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Research Article

BIOACTIVE AND NUTRACEUTICAL COMPOUND MANIPULATION IN *HIBISCUS SABDARIFFA* L. LEAVES: A COMMON UNDERSHRUB CONSUMED BY THE BODO TRIBES OF BTC, ASSAM, INDIA

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ABSTRACT
Hibiscus sabdariffa L. is a gregarious under shrub, which is traditionally consumed by the Bodo tribes of Assam, India as a major constituent of food. Ethno medicinally the leaves, flowers and fruits are used for stomachic, in high blood pressure, cough and for diuretic purposes. A wide range of chemical compounds including hibiscin, anthocyanins, flavonols and sterols has been isolated from *Hibiscus sabdariffa*. Phytochemical constituents isolated from these plants include flavonoids, tannins, steroids, phenols, triterpenoids and alkaloids. Proximate analysis showed the presence of various trace elements with high nutritive values. The present review summarizes the information concerning the botany, ethno pharmacology, phytochemistry and pharmacological activities of the plant.
Keywords: *Hibiscus sabdariffa* L., Phytochemical constituents, Pharmacological, Ethno botany.

INTRODUCTION

Food plants and culinary herbs are known to contain myriad phytochemicals with medicinal properties[1]. Phytochemicals are natural bioactive compounds found in plants which work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions[2]. These phytochemicals acting individually or synergistically helps to reduce the risk of a variety of chronic and inflammatory conditions. These includes atherosclerosis and stroke, myocardial infarction, certain types of cancers, diabetes mellitus, allergy, asthma, arthritis, Crohn's disease, multiple sclerosis, osteoporosis, psoriasis, septic shock, AIDS and neurodegeneration. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrate and phenolic compounds[3]. Similarly nutraceutical is a term that is a combination of nutritional and pharmaceutical that refers to the compounds within foods that act as medicines. Many of the secondary metabolites from plants play major role as bioactive and nutraceutical compounds which includes polyphenols, phytosterols, phytates and polysaturated fatty acids. Other major nutraceutical compounds investigated by various workers include carotenoids as antioxidants and polyketides. *Hibiscus sabdariffa* also locally known as 'Mesta' or 'Meshta' on the Indian Subcontinent[4,5] is an erect, gregatious under shrub or annual shrub belonging to the family Malvaceae. Stems are reddish in color, mostly branched, and up to 3.5 m tall, with a deep penetrating taproot. Leaves are variously colored, dark green to red, alternate, glabrous, long petiolate, palmately divided into 3–7 lobes, with serrate margins. Flowers large, short peduncled, red to yellow with dark center. Capsules are ovoid, beaked, hairy, 5 cm long and 5.3 cm wide. This plant has drawn the attention of food, beverage and pharmaceutical manufacturers because of its commercial potential as a natural food and coloring agent that can replace some synthetic products[6]. It is commonly known as Mwitha gaza in Bodo and Mesta tenga or tengamora in Assamese. The plants grows in warm and humid tropical climate during the month of April- August and it is exceptionally susceptible to frost and mist[7,8]. The temperature range within which *H. sabdariffa* thrives is between 18 and 35ºC, with an optimum of 25ºC. Growth of the plant ceases at 14ºC[9]. In tropical and subtropical regions, an altitude 3000 ft. (900 m) above sea level is suitable for growing this plant with an annual rainfall between 400 and 500 mm. Ethno botanical information of *Hibiscus sabrariffa* plant revealed diuretic, diaphoretic, uricosuric, antibacterial, antifungal agent, mild laxative, sedative, antihypertensive, anti tussive, gastrointestinal disorder treatment, hypercholesterolemia treatment, kidney stone treatment, liver damage treatment, agent for decreasing the viscosity of the blood, and agent for treating the after effects of drunkenness[10,11]. It is used to control high blood pressure and its leaves are used as a source of high nutrition. Extractions of *Hibiscus sabdariffa* have been used medicinally to treat colds, toothaches, urinary tract infections and hangovers. The leaves have also been applied as a poultice to treat sores and ulcers, besides being used as an anti scorbutic for the treatment of scurvy, a refrigerant to relieve fevers, an emollient, a diuretic, and a sedative[12]. The Bodo tribes of Bodoland, BTC, Assam (India) consume the leaves of *Hibiscus sabdariffa* cooked along with chicken, fish or pork as one of their traditional cuisines.

Taxonomy of the plant

Kingdom	Plantae
Division	Tracheophyta
Superdivision	Spermatophytina
Infradivision	Angiospermae
Class	Magnoliopsida
Superorder	Rosanae
Order	Malvales
Family	Malvaceae
Genus	*Hibiscus*
Species	*Hibiscus sabdariffa* L.roselle

Vernacular names

Assamese: Tengamora
Nepali: Belchanda
Bodo: Mwitha
Bengali: Chukor, Lal mestha
Telugu: Gongura
English name: Indian Sorrel, Jamaica Sorrel, Natal Sorrel, Red Sorrel, Rosella, Rozelle Hemp.

