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Bioactive and Nutraceutical Compound Manipulation from the Leaves of Some Wild Edible Medicinal Plants in Chirang District of Assam, India.

Jahnovi Brahma*[1] and Dhananjoy Narzary[2]

[1]Department of Botany, Science College, Kokrajhar, India
[2]Department of Botany, Kokrajhar Govt. College, Kokrajhar, India

***Corresponding author e-mail:** jahnovibrahma6@gmail.com

ABSTRACT

Background: Wild edible plants are important sources of minerals, fibers and vitamins with nutritional factors that could help prevent and treat several important diseases. Although the potential of biodiversity in Chirang district has been long recognized, yet a systematic effort to record individual plant species with defined bio-active molecules has been lacking. Since this district is rich in ethnic community, traditional knowledge may reveal many socio economically important plants with interesting uses and relationships.
Objectives: The study attempts to highlight the nutritional value and phytochemical analysis of the wild edible food plants consumed by the Bodo communities of Chirang district. The study of nutritional value may reveal the knowledge about its edibility, habitat, distribution, harvesting time and uses of the traditionally used wild edible plant species which is still maintained among the Bodo communities of Chirang district.
Method: Extensive and intensive ethno botanical surveys were conducted in different regions and forest fringe areas of Chirang district. Determination of total fats, proteins, phytochemicals and trace elements along with reducing sugars were done both in aqueous extract and on powdered specimen using standard procedures in the leaves of some wild edible medicinal plants like *Lippia alba, Lasia spinosa, Gonostegia hirta, Blumea lanceolaria, Hibiscus sabdariffa, Hibiscus cannabinus, Cayratia trifolia* and *Ipomoea aquatica* consumed by the Bodos of Chirang district. Information from villages were gathered with the help of local interpreter by consulting village elders through informed semi structured questionnaire.
Result: The results of the phytochemical studies revealed the presence of phenols, alkaloids, saponins, tannins, steroids, flavonoids, terpenoids and cardic glucoside in most of the samples. Nutritive values and the presence of these phytochemicals proves that these plants are potential medicines. Data for relative mineral concentration from each plant when compared shows that all these plants plays an

important role in traditional medicine system and can be used as potential sources of good health support for humans.

Conclusion: This study attempts to highlight the nutritional value and phytochemical analysis of the wild edible food plants consumed by the Bodo communities of Kokrajhar district. The study of nutritional value may reveal the knowledge about edibility, habitat, distribution, harvesting time and uses of the traditionally used wild edible plant species which is still maintained among the Bodo communities of Kokrajhar district.

Keywords- Phytochemicals, Mineral analysis, Leaves, Medicinal plants, Bodos, Cirang district.

INTRODUCTION

Nutraceutical is a term which is formed by the combination of 'nutritional' and 'pharmaceutical' word that refers to the compounds within foods that acts as medicines. Many secondary metabolites derived from plants play major roles as bioactive and nutraceutical compounds[1]. Food plants such as culinary herbs and spices are known to contain myriad phytochemicals with medicinal properties[2]. Phytochemicals are not essentially considered necessary for normal body function as their absence does not lead to deficiency conditions like those of conventional vitamins, but are associated with the prevention of chronic diseases associated with aging as it acts individually or synergistically and help reduce the risk of a variety of chronic and inflammatory conditions. These include atherosclerosis and stroke, myocardial infarction, cancers, diabetes mellitus, allergy, asthma, arthritis, Crohn's disease, multiple sclerosis, Alzheimer's disease, oesteoporosis, psoriasis, septic shock, AIDS, menopausal symptoms and neurodeneration. On a molecular level, phytochemical effects include the suppression of growth factor expression or signaling, activation of apoptosis, downregulation of antiapoptotic proteins, suppression of phosphatidy-linositol-3- kinase, signaling pathways and downregulation of aniogenesis through inhibition. Tanins are water soluble oligomers rich in phenolic groups capable of binding or percipitating certain water soluble proteins[3]. They are bitter in taste and their presence in plants could quicken the healing of wounds and burns[4]. Alkaloids are plant derived pharmacologically active, basic compounds derived from amino acids that contain one or more heterocyclic nitrogen forms. They are used as basic medicinal agents due to their chemoprotective, analgesic, antiplasmodic and anti- bacterial properties[5]. Flavonoids are potent water soluble antioxidant free radical scavengers which prevents oxidative cell damage, have strong anticancer activity and protect against carcinogenesis[6]. Steroids are hydroxylated compounds having profound importance as hormones, coenzymes, provitamins and are effective against cardiovascular disease[7].

