Nutritional and biochemical properties of natural sweeteners of six cultivars of 
**Stevia rebaudiana** Bertoni leaves grown in Morocco

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**Abstract**
In order to know the variability of a breeding on six cultivars from different areas in Morocco, **Stevia rebaudiana** leaf was investigated for its proximate composition, mineral content and biochemical screening. Further, stevia plants are a good source of carbohydrates, protein and crude fiber contents were found to be (51.50 – 56.72%), (11.75 – 16.23%) and (17.43 – 19.13%) on dry weight basis, respectively. In this order, stevia leaves also contained (12.47 to 17.35 %) on dry weight basis for total soluble carbohydrates. Minerals content analysed by spectrometric ICP-AES showed that the high content of K, Ca, Mg, Na of stevia were present in the leaf; whereas, Mn, Fe, Cu, Zn, and Cr were found as trace amount. The responsible compounds for the natural sweetness of stevia leaves include a diversity of diterpenoid glycosides derived from a steviol skeleton. These steviol glycosides also exhibit a low calorific value as well as promising therapeutic applications. The stevioside was extracted by hot water (60 °C) from the dried stevia leaves and analyzed by HPLC. Our results showed that the stevioside content was obtained at (6.26 - 10.10 %) on dry weight basis. So, stevia leaf produces a variety of high potential natural-source and low calorie sweetener. These results strongly suggest that due to its all favourable properties; stevia could be used in either food or cosmetic and pharmaceutical products.

1. **Introduction**
There is an increasing interest in **S. rebaudiana** Bertoni leaves as they are a good source of non-caloric sweeteners due to the presence of steviol glycosides that has a great potential in food industry as a strategy to reduce sugar consumption [1].
Moreover, **S. rebaudiana** is a perennial shrub of the Asteraceae (Compositae) family native to certain regions of South America (Paraguay and Brazil). It is often known as “the sweet herb of Paraguay” [2]. The plant is also cultivated in China and Southeast Asia [3]. Stevia sweeteners, crude extract from leaves, are used to sweeten soft drinks, soy sauce, yogurt, and other foods in Japan, Korea and Brazil [4]. The dry extract from the leaves of stevia contains flavonoids, alkaloids, chlorophylls, xanthophylls, hydroxycinnamic acids (caffeic, chlorogenic, etc.), oligosaccharides, free sugars, amino acids, lipids and trace elements [5].
Stevia, the common name for the extract steviolide from the leaves of **S. rebaudiana Bertoni**, is new promising a wide range of foodstuff on the world market and is a natural, sweet-tasting calorie-free botanical that may also be used as a sugar substitute or as an alternative to artificial sweeteners [6]. The natural sweeteners of stevia leaves, called steviol glycosides, are diterpenes, isolated and identified as stevioside, steviolbioside, rebauisidose A, B, C, D, E, F and dulcoside [7].
The study of Mohammad *et al* [8] identified nine amino acids in stevia leaves, namely glutamic acid, aspartic acid, lysine, serine, isoleucine, alanine, proline, tyrosine and methionine. Abou-Arab *et al* [9] found still more amino acid in the stevia leaves. Altogether seventeen amino acids were determined and classified as essential and non-essential amino acids.
In *S. rebaudiana* roots and leaves, inulin-type fructooligosaccharides, a naturally occurring plant polysaccharide with important functional properties related to prebiotics, dietary fiber, role lipid metabolism and diabetes control, have been isolated by Braz de Oliveira *et al* [10]. In the leaf oil of stevia, Tadhani and Subhash [11] identified six fatty acids using methyl ester standards. Palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids were identified in the leaf oil.

