Pharmacognostic, phytochemical, Chemical and functional studies of leaves of *Stevia rebaudiana* Bertoni

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**Abstract**

*Stevia rebaudiana* (Bert) is a plant of compositae family and native to Paraguay, its sweetness and calorie free property increased its demand tremendously. This Plant is a boon to us from the nature as it not only imparts the sweetness but also maintain the normal blood sugar level. That's why leaves are being used in homemade recepies and also in allied industries for diabetics. The investigation carried out by us was on the Pharmacognostical, Phytochemical and Physiochemical aspects of the leaf of Stevia. Morphological studies of root showed the presence of various diagnostic characters. Proximate content was determined for quality standard. During the course of the experimental work the leaves part showed the presence of various phytoconstituents like tannins, reducing sugars etc. Functional properties were also determined to establish the potential use of *Stevia* leaves in food processing. This information will be of use for further pharmacological, therapeutic and food evaluation of the species and will help in standardization for quality, purity and sample identification.


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1. Introduction

*Stevia rebaudiana* belongs to the Asteraceae family (Ray, 2008; Megeji et al., 2005). It and *Stevia* phlebophylla are the only members of the 230 species of this genus to produce Steviol glycosides (Kinghorn and Soejarto, 1985; Brandle and Telmer, 2009). *Stevia* rebaudina is ingenous of Paraguay (Savita et al., 2004) and the native Guarani tribe has used the leaves of this herb for centuries due to the unique sweetening property of its leaf (Singh and Rao, 2005; Panpatil and Polasa, 2008; Chengguo et al., 2009). Their sweetness is due to six diterpene glycoside (Panpatil and Polasa, 2008; Chengguo et al., 2009). *Stevia rebaudiana* is also known as honey yerba, honey leaf (Hawke, 2003), Cheeni Tulsi and Mau Tulas. Apart from being a good source of protein, ash and crude fiber, it has a lower calorie and glycemic index as compared to common sugar (Savita et al., 2004). *Stevia* is fast growing as an alternative to artificial sweeteners. Leaves of this plant produces zero-calorie ent-kaurene diterpene glycosides, a non nutritive, high potency sweetener which is 300 times sweeter than sucrose (Chengguo et al., 2009; Kumar and Oommen 2008; Megeji, 2005). *Stevia* has a number of health benefits. It can help in the treatment and management of obesity. *Stevia* has negligible effect on blood glucose and may even enhance the levels of a patient’s glucose tolerance. It can help alleviate high
blood pressure. These beneficial medical factors help make stevia an attractive alternative to sugar, and a natural sweetener for diabetics and others who may be on a carbohydrate-controlled diet.

2. Objective of the study

The current article describes some pharmacognostical, chemical, phytochemical and functional characteristics studied. The main objective of this study is to supplement constructive information with regards to its characterization, identification and standardization of the leaves of Stevia rebaudiana Bertoni.

3. Material and Method

3.1 Collection and identification of plant

The fresh leaves were plucked from the plants grown in pots at the Centre for Food Technology, Jiwaji University, Gwalior. The fresh leaf of Stevia was plucked after three month for the analysis as the stevioside content (sweetness) is highest just before flowering of the plants i.e. after three months (Plate 1). Stevia is a semi humid subtropical plant that prefers a well drained fertile sandy loam or loam soil, high in organic matter with ample supply of water. The height of mature plant of Stevia ranges from 65 cm (26 inch) to 180 cm (72 inch) when cultivated on a fertile soil. It has a sessile, alternate lance, lanceolate to oblanceolate leaves, serrated above the middle. High leaf production is seen under high light intensity and warm temperature. Short days induce flowering. Time of harvesting depends on land type, variety and growing season. The harvest usually starts in August and ends before the first frost.

3.2 Justification of Research

The Pharmacognostic, phytochemical screening will help in characterisation and identification of stevia leaves. The chemical and functional properties studied will help in further utilization of stevia leaves in food processing.

3.3 Morphological examination

Morphology is the study of the form of an object while morphography is the description of that form. The macroscopical characters were done by the method given by Bawane et al., 2013. The colour of Stevia leaves was examined under a diffuse day light and artificial light source. Odour was examined by taking a small piece of leaf in the palm of hand, and also pieces of it slowly and repeatedly inhaled along with the air over the leaf. Taste was extremely sweet. Physical properties were analyzed for the fresh leaf while the dry leaves were analyzed for chemical composition, functional properties and phytochemical screening.

3.3.1 Physical properties

The leaf length to width ratio was calculated by the method Bawane et al., 2013. Fifteen leaves were plucked and the longest and widest part of the leaf measured by the use of scale. The leaves were placed on a graph paper and marked the longest and widest part of the leaf. The distance was then measured in cm.

