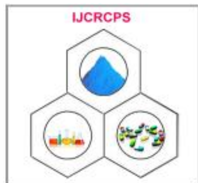

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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, MINERAL COMPOSITION AND NUTRITIVE ANALYSIS OF THE LEAVES OF *GONOSTEGIA HIRTA:* AN IMPORTANT UNDERSHRUB CONSUMED BY THE BODOS OF KOKRAJHAR DISTRICT, ASSAM.

JAHNOVI BRAHMA[1]* AND DR. BINAY SINGH[2]

[*1]Department of Botany, Science College, Kokrajhar, Assam, India
[2]Department of Forestry, NERIST, Itanagar. Arunachal Pradesh, India
*Corresponding author e-mail: jahnovibrahma6@gmail.com

Abstract

The chemical nutrient composition and the phytochemical content of the leaves of *Gonostegia hirta*, an underutilized leafy vegetable were analyzed using standard methods. The results of proximate composition analysis showed that the leaves contained moisture (85kcal/100g), crude fat (0.61kcal/100g), ash (35kcal/100g), crude protein (21.3kcal/100g) and carbohydrate (42kcal/100g). Results of the mineral analysis showed that it contains the following essential minerals: iron (21.99±0.17ppm), manganese (3.0±0.05ppm), copper (0.487±0.01ppm), magnesium (3.266±0.07ppm), zinc (9.571±0.12ppm) and molybdenum (5.40±0.88ppm). The concentration of iron shows that *Gonostegia hirta* leaves could help in boosting of blood level in anaemic conditions. The phytochemical screening of the leaves revealed the presence of tannins, saponins, phenol, reducing sugars and triterpenoids. These results rank the leaves of *Gonostegia hirta* among the best in terms of essential nutrients composition when compared with those of other edible leaves in literature.

Keywords: *Gonostegia hirta,* Phytochemical, Nutritional analysis.

Introduction

Plants produce an amazing array of organic chemicals with an enomerous diversity of structural types which are essential for plant growth and development and are widely used by humans and other animals as food sources. The useful products obtained from plants directly or indirectly, demonstrates their importance to man. Plants serve as a source of food (Saka and Msonthi, 1994; Katsayal et al., 2004; Kawo, 2007), medicinal products (Adoum, 1997; Ezeamuzie et al., 1996; Careres et al., 1997), energy (Oladele and Yisa, 1989) and shelter to man and his livestock (Ogunkunle and Oladele, 2004). These biologically active compounds are not only necessary for the well being , survival and evolution of the plants that produce them, but also for humans, who have exploited them for industrial use, pharmaceutical, biotechnology and nanotechnology use.

Gonostegma hirta also popularly known as 'Sumlouthe' by the Bodo tribes of Kokrajhar district, Assam is a creeping perennial herbs, sometimes sub shrubs, with leaves 5-8cm long, often prostrate, 50-100(-160) cm, monoecious or dioecious. Stems 4-angled distally, pubescent with leaves opposite, stipules broadly ovate, ca. 2.5 mm; petiole 1-4 mm; leaf blade narrowly lanceolate, rarely narrowly ovate or elliptic, (1.2-) 3-10 × (0.7-) 1.2-2.8 cm, herbaceous or thinly papery, 3(or 5)-veined, adaxial surface sparsely strigillose or sub glabrous, adaxial surface sparsely pubescent along veins or sub glabrous, base subcordate or rounded, apex acuminate or acute. Glomerules often bisexual or sometimes unisexual, 2-9 mm in diam. Male flowers: pedicel 1-5 mm; buds ca. 2 mm in diam.; perianth lobes 5, oblanceolate, 2-2.5 mm, apex acute. Female flowers sessile; perianth tube ovoid,

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ca. 1.6 mm, longitudinally 10-winged, apex 2-toothed. Achene white to black, ovoid, ca. 1.4 mm. The plants are depurative and febrifuge. The roots, the leaves or the whole plant are used to treat boils and abscesses, abdominal cramps in females, leucorrhoea, to treat bone dislocations and fractures (Flora of China).It is commonly used by the local people as pot vegetable and is given to lactating mothers.