Kim *et al* [12] studied the amounts of water-soluble vitamins in the stevia leaf and callus extracts, and determined that the contents of folic acid, vitamin C and vitamin B2 in the leaf extracts were significantly higher than those of the callus extracts. In the leaf extract, folic acid was found to be the major compound, followed by vitamin C. In the callus extract, vitamin C was the major compound, followed by vitamin B. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body [13]. The phytochemicals present in *S. rebaudiana* are austroinullin, b-carotene, dulcoside, nilacin, rebaudoxides, riboflavin, steviol, stevioside and thiamine [14]. The presence of macro and micronutrients in foods is important for the development and maintenance of vital body functions. They are involved in all aspects of growth, health and reproduction, participating also in the formation of cells, tissues and organs [15].

*S. rebaudiana* extracts have been suggested to exert beneficial effects on human health, including antihypertensive, antimicrobial, antiobesity and antioxidant activities and also thought to influence glucose metabolism and renal functions [16]. *S. rebaudiana* does not lower blood glucose levels in normal subjects [17]. Diabetes mellitus is a major public health problem in the world, the prevalence of diabetes mellitus is increasing with ageing of the population and lifestyle changes associated with rapid urbanization. The World Health Organization estimates that 3% of the world’s populations (194 million) have diabetes and is expected to double (6.3%) by the year 2025. In Morocco, the statistics are alarming. According to the latest figures from the Ministry of Health issued on the occasion of World Diabetes Day, Morocco has more than 2 million diabetes in over 20 years.

The inhibition for the corrosion of mild steel in sulphuric acid solution by the extract of Stevia rebaudiana leaves has been studied using electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization techniques. Inhibition was found to increase with increasing concentration of the leaves extract. The effect of temperature on the corrosion behavior of mild steel in 0.5M H2SO4 with addition of extract was also studied [18]. Introduction of green corrosion inhibitor can stop the use of expensive and harmful synthetic inhibitor [19]. In many Moroccan regions, stevia Leaves powdered are just used to sweeten tea and the tisanes. Until now, nobody has investigated the potential nutritive of biochemical constituents of stevia leaves in Morocco. Therefore, the main aim of this study is to evaluate the biochemical characteristics that were investigated in six different areas of Morocco.

## 2. Material and Methods

### 2.1 Raw Materials

The stevia leaves (*Stevia. rebaudiana* Bretoni), were collected from different areas in Morocco (Table 1, Figure 1) at Agadir (30°30’ N, 9°33’ W), Berkane (34°55’ N, 2°19’ W), Larache (35°09’ N, 6°08’ W), Marrakech (31°37’ N, 8°30’ W), Rabat (34°01’ N, 6°51’ W) and Sefrou (33°49’ N, 4°51’ W). The stevia plants that reached the maximum growth stage (mature stage before flowering) were harvested at August 2014 by cutting the plant at 5 - 10 cm from the ground. The brown and yellow leaves were removed. The plants were also dried under an aired greenhouse at temperature ranged from to 25 - 30°C for 48 - 72 h.

<table>
<thead>
<tr>
<th></th>
<th>Soil characteristics</th>
<th>Bioclimat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agadir</strong></td>
<td>Sandy</td>
<td>Arid</td>
</tr>
<tr>
<td><strong>Berkane</strong></td>
<td>Very fine silty</td>
<td>Semi-arid</td>
</tr>
<tr>
<td><strong>Larache</strong></td>
<td>Sandy loam</td>
<td>Sub-humid</td>
</tr>
<tr>
<td><strong>Marrakech</strong></td>
<td>Sandy clay</td>
<td>Semi-arid</td>
</tr>
<tr>
<td><strong>Rabat</strong></td>
<td>Sandy loam</td>
<td>Sub-humid</td>
</tr>
<tr>
<td><strong>Sefrou</strong></td>
<td>Silty clay</td>
<td>Sub-humid</td>
</tr>
</tbody>
</table>

