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The nutraceutical studies of *Gonostegia hirta* (Blume) Miq a traditional vegetable from Northeast India

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Abstract
The paper deals with the taxonomic details, geographical distribution, and nutritional aspects of *Gonostegia hirta* used as a vegetable in states of North-east India. This plant is also used as traditional medicine in the treatment of asthma. The plant is also reported from other tropical parts of India. A study was conducted to analyse the nutritional potential of leaves of *G. hirta* by evaluating carbohydrates, fat, proteins, energy, total ash, and water content by standard methods. However, UHPLC profiling as well as HPTLC was performed using an in-house protocol developed at Patanjali Research Institute, Haridwar, Uttarakhand. The presence of flavonoids (0.29%.), and phenols (0.021%.), indicates their nutritional and medicinal importance. The biochemical compounds per 100g, like carbohydrates (11.33g), proteins (0.134g), fats (0.65g), total ash (02.72g), moisture content (83.96g), and energy level (56.53kcal). It represent its appreciable nutritional value, especially as a low-fat diet. The study was performed with the objective of nutritional composition analysis, qualitative analysis of secondary metabolite so that it can be recommended for its maximum utilization for edible purposes.

Keywords: Nutraceutical, flavonoids, polyphenols, *Gonostegia hirta*

Introduction
Gonostegia hirta (Blume) Miq belongs to the family Urticaceae comprising of about 6 species widely distributed throughout tropical and temperate regions of Asia to North Australia. In India, 5 species are widely distributed [1]. The species of genus *Gonostegia* Turcz. are widely distributed and mainly leaves are used as vegetables. The nutritional attributes of *Gonostegia hirta* from North-eastern states of India like Arunachal Pradesh, Assam, Nagaland, and Manipur are well known however, the nutritional aspects of the tender shoots were yet to be studied. Accordingly, the present study was carried out. It has been reported that the tender shoots contain minerals like calcium, iron, magnesium, phosphorus, vitamins A, and vitamin C and are eaten as vegetables [2-3]. It is used medicinally in India [4-6] and in Bhutan [7].
The present investigation shows the presence of alkaloids, flavonoids and phenolics in the tender shoot which are well acknowledged for their health-promoting activities and also helpful in maintaining the metabolic functions in the body [8, 9].

Medicinal uses
The plant is also used for medicinal purpose [Table 1]. It is considered as depurative and febrifuge. The raw paste of the whole plant is used to relieve inflammation of the skin due to fire burns among the local women [10]. In case of sore throat and cough the leaves are rubbed on the throat of a person giving numbing sensation and also eaten. The crushed leaves are applied as a poultice on ulcers. An infusion of the whole plant is drunk by children suffering from atrophy and indigestion [6]. Due to its slippery and soft nature, it acts as a laxative ideal for clearing constipation. The preparation of Oyik is given to a pregnant woman during her gestation period to enable easy delivery of the child [10]. The shoot promotes lactation amongst lactating ladies [4]. The Monpa people in south-eastern Tibet use this species as a leafy vegetable as well as a functional food to provide energy for children and elders [8].

Table 1: Medicinal uses of *Gonostegia hirta*

Whole Plant	Boils, abscesses, abdominal cramps, leucorrhoea in females[5], bone dislocation, fractures, atrophy, indigestion [6], inflammation of skin [10].
Leaves	Sore throat, ulcers [9] .