In order to boost their nutrition levels, most rural dwellers in many parts of Kokrajhar district, especially the Bodo communities have resorted to the administration of the leaf extract of Gonostegia hirta as the cheapest source of multivitamins. This study therefore focuses on the phytochemical screening, mineral analysis and the proximate analysis of *Gonostegia hirta* leaves with a view to assessing its nutritional potentials in relation to its ethno medicinal uses.

Scientific Classification

KINGDOM : Plantae
PHYLUM : Magnoliophyta
CLASS : Angiospermae
SUBCLASS : Fabids
ORDER : Rosales
FAMILY : Urticaceae
GENUS : Gonostegia
SPECIES : hirta

Materials and Methods

Sample collection

Fresh plant samples were collected from the field and herbarium was prepared. The herbarium was identified for authenticity by the experts of Department of Botany, Gauhati University, Assam. Fresh tissues of the plants are made free from dust and other foreign material by washing either with distilled water or tap water. The washed plant samples are then placed on filter paper sheets for soaking the moisture followed by putting them in separate open mesh or perforated bags for air drying for 1-3 days. Then the plant samples are dried in a hot air oven at 65 $^oC\pm 2^oC$ for 48 hours. The samples are afterwards grinded in an electrical stainless steel grinder using 0.5mm sieve. Each sample are again put in oven and dried for few hours more for constant weight. They are then stored in paper bags for further analysis. Dried powder is soaked in distilled water for 72 hours with occasional stirring. The mixture is then filtered and the filtrate is taken for experiments whenever applicable (Bhandari et al., 2007).

Digestion of plant sample

Digestion of the plant sample is a pre-requisite step to determine total nutrients present in plants. In plants the nutrients exist in various organic combinations such as carbohydrates, proteins, fats e.t.c and digestion of plant material releases them in mineral forms (Sangwan et al., 2007). The usual weight of plant sample taken for digestion for total elemental analysis is 0.5g. and digestion of plant samples is done by two methods-

i) Dry ashing – It is done by igniting the plant material in a muffle furnace at 550-600 ^oC followed by the extraction of ignited material in dilute acid (HCL or HNO_3).

ii) Wet oxidation – It involves digestion of plant material in a mixture of two or three concentrated oxidizing acids of $HClO_4$, H_2SO_4 and HNO_3.

The plant samples for the determination of nutrient elements like K, Na, Ca, Mg, Cu, Zn, Fe, Mn, Mo and B which do not volatilize at high temperature can be digested by dry ashing in furnace using suitable silica, porcelain or platinum crucible and extracting them in dilute HNO_3.

Proximate composition

Determination of crude protein

The crude protein was determined using micro Kjeldahl method (Matissek et al., 1989). 2g of sample material was taken in a Kjeldahl flask and 30ml concentrated sulphuric acid (H_2SO_4) was added followed by addition of 10g potassium sulphate and 1g copper sulphate. The mixture was heated gently and then strongly once the frothing had ceased. When the solution became colourless or clear, it was heated for another hour, allowed to cool, diluted with distilled water (washing the digestion flask) and is then transferred to 800 ml Kjeldahl flask. Three or four pieces of granulated zinc and 100 ml of 40% caustic soda is added and the flask is connected with the splash heads of the distillation apparatus. Next 25 ml of 0.1N sulphuric acids will be taken in the receiving flask and is then distilled. When two thirds of the liquid had been distilled, it is then tested for completion of reaction. The flask is removed and titrated against 0.1N

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caustic soda solution using methyl red indicator for determination of Kjeldahl nitrogen which in turn gives the protein content. The nitrogen percent is calculated by the following formula-

$$N\% = \frac{1.4\,(V_1 - V_2) \times \text{Normality of HCL}}{\text{(dilution) Weight of the sample}} \times 250$$