Length to width ratio = \( \frac{\text{Average Length}}{\text{Average width}} \)

3.4 Functional Properties

3.4.1 Determination of bulk density

Bulk density was measured by the method of Narayana and Narasinga, 1984. A 25 ml measuring cylinder was weighed and the weight recorded as \( W_1 \). 10 gm of sample was transferred into the measuring cylinder. For the bulk density determination, the sample was gently tapped to eliminate spaces between the sample and then weighed (\( W_2 \)). The level was also noted to be the volume of the sample and then weighed. The study was conducted in triplicate. The bulk density was calculated by the formula:

\[
\text{Bulk density (g/ml)} = \frac{W_2 - W_1}{25}
\]

3.4.2 Determination of water and oil absorption capacity

Water absorption capacity was determined using the method of Sathe and Salunkhe, 1981 with slight modifications. 10 ml of distilled water was added to 1.0 g of the leaf powder in a beaker. The suspension was stirred using a magnetic stirrer for 5 min. The suspension obtained was there after centrifuged at 3555 rpm for 30 min and the supernatant measured in a 10 ml graduated cylinder. The density of water was taken as 1.0 g/cm\(^3\). Water absorbed was calculated as the difference between the initial volume of water added to the sample and the volume of the supernatant. The result was reported in ml.
Table 1: Organoleptic evaluation of fresh leaf

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour : Upper Surface</td>
<td>Green</td>
</tr>
<tr>
<td></td>
<td>Lower Surface</td>
<td>Light green</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Very sweet</td>
</tr>
<tr>
<td>4</td>
<td>Texture</td>
<td>Rough</td>
</tr>
</tbody>
</table>

Table 2: Morphological study of Stevia leaf

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>1 month old</th>
<th>2 month old</th>
<th>3 month old</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaf length</td>
<td>Range</td>
<td>3.4 – 4.9</td>
<td>4.8 – 6.5</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>4.3&quot;</td>
<td>6.0&quot;</td>
</tr>
<tr>
<td></td>
<td>CD at 5% level</td>
<td></td>
<td>0.63</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Organoleptic Analysis of Dried Stevia Leaf

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Colour : Upper Surface</td>
<td>Light green</td>
</tr>
<tr>
<td></td>
<td>Lower Surface</td>
<td>Whitish green</td>
</tr>
<tr>
<td>2</td>
<td>Odour</td>
<td>Characteristic</td>
</tr>
<tr>
<td>3</td>
<td>Taste</td>
<td>Very Sweet, Sweeter than fresh leaf</td>
</tr>
<tr>
<td>4</td>
<td>Texture</td>
<td>Rough</td>
</tr>
</tbody>
</table>

Table 4: Phytochemical Screening of Stevia leaf

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical constituents</th>
<th>Result</th>
<th>+ve/ -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>Very high concentration</td>
<td>++++</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>Turbid solution resulted</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>Foam persist for 12 min</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Reducing Compounds</td>
<td>Small amount of ppt formed</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 5: Functional properties of Stevia leaf

<table>
<thead>
<tr>
<th>S.No</th>
<th>Functional Properties</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Packed Bulk Density</td>
<td>0.49 gm/ml</td>
</tr>
<tr>
<td>2</td>
<td>Water Absorption Capacity</td>
<td>5.27 ml/gm</td>
</tr>
<tr>
<td>3</td>
<td>Oil Absorption Capacity</td>
<td>3.15 ml/gm</td>
</tr>
<tr>
<td>4</td>
<td>Emulsification Capacity</td>
<td>5.64 ml/gm</td>
</tr>
</tbody>
</table>

Table 6: Chemical Analysis of Dried Stevia Leaf

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chemical Analysis</th>
<th>Fresh weight basis</th>
<th>Dry weight basis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture</td>
<td>9.72</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>Fat</td>
<td>2.34</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>Protein</td>
<td>10.22</td>
<td>9.22</td>
</tr>
<tr>
<td>4</td>
<td>Ash</td>
<td>10.73</td>
<td>9.68</td>
</tr>
<tr>
<td>5</td>
<td>Fiber</td>
<td>13.25</td>
<td>11.96</td>
</tr>
<tr>
<td>6</td>
<td>Total Carbohydrate</td>
<td>52.75</td>
<td>47.62</td>
</tr>
<tr>
<td>7</td>
<td>Energy</td>
<td>272.88</td>
<td>246.35</td>
</tr>
<tr>
<td>8</td>
<td>Vitamin C (mg/g)</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>9</td>
<td>Calcium (mg/g)</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>10</td>
<td>Acidity</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>pH</td>
<td>4.82</td>
<td></td>
</tr>
</tbody>
</table>
Oil absorption capacity is attributed mainly to
the physical entrapment of oils. It is an
indicator of the rate at which protein binds to
fat in food formulations (Akubor et al., 2013).
The same procedure was repeated for oil
absorption except that oil was used instead of
water. The oil used was soybean oil. The
density of the oil was taken as 0.92gm/ml.