**Table1.** Geographical characteristics of the regions studied
2.2 Experimental
Harvested leaves were blended to powder form with a high speed blender (Microtron MB 550), KINEMATICA AG (Switzerland) and were kept in glass vials to be stored at 4 ± 1°C in a refrigerator until used. Moisture, contents of stevia leaves samples were established according to (AOAC, 1990) [20] method; the ground samples were dried in an oven at 105°C for 24 h. The percentage of loss in weight was expressed as the moisture content. The dry matter of harvested plants was determined by difference [20]. Ash was determined by combustion of the samples in a muffle furnace at 550°C for 12 h [20]. The sugar content was established by using the Bertrand method [20]. This dosage consists of collecting the precipitate of the copper (II) oxide formed by reduction of the cupro-alkaline liqueur in presence of reducing sugars, and to measure it out by manganimetry. Bertrand’s tables give the directly correspondence between the volume of KMnO4 (0.1N) used and content of glucose of the trial hold. The fat content of the samples was extracted with hexane in a Soxhlet apparatus. Proteins content was determined from the nitrogen content by Kjeldahl method using factor 6.25 and calculated as N x 6.25 [21]. Crude fiber is loss on ignition of dried residue remaining after digestion of sample with 1.25% H2SO4 and NaOH under specific conditions [20]. Total energy (Calorific value) was determined according to the method described by Sukker [22] using the Atwater factor. By this determination, 1g of carbohydrate provides 4 kcal; 1g of protein provides 4 kcal and 1 g fat provides 9 kcal. Conventional extraction method of stevioside was performed with 1g of stevia leaves, by adding 40 ml of distilled water maintaining at 60 °C for 120 min with magnetic. Extracts were diluted if necessary and the amount of stevioside were determined by HPLC. All the extracts were diluted with deionized water and filtered through a 0.45 µm cellulose membrane filter (Xilab, Belgium) before being subjected to HPLC analysis. The HPLC system consisted of a Dionex P680 pump, a Dionex ASI-100 automated sample injector, a Dionex Ultimate 3000 Column Compartment (Thermo Scientific, France), a diode array detector (DAD) (Hewlett Packard, France) and piloted by a Chromelon chromatography data system (Thermo Scientific, France) to obtain chromatographic profiles of the extracts. Separation was performed with the mobile phase consisted of 0.1% aqueous formic acid (solvent A) and acetonitrile (solvent B) (69:31 v/v) as isocratic at flow rate of 1 ml/min for 30 min. Samples were injected (20 µl) onto an apolar reversed-phase C18 column (250 - 4.6 mm ID, 5µ,
Phenomenex, Le Pecq, France) at 40°C. Chromatograms were recorded at 200nm. The standards used in the experiments were weighed and dissolved in deionized water. Calibration curves were generated with concentrations ranging from 50 to 500 mg/l of stevioside.

For mineral analysis, samples were incinerated in muffle furnace at 550°C for 12h. Ashes were quantified gravimetrically. The ashes were diluted with hydrochloric acid 0,1N and with distilled and demineralised water. The dosage of the studied trace elements (Ca, K, Mg, Na, Mn, Fe, Cu, Zn and Cr) was created by the water method regia and analysis by spectrometric Plasma Atomic Emission Inductively Coupled argon (ICP-AES). The water method regia's policy sample dissolution in a mixture of hydrochloric and nitric acid. Then analyzed by ICP-AES. The used apparatus was an ICP-AES (Varian-Vista), equipped with an ultrasonic nebulizer Cetac. The main analytical parameters of the device are:

- RF power: 0.7 - 1.5 kilowatt (kW 1.2-1.3 for axial);
- Flow rate of plasma gas (Ar): 10.5- 15 l 1/ min (radial), 15 l/ min (axial);
- Auxiliary gas flow (Ar): 15 l 1/ min (axial);
- Viewing size: 5-12 mm;
- Copy and playback time: 1-5s (maximum 60 s);
- Copy Time: 3 s (maximum 100 s).

2.3 Statistical Analysis

Statistical analyses were conducted using SPSS (Statistical Program for Social sciences) version 20.0 for window. All analyses were performed in triplicate and data reported as means ± standard deviation (SD).

3. Results and Discussion

The analysis of variance (ANOVA) showed a variability of biochemical characters measured highly significant (p <0.01) than the threshold 0.05 (Table 2).

