighed. The percentage of total ash was calculated using equation 2.

Ash (%) = $W_3\text{-}W_1/W_2 \times 100$ 2

Where; W_1 = Weight of empty crucible, W_2 = Weight of fresh samples, and W_3=weight of ashed samples and crucibles.

Protein: The crude protein was determined using the Kjeldahl method [5]. Sample 2 g was introduced into the digestion flask called Kjeldahl digestion flask (KDN-20C, China) at 380°C for 6 hours until the mixture by adding 10 mL of concentrated sulphuric acid (H_2SO_4), a mixture of 2.5 g of copper sulfate ($CuSO_4$), Potassium sulfate (K_2SO_4), and Titanium dioxide (TiO_2). The digest was filtered using distilled water and connected for distillation to collect ammonia for an hour to by adding 20 mL of 40% NaOH solution. The distillates 200 mL were collected in a 250 mL flask containing 20 mL of 0.2 NH_2SO_4 and methyl red indicator. The collected ammonia was estimated by back titration when the color change from red to yellow during reaction made with 20 mL of 0.1 N NaOH and 0.2 N H_2SO_4. Total nitrogen and crude protein content of the sample was then determined and calculated using equations 3 and 4, respectively.

Nitrogen (%)= (M of N_2 × acid conc. (0.02 m) × volume made × titer value)/ (1000 × W_1) × 100.....3

Crude protein (%) = % Nitrogen × 6.25 4

Where, W_1=Sample weight, M=Molar mass

Crude fat: The crude fat content was determined by the Soxhlet method [5] . The sample 2 g of the sample was weighed into the thimble and extracted with 200 mL of petroleum ether for 6 hrs. The solvent-free fat in the flux was dried in an oven for an hour at 105°C, cooled in desiccators, and fat content was then calculated using equation 5.

Crude fat (%) = $(W_e+W_f)\text{-}W_f/W_s \times 100$.....5

Where We =Weight of extract, Wf= Weight of flux and Ws= Weight of sample

Crude fiber: The crude fiber was determined according to Sabir et al. [5]. The samples 2 g were digested for 30 using 150 mL of hot 0.2N H_2SO_4. The acid was drained and the sample was washed with hot deionized water. Finally, the fiber was extracted

and dried by moistening with a small portion of acetone which was then allowed to drain. The sample was incinerated at 550°C for 3 hrs. Until all carbonaceous matter was burnt. The crucible containing the ash was cooled in the desiccators and weighed. The percentage of crude fiber was calculated using equation 6.

% Crude fiber = W_2-W_3/W_1 × 100 6

Where: W_1 = Weight of sample used W_2 = Weight of sample and crucible before ashing W_3= Weight of crucible and ash.

Carbohydrate (CHO): The Carbohydrate Content of the milled leaves of *Stevia* was determined by difference as described by Ngoddy et al. [6] and calculated using equation 7.

% CHO = 100 - % (Ash + Protein + Fat + Crude Fiber + Moisture) 7

Mineral content: The mineral contents such as - Sodium (Na), Potassium (K), Magnesium (Mg), Calcium (Ca), Zinc (Zn), Iron (Fe), Copper (Cu) and Manganese (Mn) of *Stevia* were analyzed as described by 7. Marcinek et al. [7] ash method using an Atomic Absorption Spectrophotometer (Spectra AA 220, USA Varian). Sample of *Stevia* 2 g was put in crucibles and then in a muffle furnace for 3 hrs. to obtain ash. The residue was dissolved in 10 mL of HNO_3: HCl (2:3 v/v) then heated until fumes disappeared. The solution was transferred separately in 250 mL volumetric flasks by filtration using Whatman filter paper No 42, then volume made up to 250 mL with distilled water. The concentration of mineral was calculated and expressed as mg/100 g as described in equation 8.

Element (mg/100g) = C × V × df/W 8

Where C=Concentration of the element in the sample solution in mg/L; V =Volume of the undiluted sample solution in mL; W=Weight of samples in grams, and df=dilution factor.

Preparation of extracts from *Stevia*

The extract was prepared according to Koh and Munoz-Mingarro et al. [8,9]. The *Stevia* infusion was boiled for 5 min at 97°C and filtered through a double-layered muslin cloth to get rid of the large particles and filtered through a filter paper (Whatman no.1). The filtered product was allowed to concentrate at 45°C for three consecutive days by evaporating excess water to obtain the dried. The dried extract were weighed and recorded as respective percentage yield. The 20 mg/mL of stock solution was prepared from 1 g of crude extract dissolved in 50 mL of methanol. The prepared stock solution was kept at 4°C in a refrigerator, to serve as the working solution for all the phytochemicals and antioxidant tests.