Water/oil absorption =
(Vol. of water/oil added - vol. of supernatant) x
density of water/oil

3.4.3 Determination of emulsification capacity
Preparation of sample was in accordance with
the method of Igene et al., 2005. 2 gm Stevia
leaf powder and 100 ml distilled water were
blended for 30 seconds in a Philip’s blender at
1600 rpm. While continuing blending
vegetable oil was added in 5 ml portions from
a burette. Blending did not stop until the
emulsion breakpoint, a separation into two
layers, was reached. Emulsification
determinations were carried out at room
temperature (28° C). Emulsion capacity was
expressed as the amount of oil emulsified and
held per gram of sample.

3.5 Phytochemical Screening
Leaves of Stevia were subjected to qualitative
phytochemical screening for the identification
of various phytochemical compounds by the
method described by Tandhani and Subhash,
2006. Tests were performed for the following
compounds viz., tannins, alkaloids, saponins
and reducing agents.

3.5.1 Test for Tannins
About 0.5g of the dried leaf powder was boiled
in 20 ml of water in a test tube and then
filtered. A few drops of 0.1% ferric chloride
was added and was observed for brownish
green or blue black coloration.

3.5.2 Test for alkaloids
1 ml of aqueous extract was stirred in 1%
aqueous hydrochloric acid on a steam bath.
Then 1 ml of the filtrate was treated with
Dragedorff’s reagent. Turbidity or precipitation
with this reagent was considered as an
evidence for the presence of alkaloids.

3.5.3 Test for saponins
5 ml aqueous extract was vigorously shaken
with 10 ml distilled water for 2 min. The
appearance of foam that persists for at least
15 min or forming of an emulsion when olive
oil was added confirmed the presence of
saponins.

3.5.4 Test for reducing Compounds
2 ml of the aqueous extract was taken in test
tubes and 5ml mixture of equal volumes of
Fehling’s solution A and B was added and
boiled for 5 min in a water bath. The test tube
was observed for brick red precipitate which
confirmed the presence of reducing sugars.

3.6 Chemical Analysis of Stevia leaves
The leaves were analysed for the following
compounds- Moisture, Fat, Protein, Ash, Fiber,
Carbohydrate, Energy, pH, Acidity, Vit C and
Calcium. Moisture was determined by AOAC,
2000 method. Fat content in the sample was
estimated Soxhlet extraction method AOAC,
2000. The protein content was determined by
Micro-Kjeldahl’s process as described in
AOAC, 2000. The protein content was determined by
AOAC, 2000. Ash content was determined by
the method described in AOAC, 2000. The
crude fiber content in various samples was
determined AACC, 1976 method. pH was
measured using a digital pH meter at room
temperature. The pH meter was calibrated
prior to sample measurement using buffer solutions of pH value 4.0, 7.0 and 9.0 Ranganna, 1994. Titrable acidity was determined by titration method and Vitamin C was determined by method given by Ranganna, 1994. Calcium content in sample was determined by procedure described by Ranganna, 1994. Carbohydrates were calculated by difference method

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

Food energy is estimated using the sum of the product of respective physiological fuel values and contents of protein, carbohydrate and fat. It was expressed in Kcal/100g.

\[
\text{Energy} = 4 \times (\text{Carbohydrate} + \text{Protein}) + (9 \times \text{Fat})
\]

4. Results and Discussion

4.1 Organoleptic and morphological evaluation of Fresh Stevia leaf

The organoleptic characteristic of mature (90 days) fresh Stevia leaves just prior to flowering is presented in Table 1. The results showed that the colour of both the sides of leaves was different. It was green on the upper surface and light green on the lower surface. The leaf had extremely sweet taste and a characteristic odour. The texture of fresh leaf was found to be rough. The finding of this investigation are in accordance with those of Bawane et al., 2013.