The results obtained by the biochemical analysis of dried stevia plants are presents in Table 2. An ANOVA analysis was performed to evaluate if there were significant. Differences in data showed that leaves had moisture ranged from 4.97 to 8.31 % in Marrakech and Agadir regions respectively. These moisture levels are similar to those reported by Goyal et al [23], and Kaushik et al [24].

The results of dry matter and the ash’s data shows that they vary widely from one area to another and ranges from 91.68 to 95.02 % and 7.37 to 11.28 % respectively. Marrakech cultivar records the highest value of dry matter and ash compared to other regions, confirming also the results found by Goyal et al [23] and Tadhani & Subhash [11], with 95.35 % for dry matter and 13.1 % for the ash.

Stevia dry mass production from the six regions has yielded ranged from 11.75 and 16.23 % for Marrakech and Sefrou respectively. Sefrou cultivar records the highest value of proteins compared to other regions and is found of the low amount of total proteins in Marrakech cultivar (Table. 2). These values were important than those found by Serio [25], Wölwer-Rieck [1] and Tadhani & Subhash [11].

The leaves stevia analysed contain quite high percentage of carbohydrates ranging from 51.50 to 56.72 % of dry matter. Agadir cultivar was also found to contain the highest value while a low value was found in Rabat and Berkane cultivars. These results are in agreement with those reported by Mishra et al [26] and Abou-Arab et al [9], in this order, stevia leaves also contained 4.06 to 5.87 %, 9.17 to 11.47 % and 12.47 to 17.35 % for reducing sugars, total sugars and total soluble carbohydrates, for Rabat and Marrakech respectively (Table 2).

The stevia leaves contained higher amounts of dietary fiber in the range of 17.43 to 19.13 % of dry matter in Rabat and Berkane cultivars respectively, which is in accordance with those reported by [23] and [16]. Thus, stevia leaves could be considered as a potential fiber to be used in food formulations. High ash content indicates that the stevia leaves are also good source of inorganic minerals.

![Table 2](https://example.com/table2.png)

<table>
<thead>
<tr>
<th></th>
<th>Agadir</th>
<th>Berkane</th>
<th>Larache</th>
<th>Marrakech</th>
<th>Rabat</th>
<th>Sefrou</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>8.31 ± 0.19</td>
<td>5.95 ± 0.36</td>
<td>7.04 ± 0.34</td>
<td>4.97 ± 0.24</td>
<td>8.24 ± 0.23</td>
<td>5.01 ± 0.22</td>
</tr>
<tr>
<td>Dry matter</td>
<td>91.68 ± 0.19</td>
<td>94.04 ± 0.36</td>
<td>92.95 ± 0.34</td>
<td>95.02 ± 0.24</td>
<td>91.75 ± 0.23</td>
<td>94.98 ± 0.22</td>
</tr>
<tr>
<td>Ash</td>
<td>8.13 ± 0.04</td>
<td>9.83 ± 0.16</td>
<td>7.37 ± 0.06</td>
<td>11.28 ± 0.08</td>
<td>10.34 ± 0.11</td>
<td>8.82 ± 0.10</td>
</tr>
<tr>
<td>Protein</td>
<td>12.36 ± 0.04</td>
<td>15.24 ± 0.15</td>
<td>14.89 ± 0.15</td>
<td>11.75 ± 0.06</td>
<td>14.90 ± 0.07</td>
<td>16.23 ± 0.04</td>
</tr>
</tbody>
</table>
From this study and other investigations, stevia leaves are a good source of carbohydrates, protein, and crude fiber which are the essential factors for maintenance of health. Beside, compared to other stevia leaves fat content varies in different studied regions found by Serio [25] and Mishra et al [26], our results are less, ranging between 3.86 to 5.78 % in Sefrou and Rabat regions respectively.