Phytochemicals

Total Phenolic Content (TPC): The total phenolic content of *Stevia* infusion extract was determined according to Shan et al. [10] using gallic acid as a standard. Folin-Ciocalteu reagent (diluted ten times) (1 mL) was added to 0.1 mL of the extract (1 mg/mL) and left for 5 min. The 1 mL (7.5% w/w) of sodium carbonate was added and the absorbance resulting blue color was measured after incubation for 90 min at room temperature at 765 nm using a UV-visible spectrophotometer (Janeway, 96500, UK). The result was calculated from gallic acid calibration curve (y = 0.0073x -0.0462, R2=0.973) and expressed as milligram gallic acid equivalent/gram of dried extract (mg GAE/g).

Total Flavonoid Content (TFC): Total flavonoid content (TFC) *Stevia* infusion extract was determined according to Ayoola et al. [11]. The extracts (1 mg/mL) were diluted with 1.25 mL distilled water and 0.75 µL 5% $NaNO_2$ was added to the mixture incubated for 6 min and 150 µL 10% $AlCl_3$ was added. The mixture was incubated for 5 min and 1 mL NaOH was added to the mixture. The absorbance of the solutions was measured using a UV-visible spectrophotometer (Janeway, 96500, UK) at 510 nm. The result was calculated using standard calibration curves obtained from Catechin (y=0.0014x+0.0192, R2=0.97 ($p<0.001$)). Results were expressed as milligram of catechin equivalents per gram of dry extract (mg CE/g).

Condensed Tannins Content (CTC): The condensed tannins content of *Stevia* infusion extract was assayed as described by Chew et al. [12]. The undiluted crude extract 0.5 mL was first mixed with 3 mL of vanillin reagent (4%, w/v, in absolute methanol), followed by the addition of 1.5 mL of concentrated HCl (37%) and stored in a dark at room temperature for 15 min. Blank was prepared by replacing 0.5 mL of the undiluted crude extract with 0.5 mL of deionized water. The absorbance of the solutions was measured using a UV-visible spectrophotometer (Janeway, 96500, UK) at 500 nm. The result was calculated from standard curve calibration obtained from Catechin (y=0.0042x+0.0331, R2=0.995 ($p<0.001$), and the results were expressed as mg catechin equivalent per 100 g dry weight sample (mg CE/100 g).

Antioxidant activities

DPPH radical scavenging activity: The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the *Stevia* infusion extract was determined as described by Brand-Williams et al. [13]. Different concentrations (50 to 1000 µg/mL) of the extracts were taken in different test tubes and the freshly prepared DPPH solution (2 mL, 0.06%, and w/v) in methanol was added into each test tubes containing 1 mL of the extract. The reaction mixture and the reference standards (ascorbic acid and BHT) were vortexed and left for 30 min in the dark at room temperature. The absorbance of the solutions and methanol (100%) used as a blank was measured using a UV-visible spectrophotometer (Janeway, 96500, UK) at 520 nm. The ability to scavenge the DPPH radical was calculated using equation 9.

Radical scavenging effect (%)= A_c-A_s/A_c×100 9

Where A_c= Absorbance of the control; A_s= Absorbance of the sample

The antioxidant activity of the extract was expressed as IC-50 (IC-50 Inhibitory Concentration 50%) and the value is the concentration in (µg/mL) of extracts that scavenges the DPPH radical by 50%.

Ferric Reducing Antioxidant Power (FRAP): The ferric reducing antioxidant power assay was carried out according to

Safdar et al. [14]. One milliliter of the *Stevia* infusion extract with a concentration of 1 mg/mL was mixed with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added to the mixture and 2.5 ml of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (0.1%). The absorbance of the solutions was measured using a UV-visible spectrophotometer (Janeway, 96500, UK) at 700 nm. The result was calculated from the calibration curve (y=0.0063x+0.148, R2=0.99 (p<0.01)) obtained from the ascorbic acid standard. The reducing power was expressed as mg of ascorbic acid equivalents/g of dried extract (mg AAE/g).

Total Antioxidant Activity (TAA): The total antioxidant activity of *Stevia* infusion extract was determined by phosphomolybdenum assay according to Safdar et al. [15]. Sample of 0.3 mL of extract (1 mg/mL) in the solution was mixed with 3 mL phosphomolybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid) in capped test tubes and incubated for 90 min in a water bath at 95°C. The absorbance of the solutions was measured using a UV-visible spectrophotometer (Janeway, 96500, UK) at 695 nm against a blank (3 mL methanol without plant extract). The total antioxidant activity was expressed as milligram butylated hydroxytoluene equivalent/gram of dried extract (mg BHTE/g) using a calibration curve (y=0.0094x+0.112, R2=0.99 (p<0.001)).

Statistical analysis

The all nutritional profile (proximate, minerals, phytochemicals, and antioxidants) of dried *Stevia* leaves and its infusion was determined in triplicate, and values were put in mean ± SD (n=2).