Protein content is estimated by conversion of nitrogen percentage to protein (AOAC, 1984). Thus,

$$\text{Protein \%} = \text{N\%} \times \text{conversion factor (6.25)}$$

Estimation of Oils and Fats

Crude fat is determined by Mojonnier method (James, 1995). The fat content is determined gravimetrically after extraction with diethyl ether ethoxyethane and petroleum ether from an ammonium alcoholic solution of the sample. About 10 gm of the sample is taken into a Mojonnier tube, to it is added 1 ml of 0.88 with 10 ml ethanol, mixed well and cooled. Then 25 ml diethyl ether is added, Stoppard the tube, shaken vigorously and then 25 ml of petroleum ether is added and the tube is left to stand for 1 hour. The extraction is repeated thrice using a mixture of 5 ml ethanol, 25 ml diethyl ether and 25 ml petroleum ether and this extraction is transferred into the distillation flask. The solvent is then distilled off and the flask is dried by heating for 1 hour at 100 ^{o}C and reweighted. The percentage of fat content of the sample is calculated by the following formula which gives the difference in the weights of the original flask and the flask plus extracted fat which represents the weight of the fat present in the original sample. Hence,

$$\text{\% of fat content of the sample} = W_2\ W_1 / W_3 \times 100$$

Where, W_1 = weight of the empty flask (g)
W_2 = weight of the flask + fat (g)
W_3 = weight of the sample taken (g)

Determination of moisture content

Since the analysis results are expressed on oven dry weight basis, it becomes necessary to determine the moisture content of air dried tissue (Wildey et al., 1979). Duplicate determinations are made on each sample of the plant tissue. The results of air dried tissue analysis are then converted to oven dry basis. 20 gm of the samples of ground air dried tissue is dried in an oven at 105°C for overnight or for 12 to 16 hour. The samples are then cooled in desiccators and weighed. The differences in weight are then taken to represent the loss of moisture and are expressed as a percentage of oven dry weight (AOAC, 2000). Hence,

$$\text{Moisture \%} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Determination of Total solids

Total solids were estimated by deducting moisture percent from hundred as described by James (1995). Therefore,

$$\text{\% of total solids} = 100 - \text{percentage of moisture}$$

Determination of Ash content percentage

For determination of ash content, me thod of AOAC (1984) will be followed. According to this method, 10 gm of each sample will be weighted out in a silica crucible, this crucible will be heated in muffle furnace at 300°C for one hour, and then it is cooled in a desiccator, waited for completion of ash and then cooled. When the ash becomes white or grayish in colour, weight of the ash content is calculated out by using the following formula-

$$\text{Ash \%} = \frac{\text{Weight of the ash sample}}{\text{Weight of the sample taken}} \times 100$$

Determination of Carbohydrates

Determination of available carbohydrates in the sample will be calculated by difference method as described by James (1995) based on Traditional Carbohydrate Determination. Thus,

$$\text{\% of carbohydrates} = 100 - (\text{Protein} + \text{Ash} + \text{Moisture} + \text{Fat})$$

Determination of nutritive value

The total energy value in kcal/100g was estimated by using the method described by FAO (2003) as shown below:

Nutritive value = 4 x percentage of protein + 9 x percentage of fat + 4 x percentage *of carbohydrate*

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Minerals and trace element analysis

Among the nine micronutrients (Fe, Cu, Zn, Mn, B, Mo, Cl, Co and Ni), Fe, Cu, Zn, Mn, Co and Ni are heavy metals. These heavy metal elements are suitably estimated on Atomic Absorption Spectrophotometer (AAS) because their atoms do not get excited under ordinary flames; hence they cannot be estimated correctly by Emission Spectrophotometer. The method gives a good precision and accuracy (Ojeka and Ayodele, 1995).The principle of the method is based on nebulising a sample solution into an air acetylene flame where it is vaporized. Elemental ions were then atomised and the atoms then absorb radiation of a characteristic wavelength from a hallow-cathode lamp. The absorbance measured, is proportional to the amount of analytic in the sample solution. As mentioned already, the level of each element in the sample solution was determined by reference to a calibration curve. The atoms of metallic elements like ZN, MN, Fe, Cu, Ni, Co which normally remain in ground state under flame conditions absorb energy when subjected to radiation of specific wavelength. The absorption of radiation is proportional to the concentration of atoms of that element. The absorption of radiation by the atoms is independent of the wavelength of absorption and temperature of the flame (Narwal et al., 2007).