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, India. 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 0.5 mm 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.

Digestion of plant sample

In plants, the nutrients exist in various organic combinations such as carbohydrates, proteins, fats etc and digestion of plant material releases them in mineral forms.[13] The usual weight of plant sample taken for digestion for total elemental analysis is 0.5 g 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.

For determination of nutrient elements like K, Na, Ca, Mg, Cu, Zn, Fe, Mn, Mo and B which do not volatilize at high temperature, they are usually digested by dry ashing in muffle furnace using suitable silica, porcelain or platinum crucible and extracting them in dilute HNO_3.

Proximate composition

Determination of crude protein

Crude protein was determined by using micro Kjeldahl method. The nitrogen in proteins or any other organic material is firstly converted to nitrogen liberated against standard acid i.e. ammonium sulphate by H_2SO_4 during digestion. This salt on steam distillation liberates ammonia which is collected in boric acid solution and liberated against standard acid. Since 1 ml of 0.1N acid is equivalent to 1.401 mg N, calculation is made to arrive at the nitrogen content of the sample. A blank determination was carried out using the same reagents and total nitrogen content is calculated from the difference between the titrates of blank and test (1 ml of 0.1 NH_2SO_4= 0.0014 g N). Thus the total nitrogen content of the sample can be calculated based on any one of the following formula

$$\text{Ng/Kg} = \frac{(\text{ml of HCl} - \text{ml Blank}) \times \text{normality} \times 14.01}{\text{Weight (g)}}$$

Multiplying total nitrogen value with 6.25 will give the crude protein content.[14]
Thus, Protein % = N% x conversion factor (6.25)

Estimation of Oils and Fats

Crude fat is determined by Mojonnier method[15]. It is determined gravimetrically after extraction with diethyl ether ethoxyethane and petroleum ether from an ammonium alcoholic solution of the sample. About 10 g 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^oC 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[16]. Duplicate determinations are made on each sample of the plant tissue and the results of air dried tissue analysis are then converted to oven dry basis. 20 g of the samples of ground air dried tissue is dried in an oven at 105^oC 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[17]. 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

The inorganic material, which does not volatize at high temperature, is called ash. For determination of ash content, method of AOAC (1984)[18] was followed. According to this method, 10 g of each dried sample are being weighted out in a silica crucible, this crucible is heated in muffle furnace at 300^oC 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 color, 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 is calculated by difference method as described by James (1995) based on Traditional Carbohydrate Determination. Thus,

% of carbohydrates = 100 – (Protein + Ash + Moisture + Fat)

Determination of nutritive value

The total energy value in kcal/100 g was estimated by using the method described by FAO (2003)[19] as shown below,

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

Minerals or 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. 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 atomized and the atoms then absorb radiation of a characteristic wavelength from a hollow-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. All experiments were carried out in triplicate and values were obtained by calculating the average of three experiments using micro soft office excel 2007 format and data are presented as Mean ± SEM[20].

Test for qualitative estimation of bioactive compounds

Powdered samples are either soaked in water for overnight or 5 g of dried samples are boiled in 50 ml of distilled water for 10 minutes and the mixture was filtered and the filterate was taken for the experiments wherever applicable.[21-24]

Test for Tannins

1 g of powdered was boiled with 20 ml distilled water for 5 minute in a water bath and was filtered while hot. 1 ml of cool filtrate was mixed with 5 ml distilled water and few drops of 10 % Ferric chloride was added and observed for any formation of bluish black or brownish green color. Occurrence of bluish black or brownish green color indicated the presence of tannins.

Test for Saponins

Froth test- 1 g of powdered sample was boiled with 10 ml of distilled water for 10 minutes. The mixture was filtered while hot and allowed to cool; then 2.5 ml of filtrate was dissolved in 10 ml of distilled water. The test tube was stoppard and shaken vigorously for 30 seconds. The test tube was allowed to stand in a vertical position and observed for the occurrence of "honey comb" froth above the surface of liquid. Frothing indicates the presence of saponin in the plant sample.

Test for Alkaloids

- Hager's test: - To 1 ml of filtrate, 3 ml of Hager's reagent (Saturated solution of Picric acid) was mixed in it and observed for the formation of a yellow precipitate. Occurrence of yellow precipitate indicated the presence of alkaloids.
- 1 g of powdered sample was boiled with water and 10 ml HCL was dissolved in it. A small quantity of picric acid was added to it. Occurrence of colored precipitate or turbidity indicated the presence of alkaloid.

Test for Flavonoids

- To 1 ml of filtrate was mixed few fragments of Magnesium ribbon and concentrated HCl was added drop wise. The mixture was allowed to stand for a few minutes until fragments of Magnesium ribbon gets completely dissolved in it. Formation of pink scarlet color indicated the presence of flavonoids.
- 1 g of powdered sample was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. After cooling a few drops of 20 % NaOH solution was added to 1 ml of the cool filtrate. A change to yellow color to colorless solution on addition of acid indicated the presence of flavonoids.