The morphological characteristics of fresh Stevia leaves are presented in Table 2. The result showed that the range of leaves of 1 month for its length and width are 3.4 to 4.9 cm, and 1.7 to 2.3 cm respectively and an average of 4.3 and 2.1cm. For the second month the leaf length and width were in the range of 4.8 to 6.5 cm and 2.1 to 2.8 cm respectively and an average of 6.0 and 2.2 cm. For the third month the leaf length and width were in the range of 6.1 to 7.0 cm and 2.3 to 3.0 cm respectively and an average of 6.52 and 2.4 cm. The result showed that the length and width of Stevia leaves showed an increasing trend. There is no significant difference between the length to width ratio of Stevia leaf for 2nd (2.32) and 3rd (2.42) month. While leaves of the first month were found to have length to width ratio of 2.02 which is significantly lower (\( P \leq 0.05 \)) than the two.

4.2 Analysis of Dried leaf

Fresh green leaves were plucked and blanched and then oven dried and shade dried. Shade drying of blanched leaves was not satisfactory as wetting of leaf influences the moisture content of leaf and make it difficult to be shade dried. The precursors of steviol glycosides are synthesized in chloroplasts so the tissues without chlorophyll pigment do not contain or contain only minor amounts of the sweet steviol glycosides (Bradle et al., 1998; Singh and Rao, 2005). Also, during the leaves drying process the structure of chlorophyll is changing and as a main consequence occurs the change in colour from green to brown. This colour change ultimately affects the change of colour during the extraction and purification process of sweeteners so light colour is preferred. The blanching process intensified the colour. Oven drying method intensified the green colour and so it was undesirable. Kaushik et al., 2010 and Kalpana and Khan, 2008 also used shade drying process for drying of Stevia leaves. Shade drying method should be preferred for drying of leaf.

The dried leaf was analyzed for organoleptic, phytochemical screening, functional and chemical properties.

4.2.1 Organoleptic Analysis of dry leaves

The results in Table 3 present the organoleptic properties of dry leaf. It showed that colour change in dry leaf was more pronounced than the fresh leaf. The colour was found to be light green on the upper surface and whitish green on the lower surface. The leaf had very sweet taste, the sweetness was more pronounced than fresh leaf and had a characteristic odour. The texture of fresh leaf was found to be rough. It was also reported by Bawane et al., 2013.

4.2.2 Phytochemical Screening

Table 4 present the results of phytochemical screening. The number of positive signs indicated the intensity of reactions that reflects the quantity present. The powered leaf subjected to preliminary phytochemical screening using chemical method showed the most abundant compounds in the leaf extract were of tannins and alkaloids followed by saponins and reducing compounds. The
results were in accordance with those reported by Tandhani and Subhash, 2006.

4.2.3 Functional Properties
The functional properties play an important role in manufacturing of products using Stevia. They determine suitability in various methods of cooking and in different aspects of handling. The functional properties viz bulk density, water absorption capacity, oil absorption capacity and emulsification capacity of Stevia leaf are presented in Table 5.

The Bulk density of Stevia determined in the present investigation was 0.49 gm/ml. It is higher than the bulk density 0.46 gm/cc and 0.443 gm/ml reported by Rao et al., 2014 and Savita et al., 2004 (a). The variation in the bulk density might be due to the agroclimatic conditions and pressure applied during tapping.

Water absorption characteristic represents the ability of the product to associate with water under conditions when water is limiting such as doughs and pastes. The results of water absorption capacity was 5.27 ml/gm however Savita et al., 2004(a) reported it to be 4.7 ml/g, which is lower than the values of the present investigation. The disparities observed could be attributed due to the varietal differences and agroclimatic conditions.

Oil absorption capacity is attributed mainly to the physical entrapment of oils. It is an indicator of the rate at which protein binds to fat in food formulations (Akubor et al., 2013). Oil absorption capacity of Stevia was 3.15 ml/gm. Savita et al., 2004(a) reported 4.5ml/gm which is higher than the values obtained in the present investigation. The variation in the values of oil absorption capacity might be due to the varietal difference.

Emulsification capacity determined in the present investigation was 5.64 ml/gm. The emulsion capacity reported by Savita et al., 2004(a) was 5 ml/gm. The disparities observed could be attributed to the method used as well as the varietal differences.

4.2.4 Proximate Analysis
The results of chemical composition of Stevia powder are presented in Table 6. The energy value analyzed was 2.7 kcal per gram which may entitled Stevia the status of low or no calorie sweetener due to its intense sweetness in comparison to other available sweeteners. The same result was given by Savita et al., 2004 (a).

Moisture: There has been a numerous correlation of the effect of moisture content and water activity which suggests that the level of water activity should be minimum to prolong the shelf life of the product. In this investigation the moisture content was found to be 9.72. which varies from the earlier reported moisture content of Stevia 7.519%, 6.9397– 7.2964%,11.31%, 7.91%, 3.46%, 7.10% and 7% by Rao et al., 2014; Bawane et al.,2013; Shivanna et al., 2013; Peter et al., 2012; Belscak-Cvitanovic et al.,2010; Kaushik et al., 2010 and Savita et al., 2004(a) respectively.