Test for qualitative estimation of bioactive compounds (Ajayi et al., 2011; De and Dey, 2010; Soni et al., 2011; Bekele, 2008; Kantamreddi et al., 2010).

After shade drying the dried plant samples were powdered in a mixer grinder and the dried powder is soaked in distilled water for 72 hours with occasional stirring. Then the mixture was filtered and the filterate was taken for the experiments wherever applicable.

Test for Tannins

1 gm of powdered was boiled with 20ml distilled water for 5 minute in a water bath and was filtered while hot. 1ml of cool filtrate was mixed with 5ml distilled water and few drops of 10% Ferric chloride and observed for any formation of bluish black or brownish green colour.

Test for Saponins

Froth test- 1gm of powdered sample was boiled with 10ml of distilled water bath for 10 minutes. The mixture was filtered while hot and allowed to cool then 2.5 ml of filtrate was diluted to 10ml with distilled water and shaken vigorously for 2 minutes. Frothing indicates the presence of saponin in the filtrate.

Test for Alkaloids

1. Hager's test: - 1ml of filtrate was taken and 3ml of Hager's reagent (Saturated solution of Picric acid) was mixed in it and observed for the formation of a yellow precipitate.
2. 1 gm of powdered sample was boiled with water and 10 ml HCL was dissolved in it. A very small quantity was mixed with picric acid. Coloured precipitate or turbidity indicated the presence of alkaloid.

Test for Flavonoids

1. 1ml of filtrate was mixed with few fragments of Magnesium ribbon and concentrated HCL was added drop wise. Pink scarlet colour indicated the presence of flavonoids.
2. 1 gm of powdered sample was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20% NaOH solution was added to 1 ml of thee cool filtrate. A change to yellow colour which on addition of acid changes to colourless solution depicted the presence of flavonoids.

Test for Phenol

2 ml of filtrate was taken, then freshly prepared 1% ferric chloride and 1 ml of Potassium ferrocyanide was added to it. Formation of bluish green colour indicated the presence of phenol.

Test for Steroids and Terpenoids

1. Salkowski test: - 1 ml of filtrate was mixed with chloroform and few drops of concentrated Sulphuric acid is added, then shaken and allowed to stand for some time. Appearance of red colour indicated the presence of steroids and formation of yellow coloured upper layer indicated the presence of terpenoids.
2. 1 ml of filtrate dissolved in 1 ml of acetic acid was added and then few drops of concentrated

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Sulphuric acid were run down the side of the test tube. The appearance of pink or pinkish brown ring or colour indicated the presence of terpenoids. The appearance of blue colour indicated the presence of steroids.

Test for reducing sugars

Benedict's test- 1mL of filterate was mixed with few drops of Benedicts reagent and boiled in water bath. The appearance of reddish brown precipitate indicated the presence of sugars.

Results and Discussion

T The present study carried out in the plant samples reveals the presence of many bioactive compounds. This prominent edible species were consumed medicinally and as vegetables by the Bodo tribes of Assam. Most of these wild edible plants are weedy and often used as leafy vegetables during food shortages. Consumption of vegetal matter from wild plants has more regularity and higher intake proportion in times of food shortages (Zemede and Mesfin, 2001). It is interesting to mention that some famine wild edible species of *Hibiscus sp., Corchorus sp., Premna herbaceae, Amorphallus sp., Lasia spinosa, Paederia foetida, Gonostegia hirta* and *Blumera lanceolaria* are domesticated and used widely as traditional leafy vegetable by the Bodos. The result of qualitative analysis of *Gonostegia hirta* is presented in Table 1. The medicinal and nutritional value of these plants lies in some chemical substances that have a definite physiological action on human body. The most important of these bioactive constituents of plants are alkaloids, terpenoids, carbohydrates and protein compounds. It is evidently witnessed that the plant is very rich in carbohydrates such as 42% in dried extraction. Carbohydrates are one of the most important components in many foods and may be present as isolated molecules or they may be physically associated or chemically bound to other molecules. Some carbohydrates are digestible by humans and therefore provide an important source of energy. Carbohydrates also contribute to sweetness, appearance and textural characteristics of many foods (FAO/WHO, 1974).