RESULTS AND DISCUSSION

Proximate composition of dried *Stevia* leaf

The proximate results of the dried *Stevia* leaf are shown in Table 1. The dried *Stevia* leaf had moisture (5.5 g/100 g), ash (8.2 g/100 g), crude protein (16.2 g/100 g), crude fat (3.8 g/100 g), crude fiber (7.9 g/100 g) and carbohydrate (58.8 g/100 g) content. This finding is similar (fat), lower (moisture, fiber, and carbohydrate), and higher (ash and protein) with that reported by. Moguel-Ordóñez et al. [16]. This finding is lower (moisture, ash, protein, and fat) and higher (carbohydrate) than the finding of Chouhan et al. [17]. The chemical composition variability of *Stevia* could be due to environmental and plant age factors [2].

Table 1: The Proximate composition of dried Stevia leaves (g/100g dB).

Sample	Moisture	Ash	Crude protein	Crude fat	Crude fiber	carbohydrate
Stevia	5.5 ± 0.5	8.2 ± 1.2	16.2 ± 0.1	3.8 ± 0.2	7.9 ± 1.0	58.8 ± 1.2

Values are mean ± SD (n=2)

The mineral content of dried *Stevia* leaf

The minerals analyses of dried *Stevia* leaf showed in Table 2. The dried *Stevia* leaves had about 359.6 mg/100 g Ca, Na (102.9 mg/100g), K (347.4 mg/100 g), mg (324.1 mg/100 g), Fe (297.9 mg/100 g), Zn (3.7 mg/100 g), Cu (1.1 mg/100 g) and Mn (9.4 mg/100 g). These study results of the mineral level of dried *Stevia* were higher than that of the finding of Abou-Arab et al. [18]. The chemical composition variability of *Stevia* could be due to environmental and plant age factors [2].

Table 2: Mineral contents of dried Stevia leaves in (mg/100g, dB).

Sample	Ca	Na	K	Mg	Fe	Zn	Cu	Mn
Stevia	359.6 ± 1.2	102.9 ± 0.1	347.4 ± 0.1	324.1 ± 0.1	297.9 ± 0.7	3.7 ± 0.1	1.1 ± 0. 1	9.4 ± 0.3

Values are mean ± SD (n=2)

Phytochemicals content of dried *Stevia* leaf infusion

The Phytochemicals contents of dried *Stevia* leaf infusion results are showed (Table 3). The dried *Stevia* leaf had a level of 5.3 ± 0.1 mg GAE*/g Total Phenolic Content (TPC), 28.6 ± 0.1 mg CE/g) total flavonoid content (TFC), and 4.6 ± 0.1 mg CE/g Condensed tannin content (CTC). Phytochemicals content of *Stevia* leaf was reported: flavonoids (44.6 CE mg/g), total phenols (47.25 GAE mg/g), tannin content (5.7 mg/g) [19-21].

Table 3: Total phenolic contents (TPC), total flavonoid contents (TFC) and condensed tannin contents of dried Stevia leaf infusion.

Sample	TPC(mg GAE*/g)	TFC (mg CE/g)	CTC (mg CE/g)
Stevia	5.3 ± 0.1	28.6 ± 0.1	4.6 ± 0.1

Antioxidant activities of dried *Stevia* leaf infusion

DPPH Scavenging activity, FRAP and total antioxidant activity measured the hydrogen, electron-donating abilities of primary antioxidants and reduction of Mo (Molybdenum) (VI) to Mo (Molybdenum) (V) in the presence of the antioxidant compound and subsequent formation of a green phosphate/ Mo (V) complex at acidic pH and higher temperature were studied, respectively according to Lim et al. [22] and the concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC-50) is a parameter widely used to measure antioxidant activity [23]. The lower the IC-50 the higher is the antioxidant activity [13]. The antioxidant properties of dried *Stevia* leaves infusion results are showed (Table 4). The dried *Stevia* leaf had a level of 0.04 ± 0.01 (IC-50, g/mL) DPPH scavenging, 6.67 ± 0.25 mg AAE/g Ferric reducing power (FRAP) and 1.92 ± 0.02 mg BHTE/g and total antioxidant (TAA). The antioxidant content of *Stevia* leaf was

reported that DPPH radical scavenging activity 71.6% and IC-50 for BHA observed (38.7 mg/mL) [17,19-21].

Table 4: Antioxidant activities of dried stevia leaf infusion.

Herbal tea	DPPH scavenging (IC-50, g/mL)	Ferric reducing power (mg AAE/g)	Total antioxidant (mg BHTE/g)
TF9	0.04 ± 0.01	6.67 ± 0.25	1.92 ± 0.02
AA	0.03 ± 0.02		

Values are mean ± SD (n=2), where AAE/g: Ascorbic acid equivalents per gram of dried extract; BHTE/g: Butylated hydroxytoluene equivalents per gram of dried extract and AA (Ascorbic acid).

CONCLUSION AND RECOMMENDATIONS

In this study, the nutritional profile (proximate, minerals, phytochemicals content, and antioxidants activities) of dried *Stevia* leaf and its infusion was done and the results were shown under result and discussion, however, there is a need to carry out further composition profile using GS-MS, HPLC, and UPLC to explore the potential chemicals present in the dried *Stevia* leaf infusion.

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