Fat: Fat % was calculated to be 2.34% (on fresh wt. basis) and 2.11% (on dry weight basis). Earlier workers reported fat to be2%, 2.49%, 4.34% and 3% by Shivanna et al., 2013; Kaushik et al., 2010; Tandhani and Subhash, 2006 and Savita et al., 2004 (a) respectively.

Protein: Protein content was 10.22% (on fresh wt basis) and 9.22% (on dry wt basis). The amount of protein reported by earlier workers was 13.92%, 5.43%, 11.07%, 20.42% and 10% by Shivanna et al., 2013; Peter et al., 2012; Kaushik et al., 2010; Tandhani and Subhash, 2006 and Savita et al., 2004 (a) respectively.

Ash: Ash content indicates inorganic remains after the organic matter has been burnt away. Ash in the present investigation was found to be 10.73%(on fresh wt. basis) and 9.68% (on dry wt basis) while earlier workers reported it to be 7.6854-9.4585%, 5.742%, 7.75% and 11% by Bawane et al., 2013; Shivanna et al., 2013; Kaushik et al., 2010; Savita et al., 2004(a) respectively.

Fiber: Crude fiber content of Stevia leaf were calculated to be 13.25% (on fresh wt. basis) and 11.96% (on dry wt basis), which was lower than that reported by Shivanna et al., 2013 (15.9%) and Savita et al., 2004 (a) (18%) Total Carbohydrate: Carbohydrate content of Stevia leaf was calculated to be 52.75% (on fresh wt. basis) and 47.62% (on dry wt basis) which was similar to that reported by Savita et al., 2004(a). According to Savita Carbohydrate is 52%. Peter et al., 2012 reported the total carbohydrate to be 78.77%, which is higher than the carbohydrate in this investigation.
Vitamins C: Vitamin C was calculated to be 0.14 mg/g (on fresh wt. basis) and 0.12 mg/g (on dry wt basis) which was in accordance with that calculated by Shivanna et al.,2013 (0.1mg/g)

Calcium: Calcium was found to be 1.57 mg/g (on fresh wt. basis) and 1.42 mg/g (on dry wt basis). It was similar to Tandhani et al, 2006 report (1.55 g%) but differ from the results of Savita et al., 2004 (a) (464.4 mg/100g)

Acidity and pH: Acidity was calculated to be 0.39 ± 0.01 while pH was found to be 4.82. The pH reported by Savita et al., 2004 (a) was 5.95.

The difference in the results determined and those reported may be attributed to the methods used, climatic variance and varietal differences.

Conclusion

The energy of Stevia leaves was found to be 2.7 Kcal per gram which may entitled Stevia the status of low or no calorie sweetener due to its intense sweetness in comparison to other available sweeteners. The presence of secondary plant products in the leaf that are biologically importance eg. Tannins, alkaloids, saponins and reducing compounds contribute to its medicinal value as well as exhibiting physiological activity. The phytochemical screening of the Stevia leaves showed that the leaves were having high concentration of Tannins and Alkaloids while a moderate amount of Saponins and low amount of Reducing Sugar. The functional properties of any food apart from its nutritional value would aid in various methods of cooking and in different aspects of handling. *Stevia rebaudiana* Bertoni is a low calorie sweetener which has a good amount of protein. It is highly nutritious and can replace sugar effectively.

Research Highlights

Phytochemical screening, physic chemical properties and functional properties has been done during the research work.

Recommendations

Present work may be recommended that after phytochemical screening of secondary metabolites and functional study of stevia leaves, their further use can be undertaken.

Funding and Policy Aspects

Present work was not supported by any funding agency and source.

Author’s Contribution and Competing Interests

Ms. Rolly Mehrotra is the principal investigator and was pursuing her PhD in Food Technology. Prof Avinash Tiwari and Prof. Dheer Singh were the co-investigator and supervised Rolly. All authors declare no conflicts of interest. This study was not funded by any funding agency.

References

A.A.C.C., 1976; Approved methods of American cereal chemists. Cereals laboratory methods, St. Parul, Minnesota, U.S.A.

A.O.A.C., 2000; Official methods of analysis, Horwits W (editor), Association of analytical chemists, Washington


Hawkw J., 2003. The Bittersweet story of the *Stevia* herb. Fact, Fiction and Fraud in modern medicine, 8(3)


Ranganna S., 1994; Handbook of Analysis and quality control for fruits and vegetable products, New Delhi, Tata Mc Graw-hill publishing company