Culinary Use

In India especially in north-eastern states, like Assam, and Arunachal Pradesh the young leaves of the plant are widely used for both culinary and medicinal purposes. The Abotani tribes of Central Arunachal Pradesh such as Adis, Nyishis, Galos, and Tagins use *Gonostegia hirta*, known as "Oyik" in the local dialect. The traditional dishes are prepared by this locally available plant and are part of the cultural tradition of the Abotani tribe. The young twigs and leaves are first thoroughly washed in running water to remove any dirt or microflora from the plant surface and then chopped into small pieces. Then these are immersed in boiling water and brought to one boil. Then as per taste the green chillies and salt are added to it and the preparation is continuously stirred with a spoon to bring a thick consistency to the soup gravy. Thereafter a pinch of sesame seeds (*Sesamum indicum*-Tanam) is added to it to bring richness and aroma to the prepared dish. This gravy is eaten with rice and other vegetarian and non-vegetarian meals served with rice. The leaves are also cooked with dried bamboo shoots and smoked beef/pork during festivals and various other occasions. The Bodo tribe of Assam consumes leaf extract as the cheapest source of multivitamins and thus boosts their nutrition level [12,13]. Not only in India but the plant is used in other countries also for their health care function. The Gelao people in Southwest China consume fruits as snacks and eat or are soaked in wine and then eat it [14]. In Borneo islands, the *G. hirta* is widely consumed daily by the local indigenous people for culinary use as a traditional vegetable as well as for medicinal use [15]. The plant possesses an aggressive growth habit and thus is very frequently available for the local people and vegetable vendors. In India, however, agro-technology is not available for the commercial production of this nutritious and medicinal plant and emphasis should be laid on cultivating it to utilize its attributes.

Materials and Methods

The distributional study of plant was conducted during various explorations. Its global positioning at selected place was furnished the with the help of GPS instrument along with the collection of samples from various markets in Northeast India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram and Manipur), where local people sell the plants in local vegetable shops. The fresh shoots of *Gonostegia hirta* were collected in October, 2022 from local markets of Itanagar. 5% mercuric chloride solution in ethanol was used for poisoning during preparation of sample vouchers and thereby mounting on herbarium sheets, as described in reference method [16] and deposited in the Herbarium of the Regional Ayurveda Research Institute, Itanagar.

Phytochemical analysis

The phytochemical screening and proximate analysis were conducted in the Chemistry Laboratory of Patanjali Research Institute, Haridwar using analytical and laboratory-grade solvents and chemicals. The samples were prepared from fresh green colored leaves of *Gonostegia hirta* paste, stored in an airtight container, well labelled, and kept in a cool, dry place for analysis.

Proximate Analysis

The proximate analysis was conducted using the standard methods as prescribed in Indian Standards (IS). Protein was estimated by using the Kjeldahl method (IS: 7219 (1973) and calculated by multiplying the evaluated nitrogen by 6.25. The fat was analysed by subjecting the shoot paste to acid hydrolysis and then re-extracting it by taking ether (AOAC 4.5.02((59.02). The difference between 100 and the sum of the moisture, protein, fat, and ash contents in the sample gave the carbohydrate content (IS:1656) present in the sample. Similarly, the total energy (percent by mass) is calculated as 9 × A + 4 (B + C), where A=percent by mass of fat, B= percent by mass of total protein, C= Percent by mass of carbohydrate (IS:14433). The total ash content in the fruits was determined using method as prescribed in IS:561 taking 2-4gm of sample. The results were expressed as Ash (% w/w) = Difference in weight of Ash/weight of sample × 100. Two gram of each sample was taken in a flat bottomed dish and kept overnight in an air oven at 100-110 ºC and weighed. The loss in weight was regarded as a measure of moisture (water content). The total sugars including reducing and non-reducing were determined using method given in IS:6287.

UHPLC-Ultra high-performance liquid chromatography profiling: The UHPLC profiling was performed by taking clear supernatant prepared from 1g of sample paste dissolved in 5 mL hydro-methanol (80 methanol: 20 water), sonicated for 20 minutes. The mobile phase used was (A) 0.1% acetic acid in water and (B) acetonitrile (HPLC-grade). A thermostatically controlled column (350 C; Shodex, C18 (4.6 × 250 mm, 5 µ) with a flow rate of 1 mL/min, injection volume of 10 µL at a wavelength 254 nm. To separate polar, mid-polar, and non-polar compounds the composition of the mobile phase was gradually changed from 0% to 95%, and then brought back to its initial composition in 80 minutes. The samples were taken in duplicate.

