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

Abdela Befa1*, Abadi Gebre2, Tesfu Bekele2

1Department of Food Science and Nutrition, Ethiopia Institute of Agriculture Research, Wondo Genet, Ethiopia; 2Department 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, 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 antibacterial 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...
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 analysing.

**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.

\[
\text{Moisture} \, \% = \frac{W_1 - W_2}{W_1} \times 100 \quad \text{equation 1}
\]

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.

\[
\text{Ash} \, \% = \frac{W_3 - W_1}{W_2} \times 100 \quad \text{equation 2}
\]

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-23C, China) at 380°C for 6 hours until the mixture by adding 10 mL of concentrated sulphuric acid (H₂SO₄), a mixture of 2.5 g of copper sulfate (CuSO₄), Potassium sulfate (K₂SO₄), and Titanium dioxide (TiO₂). 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 NH₂SO₄ 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 H₂SO₄. Total nitrogen and crude protein content of the sample was then determined and calculated using equations 3 and 4, respectively.

\[
\text{Nitrogen} \, \% = \frac{M \times \text{acid conc.} \times \text{volume made} \times \text{titer value}}{1000 \times W_1} \times 100 \quad \text{equation 3}
\]

\[
\text{Crude protein} \, \% = \text{Nitrogen} \times 6.25 \quad \text{equation 4}
\]

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.

\[
\text{Crude fat} \, \% = \frac{(W_e + W_f) - W_f}{W_s} \times 100 \quad \text{equation 5}
\]

Where \(W_e\) = Weight of extract, \(W_f\) = Weight of flux and \(W_s\) = 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 H₂SO₄. 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.

\[ \% \text{ Crude fiber} = \frac{W_2 - W_3}{W_1} \times 100 \]

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.

\[ \% \text{ CHO} = 100 - (\% \text{ Ash} + \% \text{ Protein} + \% \text{ Fat} + \% \text{ Crude Fiber} + \% \text{ Moisture}) \]

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 HNO3: 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) = \( C \times V \times df/W \)

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 cool in the desiccators and weighed. The residue was dissolved in 10 mL of 6 N HCl (37%) and stored in a dark at room temperature for 15 min. Then the solution was filtered through a filter paper No 42, then volume made up to 250 mL with distilled water. The concentration of crude extract was measured and expressed as mg/100 g as described in equation 9.

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 (%) = \( A_c - A_s / A_c \times 100 \)

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 FeCl₃ (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 BHT/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>
<thead>
<tr>
<th>Sample</th>
<th>Moisture</th>
<th>Ash</th>
<th>Crude Protein</th>
<th>Crude Fat</th>
<th>Crude Fiber</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stevia</td>
<td>8.2 ± 0.5</td>
<td>16.2</td>
<td>3.8 ± 0.1</td>
<td>7.9 ± 0.7</td>
<td>58.8 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Samp le</th>
<th>Ca</th>
<th>Na</th>
<th>K</th>
<th>Mg</th>
<th>Fe</th>
<th>Zn</th>
<th>Cu</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stevia</td>
<td>± 1.2</td>
<td>± 0.3</td>
<td>± 0.1</td>
<td>± 0.1</td>
<td>± 0.7</td>
<td>± 0.1</td>
<td>± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Sample</th>
<th>TPC (mg GAE/g)</th>
<th>TFC (mg CE/g)</th>
<th>Condensed Tannin (mg CE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stevia</td>
<td>5.3 ± 0.1</td>
<td>28.6 ± 0.1</td>
<td>4.6 ± 0.1</td>
</tr>
</tbody>
</table>

**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 BHT/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.**

<table>
<thead>
<tr>
<th>Herbal tea</th>
<th>DPPH scavenging (IC-50, g/mL)</th>
<th>Ferric reducing power (mg AAE/g)</th>
<th>Total antioxidant (mg BHTE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TF9</td>
<td>0.04 ± 0.01</td>
<td>6.67 ± 0.25</td>
<td>1.92 ± 0.02</td>
</tr>
<tr>
<td>AA</td>
<td>0.03 ± 0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 GC-MS, HPLC, and UPLC to explore the potential chemicals present in the dried Stevia leaf infusion.

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