Test for Phenol

To 2 ml of filtered solution of the aqueous macerate of the plant material, 3 drops of a freshly prepared mixture of 1 ml of 1 % ferric chloride and 1 ml of potassium ferrocyanide was added. Formation of bluish-green color was taken as positive for presence of phenolic compounds.

Test for Steroids

Salkowski's test: 1 ml of the aqueous solution was dissolved in 2 ml chloroform in a test tube. Concentrated sulfuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown color at the interface indicated the presence of a steroid ring (i.e. the aglycone portion of the glycoside).

Test for Terpenoids

To 1 ml of the filtrate was dissolved 1ml of acetic acid and then a few drops of concentrated Sulphuric acid were allowed to run down the side of the test tube. The appearance of pink or pinkish brown ring or color indicated the presence of terpenoids. The appearance of blue color indicated the presence of steroids.

Test for reducing sugars

Benedict's test- To 0.5 mL of the filterate, 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. Appearance of reddish brown precipitate indicated the presence of sugar.

RESULT AND DISCUSSION

Bioactive compounds are constituents that are found in certain foods. A wealth of scientific literature from numerous types of epidemiological and case controlled studies have identified the potential relationships between bioactive compounds (or "functional" components) and their protective effects against hypertension, cardiovascular disease, cancer, and other health conditions. Phytochemical analysis of the plant extracts of *Hibiscus sabdariffa* (Table 1) revealed the presence of alkaloids, tannins, saponins, flavonoids, phenols and steroids.

Table 1: Qualitative estimation for presence of various phytochemicals in *Hibiscus sabdariffa* leaves

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 value (k.cal/100g) of *Hibiscus sabdariffa* leaves

S. No.	Proximate composition	Result
1.	Protein	15.18
2.	Moisture	91.5
3.	Fat	1.83
4.	Ash	35
5.	Total solids	8.5
6.	Carbohydrates	43.51
7.	Nutritive value	251.23

Table 3: Various amounts of micronutrients by Atomic Absorption Spectrometer (AAS)

S. No.	Specimen name	Elements					
		Zn	Mg	Mo	Cu	Fe	Mn
1.	*Hibiscus sabdariffa*	8.838 ± 0.09	6.433 ± 0.26	ND	0.431 ± 0.03	22.50 ± 0.25	1.986 ± 0.01

Where all concentrations in ppm (parts per million), ND = Not detectable

The presence and effects of these biologically active compounds have been reported in related plants with potential medicinal properties[25-27]. These chemical compounds are known to display inhibitory activities against many microorganisms[28-30]. Herbs that contain tannins are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery[31]. Flavonoids and tannins are antioxidants that improve immune function, prevent heart diseases and cancers[32]. The ingestion of the aqueous solution of saponins is harmless to man[33]. Similarly one of the largest groups of chemicals produced by plants are alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications[34]. Proximate analysis witnessed that the plant is very rich in carbohydrates such as 43.51 % 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 that also contributes to sweetness, appearance and textural characteristics of many foods[35]. The human nutritional requirements obtained from plant kingdom were also described by FAO/WHO (1974)[36]. An appropriate amount of protein in *Hibiscus sabdariffa* leaves were observed as 15.18 %, ash percent of 35 %, moisture percent of 91.5 %, total solids of 8.5 % and fat percent of 1.83 % respectively. 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. 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. The estimated total energy value in the leaves of *Hibiscus sabardiffa* per 100 g was 251.23 kcal/100 g. 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 and copper. These minerals were found in the order of Iron > Zinc > Magnesium >Manganese > Copper (Table 3). The iron content of *Hibiscus sabardiffa* leaves is 22.50 ± 0.25 ppm which is quite high than some cultivated vegetables such as spinach (1.6 mg/100 g)[37] and its daily intake in our diet could help in boosting the blood level especially in anaemic conditions. The content of zinc and manganese were 8.838 ± 0.09 ppm and 1.986 ± 0.01 ppm which is adequate when compared with the recommended dietary allowances. Manganese acts as activator of many enzymes 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 (6.433 ± 0.26 ppm) is therefore adequate and hence could be of advantage in the improvement of healthy conditions of an individual. The content of copper in *Hibiscus sabardiffa* leaves was found to be 0.431 ± 0.03 ppm. Copper plays an important role in haemoglobin formation and also contributes in iron and energy metabolism. Thus from the above study it is clear that the plant *Hibiscus sabdariffa* can be used as wild food plants and potential source of drugs.

CONCLUSION

The plants studied here can be seen as a potential source of useful drugs. It justifies the folklore medicinal uses and the claims about the therapeutic values of this plant as curative agent and we therefore suggest further the isolation, identification, purification, characterization and elucidation of the structure of the bioactive compounds of *Hibiscus*

sabdariffa L. that would be obtained with a view to obtain useful chemotherapeutic agent.

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