HPTLC-High-performance thin layer chromatography fingerprinting: 1 g of sample was dissolved in 5 mL methanol sonicated for 20 min and centrifuged. The clear supernatant was used for analysis. The mobile phase used was (A): chloroform: ethyl acetate: formic acid (4.5: 4.0: 1.5 v/v/v) (A) under 366 nm and (B) under white light after derivatization by anisaldehydes.

Qualitative analysis of total flavonoids and total polyphenols: The qualitative analysi of total flavonoids and total polyphenols was conducted using UV spectrometry. 20 mg of Quercetin dihydrate and 20 mg of gallic acid were taken separately and further dissolved in 20 mL of Milli Q water each. Further diluted from 1 mL to 10 mL with Milli Q water to prepare a concentration of 100 ppm and further diluted to prepare different concentrations of standard to plot linearity. The leaves were crushed in pestle mortar to make a paste. The paste was used for proximate analysis. About 650 mg of the sample was dissolved in 10 mL milli Q water, the solution was sonicated for 20 minutes, and centrifuged, clear supernatant was used for the analysis.

Total Flavonoids: About 1mL of sample was mixed with 0.4 mL of 10% Aluminum chloride ($AlCl_3$). Then 0.4 mL of 3M Sodium acetate (CH_3COONa) and 3 mL ethanol was added to the mixture. The mixture was kept at room temperature in the dark for 30 minutes. Then the absorbance was recorded at 450 nm taking Quercetin as standard.

Total Phenols: 1mL of the sample was mixed with 1 mL of Folin-Ciocalteu reagent (1:10 with distilled water). Then the tubes were incubated at room temperature for 5 minutes. Then 1 mL of 10% Sodium Carbonate (Na_2CO_3) was added to the

mixture and kept at room temperature in the dark for 60 minutes. The absorbance was recorded at 760 nm taking gallic acid as standard.

HPTLC-High performance thin layer chromatography fingerprinting

The HPTLC fingerprinting was performed using an in –house protocol.10 g of sample was transferred to an extraction flask and refluxed using methanol as solvent at 70ºC for 1 hr. After that sample was filtered and dried using a rota evaporator. The dry sample was dissolved in 5 mL methanol and taken for analysis. The mobile phase was of Chloroform: ethyl acetate: formic acid (9:7.8: 1 v/v/v). The saturation time was 15 minutes with a migration distance of 70mm giving band length of 8mm visualized at 254 nm 366 nm and white light.

UHPLC profiling

The UHPLC profiling was performed by taking clear supernatant prepared from 1g of sample paste dissolved in 5 mL hydro-methanol (80 methanol: 20 water), sonicated for 20 minutes. The mobile phase used was (A) 0.1% acetic acid in water and (B) acetonitrile (HPLC-grade). A thermostatically controlled column Shodex, C18 (4.6 × 250 mm, 5 µ) with a flow rate of 1 mL/min, injection volume of 10 µl at a wavelength 254 nm. To separate polar, mid-polar, and non-polar compounds the composition of the mobile phase was gradually changed from 0% to 95%, and then brought back to its initial composition in 80 minutes, the samples were taken in duplicate.

Results and Discussion

The fresh shoots of *Gonostegia hirta* procured from local market of Itanagar was identified by matching the herbarium sheets with the authentic herbarium sheetk kept in herbarium of Regional Ayurveda Research Institute, Itanagar. Plant is an annual, prostrate or semi-erect to erect 30-90 cm tall, pubescent to glabrescent herb. Flowers greenish, in dense axillary fascicles. Calyx in male flowers is deeply 5-lobed; in females shallowly 4-lobed. Stamens short, antisepalous. Achenes enclosed by persistent ribbed calyx.

Botanical description of shoots

Leaves opposite, sessile or with a short petiole; lamina narrowly ovate-lanceolate, sparsely appressed hairy on both sides, rounded or cordate at the base, entire, acute; stipules ovate, acuminate (Figure 1).