The extract prepared from the leaves of S. rebaudiana contains sweet steviol glycosides, mainly stevioside. After HPLC investigations, our results showed that stevioside varies between 6.26 to 10.10 % in Agadir and Sefrou regions respectively. These results of stevioside are similar to those reported by Goyal et al [23], and Kinghorn & Soejarto [4].

Stevia, the common name for the extract stevioside from the leaves of S. rebaudiana Bertoni, is a new promising renewable raw food stuff on the world market and is a natural, sweet-tasting calorie-free botanical that may also be used as a sugar substitute or as an alternative to artificial sweeteners [6]. Stevioside has the chemical formula of a diterpene glycoside (C_{38}H_{60}O_{18}) and, as an active component in stevia leaves is responsible for the edulcorant properties. Its use has been approved in Brazil, Argentina and Paraguay as well as in China, Korea and Japan. These molecules are highly stable in aqueous solutions within a broad range of pH and temperature [9]. And also stevioside, an abundant component of S. rebaudiana leaf, has become well-known for its intense sweetness (250–300 times sweeter than sucrose) and is used as a non-caloric sweetener in several countries. A number of studies have suggested that, beside sweetness, stevioside and rebaudioside A, may also offer therapeutic benefits, as they have anti-hyperglycemic, anti-hypertensive, anti-inflammatory, anti-tumor, anti-diarrheal, diuretic, and immunomodulatory actions [27].

Stevia leaves have recorded a higher value of proteins, crude fiber and carbohydrate comparing to different aromatic and medicinal plants. However, the quantity of fat was so low comparing to these plants (Table 3).

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Fat</th>
<th>Crude fiber</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.50 - 56.72</td>
<td>3.68 - 5.78</td>
<td>17.43 - 19.13</td>
<td>7.37 - 11.28</td>
</tr>
<tr>
<td>25</td>
<td>2 - 3</td>
<td>ND</td>
<td>5</td>
</tr>
<tr>
<td>27.5</td>
<td>6.5</td>
<td>0.8</td>
<td>3.6</td>
</tr>
<tr>
<td>1.16</td>
<td>0.18</td>
<td>1.6</td>
<td>ND</td>
</tr>
<tr>
<td>ND</td>
<td>ND</td>
<td>5.3</td>
<td>5.6</td>
</tr>
</tbody>
</table>

ND, not determined.

Data presented in Table 4 indicates the mean concentrations of macro minerals (Potassium, Calcium, Sodium and Magnesium) and micro elements that is heavy metals (Copper, Manganese, Chromium, Iron and Zinc) determined in dried stevia leaves.

The stevia leaves are considered as a good source of minerals. Our results showed that the stevia leaves are rich in potassium, detected at high content: 1421.24 to 2787.11 mg/100 g, followed by calcium: 579.68 to 734.57 mg/100 g and Sodium: 69.87 to 190.14 mg/100g. The relatively lowest level of major minerals was Magnesium, detected at concentration 179.57 to 198.18 mg/100 g.

Regarding to heavy metals, the leaves contained lower amount of Mn, Fe, Cu, Zn, and Cr which recorded 6.48 to 12.73, 5.73 to 35.44, 0.35 to 1.08 and 1.71 to 5.32 and 0.39 to 2.51 mg/100 g on dry weight basis, respectively.

**Table 3.** Stevia biochemical compounds comparison to different aromatic and medicinal plants (mg/ 100 g) of dried weight