An appropriate amount of protein in *Gonostegia hirta* plant products were observed as 21.3%, ash percent 35%, moisture percent 85%, total solids 15% and fat percent of 0.61%. FAO/WHO (1974) described the human nutritional requirements obtained from plant kingdom. Ash is the inorganic residue remaining after water and organic matter has been removed by heating in presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. Analytical techniques for providing information about the total mineral content are based on the fact that the minerals can be distinguished from all the other components within a food in some measurable way (Jain et al., 1992 and Nielsen,S.S., 1998).Similarly total solids are measure of the amount of material dissolved in water such as carbonate, bicarbonate, chloride, sulphate, phosphate, nitrate, calcium, magnesium, sodium, organic ions and other ions (American Public Health Association, 1998).

The estimated total energy value in the leaves of *Gonostegia hirta* per 100g was 258.69 kcal/100g. This high calorific value is an indication that it can be recommended to individuals suffering from overweight and obesity (Table 2). The inorganic mineral analysis of the leaves showed that it contained magnesium, iron, zinc, manganese, copper and molybdenum (Table 3).

The iron content of *Gonostegia hirta* leaves is 21.99±0.17 ppm which is quite high than some cultivated vegetables such as spinach (1.6mg/100g) (Turan et al., 2003) and its daily intake in our diet could help in boosting the blood level especially in anaemic conditions.

The content of zinc and manganese is found to be 9.571±0.12 ppm and 3.00±0.05ppm which is adequate when compared with the recommended dietary allowances (WHO, 1999). Manganese acts as activator of many enzymes (Mc Donald et al., 1995) while zinc is involved in normal functioning of immune system.

Magnesium exists primarily as an intracellular constituent in the body and its requirement is estimated to be 0.2-0.6% of the dry weight of animals. The level of Magnesium in this study (3.266±0.07 ppm) is therefore adequate and hence could be of advantage to the improvement of healthy conditions of an individual (Maynard et al., 1979).

The content of copper and molybdenum in *Gonostegia hirta* leaves is found to be 0.487±0.01ppm and 5.400±0.88 ppm. Copper plays a role in haemoglobin formation and it contributes to iron and energy metabolism. Similarly molybdenum is a key component in many biochemical processes

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Table1. Qualitative analysis of the phytochemical analysis of *gonostegia hirta*

S.no.	Phytochemical	Result
1.	Alkaloid	--
2.	Flavonoids	--
3.	Tannin	++
4.	Saponins	++
5.	Steroid	--
6.	Terpenoids	+
7.	Phenols	++
8.	Reducing sugars	++

Where + = Present, + = Trace, - = Absent

Table 2. Proximate composition and nutritive quantity (k.cal/100g) is given as follows.

Sl.No.	Proximate composition	Result
1.	Protein	21.3
2.	Moisture	85
3.	Fat	0.61
4.	Ash	35
5.	Total solids	15
6.	Carbohydrates	42
7.	Nutritive value	258.69

Table 3. Various Amounts of micronutrients by Atomic Absorption Spectrometer (AAS) [where all concentrations in ppm (parts per million), ND = Not detectable].

Sl. No	Specimen name	E L E M E N T S					
		Zn	Mg	Mo	Cu	Fe	Mn
1.	*Gonostegia hirta*	9.571±0.12	3.266±0.07	5.400±0.88	0.487±0.01	21.99±0.17	3.00±0.05

and acts as a cofactor in many enzymes that catalyze the conversion of one compound into another one within the cell and is involved in detoxifying sulfites which would be a great treatment for people who suffer of asthma attacks due to reactions to sulfites (Kister et al., 1999). From the above study it is clear that consumption of the plant *Gonostegia hirta* is adequate to supply the daily nutrient requirements for children and lactating mothers.

Conclusion

It was concluded that edible wild plant utilization should be considered not only as food resource but also as traditional cultural heritage. Here the signifance and value of local food and local cuisine were revaluated. The present studies revealed that these leaves may lend credence to its use for therapeutic potentials. Since the leaves contains appreciable amount of macronutrients and micronutrients, it can favorably compete well with other conventional edible leaves. Thus the leaves of *Gonostegia hirta* could help fulfill the growing demands of plant based foods for human nutrition.

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