Fig 1: Plant of *Gonostegia hirta* in its habitat

Global positioning of *Gonostegia hirta*

The plant was commonly found at various location in North Eastern states of India during our expeditions. In Arunachal it was recorded from Nirjuli [27^{0}13.14'N, 93^{0}74.32'E, and Pasighat [28^{0}07'124N, 95^{0}33'327E] Harmutti [(27° 7. 25' N; 93° 51.28' E] in Assam; Kohima [27^{0}06.24.91'N, 93^{0}57.96'E] in Nagaland; Imphal [24^{0}48.28'N, 93^{0}56.39'E] in Manipur and Kolasib [24^{0}13.25'N, 92^{0}40.43'E] in Mizoram.

Proximate Analysis

Gonostegia hirta is considered to be as locally available wild plant commonly found in North east India and is very commonly utilized by people for edible and medicinal purposes and plays an important role in diet of local people. However, there is not much relevant research focusing on nutritional aspects of this plant, although there are few report available on its chemical composition and functional properties. The present study is aimed to meet the void in the research. The results of the nutritional values and health related chemical constituents of leaves (proximate analysis) are presented [Table 2].

Table 2: Nutritional composition per 100g

S. No.	Content	Value (gram)
1.	Protein: (%w/w)	0.134g
2.	Fat: (%w/w)	0.65g
3.	Carbohydrate	11.33g
4.	Total Ash: (%w/w)	02.72g
5.	Moisture: (%w/w)	83.96g
6.	Energy	56.53 Kcal

The total flavonoids and polyphenols in *G. hirta* were 0.029% (w/w) and 0.021% (w/w) when compared with the respective standards. The UHPLC profile indicates the presence of non-polar compounds in the leaves of *G. hirta* [Table 3].

Table 3: Total flavonoids and polyphenols in the shoots of *Gonostegia hirta*

S. No.	Parameters	Value
1	Total Flavonoid, equivalent to Quercetin (% w/w)	0.029
2	Total Polyphenol, equivalent to Gallic acid (% w/w)	0.031 2

In a study, the total phenolics (1.20 ± 0.10 mg GAE/g DW) in *G. hirta* were found to be 2.43 times higher than that found in spinach (0.493 mg GAE/g DW) and about 5.53 times higher than that of green lettuce (0.217 mg GAE/g)[17]. The reason for the discrepancy in the phenolic content in various samples may be due to sample collection at different plant development stages, geographical variations of picking regions, and extraction methods involved. The various studies conducted by various researchers reveal the presence of 47 phenolic acid compounds by UPLC-MS/MS, accounting for 11.24% of the total. The presence of phenolic compounds in plants is mainly responsible for the antioxidant, anti-microbial nutritional, and other various pharmacological activities of plants. In a study, the total flavonoids in *G. hirta* were found to be 2.85 times higher than that of *Brassica juncea*. In plant species, more than 6000 phytoconstituents have been discovered responsible for various types of activities in plants including protecting them from pathogens, and regulating their growth along numerous pharmacological activities [18,19]. Overall *G. hirta* possesses a high content of flavonoids, and phenolics and can be an important source of antioxidants if consumed dietary meal and can be some good food supplement rich in various phytochemicals [Figure 2-5].