<table>
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<tbody>
<tr>
<td>Carbohydrates</td>
<td>51.50 - 56.72</td>
<td>25</td>
<td>27.5</td>
<td>1.16</td>
<td>ND</td>
</tr>
<tr>
<td>Proteins</td>
<td>11.75 - 16.23</td>
<td>15</td>
<td>5.4</td>
<td>0.2</td>
<td>6.5</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>17.43 - 19.13</td>
<td>ND</td>
<td>7</td>
<td>0.8</td>
<td>5.3</td>
</tr>
<tr>
<td>Fat</td>
<td>3.68 - 5.78</td>
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<td>6.5</td>
<td>0.18</td>
<td>1.6</td>
</tr>
<tr>
<td>Ash</td>
<td>7.37 - 11.28</td>
<td>5</td>
<td>3.6</td>
<td>ND</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* Calculated by difference.
- All values are means of triplicate determinations ± standard deviation (SD).
- Means within columns with different letters are significantly different (P < 0.05).
The mineral pattern depends on the fruit origin and factors of cultivation site. Similar results have been reported in the literatures [1, 11, 23, 26, and 32]. But we found that the mineral Sodium in the region of Marrakech, thus the mineral Manganese in the region of Marrakech, and also Chromium in Sefrou and Larache, generally higher than those reported by Erna [32].

Minerals have many important functions in the human body. The main elements are sodium, magnesium, phosphorus, sulphur, chlorine, potassium, and calcium which are classified as macronutrients while the minor elements, considered as micronutrients, are chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum and iodine [15, 33]. Stevia contains substantial amounts of these important nutrients, which further establishes it as a mineral loaded ingredient needed to protect the body, regulate and maintain the various systems [35] and in prevention of free radical mediated diseases at each level. Iron is an essential element for synthesis of hemoglobin. The higher amount of iron in stevia leaves is again useful in maintenance of normal hemoglobin level in the body. Moreover, stevia leaves could also be used to prepare various sweet preparations for combating iron deficiency as anemia. Which is the major nutritional disorder of developing countries [9].

Nutrient composition of stevia (Table 5) which was analyzed on dry weight basis indicated that energy value being analyzed were found to be (3.05 - 3.17 Kcal /g) which may be entitled as the status of low calorific sweetener due to its intense sweetness in comparison to other available low calorie sweeteners. These results are in agreement with those reported by [26] and [36]; they reported that total energy were (2.7 and 3.6 Kcal/g) respectively. Intense sweetness includes acesulfame potassium (Calorie free), aspartame (4.0 Kcal/ g), saccharin (1.7 Kcal/g) and sucralose (0.9 Kcal/g) [37]. Calorie contribution to the diet by commonly used sucrose being 4 Kcal /g, while stevia leaves could also be used to prepare various sweet preparations for combating iron deficiency as anemia. Which is the major nutritional disorder of developing countries [9].

### Table 4: Minerals content (mg 100 g⁻¹) of dried stevia leaves from different geographical areas in Morocco

<table>
<thead>
<tr>
<th></th>
<th>Agadir</th>
<th>Berkane</th>
<th>Larache</th>
<th>Marrakech</th>
<th>Rabat</th>
<th>Sefrou</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1421.24 ± 0.17</td>
<td>2551.34b ± 0.08</td>
<td>1633.25b ± 0.10</td>
<td>2787.11a ±0.03</td>
<td>1940.71b ±0.06</td>
<td>1987.19b ±0.76</td>
</tr>
<tr>
<td>Ca</td>
<td>579.68d ± 0.43</td>
<td>606.71b ± 0.03</td>
<td>733.77a ± 0.16</td>
<td>734.57a ±0.14</td>
<td>599.74d ±0.40</td>
<td>598.77d ±0.37</td>
</tr>
<tr>
<td>Na</td>
<td>97.83e ± 0.07</td>
<td>69.87d ± 0.05</td>
<td>185.18b ±0.11</td>
<td>123.28c ±0.13</td>
<td>190.14a ±0.07</td>
<td>108.31d ±0.19</td>
</tr>
<tr>
<td>Mg</td>
<td>182.90d ± 0.55</td>
<td>184.21de ± 0.07</td>
<td>188.42b ± 0.26</td>
<td>185.36c ±0.06</td>
<td>198.18a ±0.08</td>
<td>179.57d ±0.14</td>
</tr>
<tr>
<td>Mn</td>
<td>7.80b ± 0.04</td>
<td>6.71d ± 0.06</td>
<td>12.73a ± 0.17</td>
<td>6.52c ±0.08</td>
<td>5.40d ±0.15</td>
<td>6.48e ±0.02</td>
</tr>
<tr>
<td>Fe</td>
<td>19.56d ± 0.33</td>
<td>35.44a ± 0.04</td>
<td>12.60d ± 0.09</td>
<td>19.89b ±0.04</td>
<td>5.73d ±0.11</td>
<td>14.33d ±0.12</td>
</tr>
<tr>
<td>Cu</td>
<td>0.35b ± 0.01</td>
<td>1.08a ± 0.01</td>
<td>0.56b ± 0.12</td>
<td>1.08a ±0.03</td>
<td>0.53b ±0.08</td>
<td>0.36e ±0.04</td>
</tr>
<tr>
<td>Zn</td>
<td>2.16e ± 0.01</td>
<td>3.28b ± 0.02</td>
<td>2.87b ± 0.01</td>
<td>2.17b ±0.02</td>
<td>5.32a ±0.11</td>
<td>1.71c ±0.09</td>
</tr>
<tr>
<td>Cr</td>
<td>0.39d ± 0.004</td>
<td>1.92a ± 0.06</td>
<td>2.38b ± 0.05</td>
<td>1.12a ±0.01</td>
<td>0.96e ±0.04</td>
<td>2.51b ±0.04</td>
</tr>
</tbody>
</table>

- All values are means of duplicate determinations ± standard deviation (SD).
- Means within columns with different letters are significantly different (P < 0.05).

### Table 5: Total energy of dried S. rebaudiana leaves from different geographical areas in Morocco (Kcal /100 g dry weight basis).

<table>
<thead>
<tr>
<th></th>
<th>Agadir</th>
<th>Berkane</th>
<th>Larache</th>
<th>Marrakech</th>
<th>Rabat</th>
<th>Sefrou</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (Kcal/100g)</td>
<td>226.88 ± 0.80</td>
<td>206.00 ± 0.76</td>
<td>218.64 ± 0.72</td>
<td>215.72 ± 0.64</td>
<td>206.08 ± 0.80</td>
<td>213.48 ± 0.60</td>
</tr>
<tr>
<td>Protein (Kcal/100g)</td>
<td>49.44 ± 0.16</td>
<td>60.96 ± 0.60</td>
<td>59.56 ± 0.60</td>
<td>47.00 ± 0.24</td>
<td>59.60 ± 0.28</td>
<td>64.92 ± 0.16</td>
</tr>
<tr>
<td>Fat (Kcal/100g)</td>
<td>35.19 ± 0.45</td>
<td>38.43 ± 0.09</td>
<td>36.45 ± 0.90</td>
<td>44.46 ± 0.45</td>
<td>52.02 ± 0.45</td>
<td>34.74 ± 0.90</td>
</tr>
<tr>
<td>Total energy (Kcal/100g)</td>
<td>311.51 ± 1.41</td>
<td>305.39 ± 1.45</td>
<td>314.65 ± 2.22</td>
<td>307.18 ± 1.33</td>
<td>317.70 ± 1.53</td>
<td>313.14 ± 1.66</td>
</tr>
</tbody>
</table>
In safety studies, no negative side effects were reported [38]. Stevia was recently approved for use as a sweetener by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives [39], and has also recently received GRAS approval from the Food and Drug Administration. However, no study to date has examined the effect stevia has on food intake and satiety levels [6].

Conclusion
After a scientific study, considering the biochemical composition like protein, total sugar, fat, stevioside and dietary fiber content. S. rebaudiana could be used as part of the nutrition for human beings. So, it could be concluded that stevia plants are a good source of carbohydrates, protein, and also could be considered as an excellent source of stevioside, crude fiber and minerals. Finally, the determined concentration of heavy metals in leaves of S. rebaudiana were in normal range level. The implication of the findings may be taken into consideration, while using the herbs for food safety in preparation of herbal products and standardized extracts, should be collected from an unpolluted natural plants.

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Reference


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