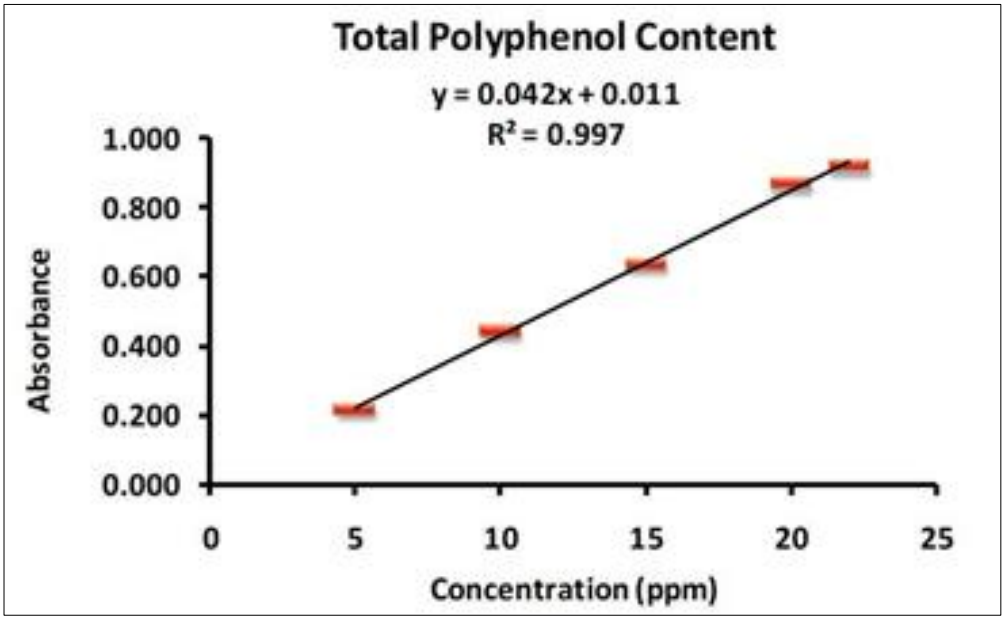

Fig 2: Linearity plot for Total Polyphenols, Gallic acid as a standard

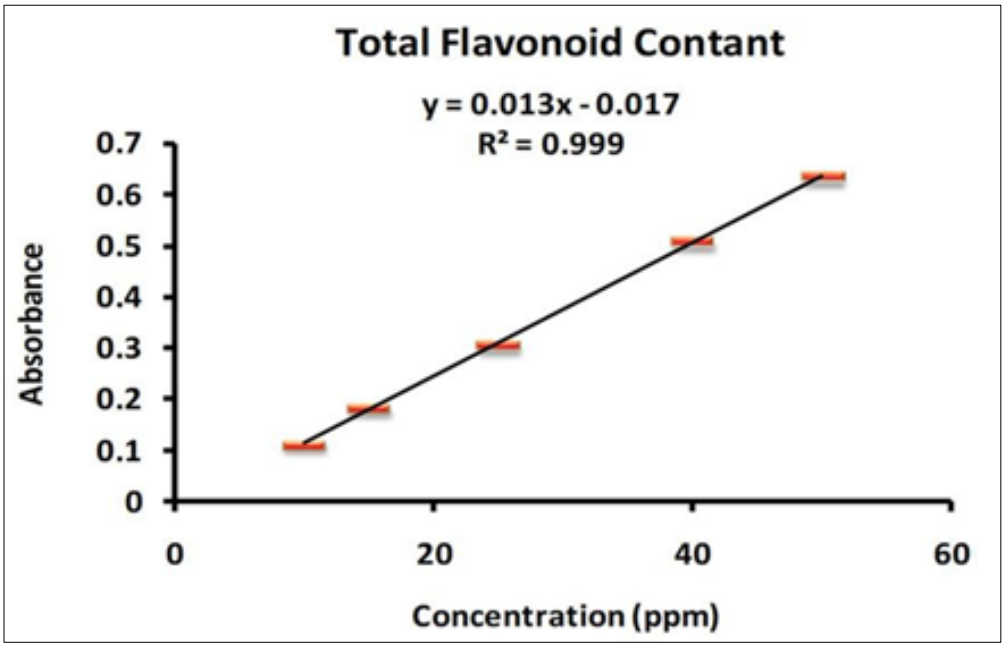

Fig 3: Linearity plot for Total Flavonoids, Quercetin as a standard

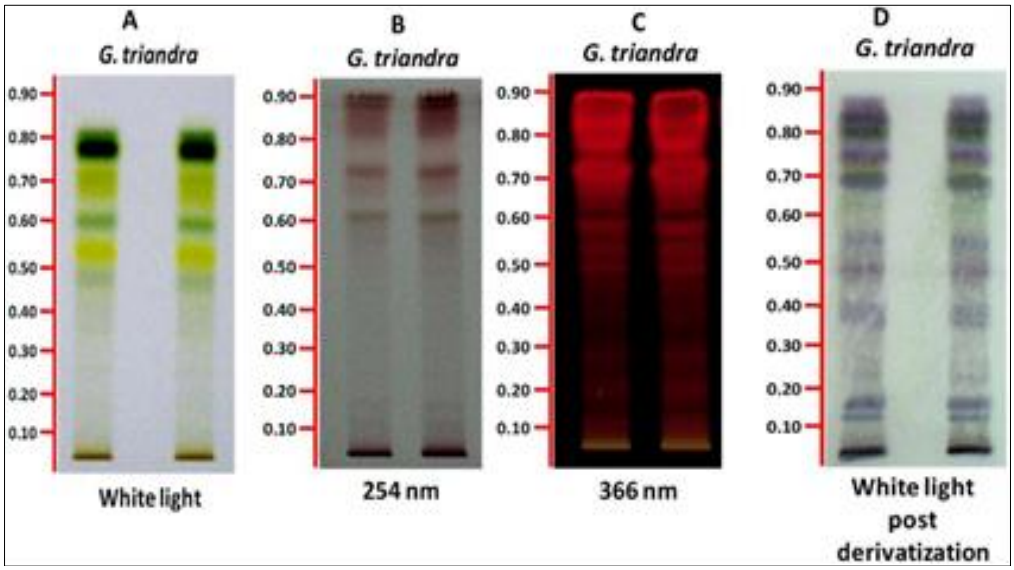

Fig 4: HPTLC fingerprinting of leaves of *Gonostegia hirta*

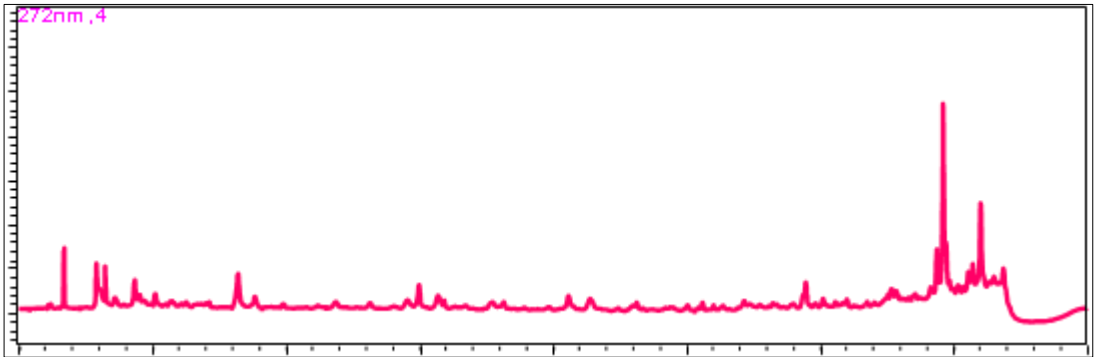

Fig 5: UHPLC fingerprint of leaves of *Gonostegia hirta*

Conclusion

The present study was aimed to evaluate the nutritional value and active phytochemicals from shoots of *Gonostegia hirta* as they are widely consumed for edible purpose and also acclaimed in traditional medicine. The presence of moderate amount of carbohydrates, proteins fats, total ash, moisture content and energy in calories are suggestive of its appreciable nutritional value, especially as a low-fat diet. The presence of flavonoids (0.29%.), and phenols (0.021%.), indicates their nutritional and medicinal importance. However other investigations are also suggestive to comprehensively evaluate the flavour, nutritional and functional values to provide a basis for the development and utilization of *Gonostegia hirta* on commercial production level so that its nutritional benefits can be perceived on large scale.

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Conflict of interest

There is no any kind of conflict of interest amongst the authors

Author's contribution: Concept and theme of the AB looked the overall guidance, RS developed concept and theme after conducting field studies and compiled the manuscript, RAJ and MJ conducted biochemical analysis and methodology of the paper, UBP prepared the technical analysis and AS and VPA supervised and draft setting.

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