Phenolic compounds are also secondary metabolites which are synthesized in plants, possess biological properties such as antioxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammatory, anti-atherosclerosis, cardiovascular protection, improvement of the endothelial function as well as inhibition of angiogenesis and cell proliferation activity[8]. Likewise cardic glycosides are organic compounds

containing a glycoside (sugar) that acts on the contractile force of the cardic muscle and helps in disrupting the function of the heart.

Similarly, trace elements like magnesium exists primarily as an intracellular constituent in the body and its requirement is estimated to be 0.2-0.6% in the dry weight of animals[9]. Copper plays an important role in haemoglobin formation and it contributes to iron and energy metabolism. Similarly molybdenum is a key component in many biochemical processes which 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 sulfates which would be a great relief for people who suffer from asthma attacks due to reactions to sulfates[10]. Intake of iron in our diet could help boosting in blood level especially in anemic conditions. Similarly, manganese acts as activator of many enzymes while zinc is involved in normal functioning of the immune system. Thus, the present work provides a preliminary investigation on the presence of phytochemicals and mineral concentrations from the extract of leaves of some wild edible medicinal plants extensively consumed as food and medicine sources by the Bodo tribes.

MATERIALS AND METHODS

All the reagents used for this analysis are of analytical grade. The major experimental plant materials used were the leaves of eight wild edible samples namely: *Lippia alba, Lasia spinosa, Gonostegia hirta, Blumea lanceolaria, Hibiscus sabdariffa, Hibiscus cannabinus, Cayratia trifolia* and *Ipomoea aquatica.* The study site is Chirang district which is one of the four districts of Bodoland territorial area located in the North eastern part of Assam. It falls under the Haltugaon forest division of Western Assam circle conservancy and lies in between 26°30'N latitude and 90°60'E longitude covering an area of 592.54 sq.km (Fig. 1). Plant specimens were identified by BSI, Shillong and voucher specimens were deposited at the Department of Forestry, North Eastern Regional Institute of Science and Technology, Itanagar.

Preparation of samples

The plant samples (leaves) were collected, washed several times thoroughly with distilled water or double distilled water and then shade dried at 25±2°C for one week. The dried plant samples were ground into powder using a medium kitchen blender and stored in airtight containers at room temperature. 100 g of each powdered sample was exhaustively extracted by soaking them in 200 ml of distilled water for 12 hours and the extracts were filtered using Whatman Filter Paper No 100. Phytochemical screening and mineral elements were analysed as described below:

Phytochemical screening

Phytochemical screening was done in aqueous extract and on powdered samples following standard procedures to trace the chemical constituents present in the samples[11-14].

Test for Tannins

The powdered sample (1gm) was boiled with 20ml distilled water for 5 minute in a water bath and then filtered while hot with Whatman filter. 1ml of this cool filtrate was then mixed with 5ml distilled water and few drops of 10% Ferric chloride was added and observed for any formation of bluish black or brownish green colour. The occurance of bluish or brownish green colour indicates the presence of tannins.

Test for Saponins

1gm of powdered sample was boiled with 10ml of distilled water bath for 10 minutes. The mixture is then filtered while

hot and allowed to cool. Then 2.5 ml of this filtrate was diluted with 10ml of distilled water, then the mixture was shaken vigorously for 2 minutes and then observed on standing for stable brake. Frothing indicates the presence of saponin in the filtrate.

Test for Alkaloids

1. Hager's test:- 1ml of aqueous filtrate was taken and to this 3ml of Hager‟s reagent (Saturated solution of Picric acid) was mixed and observed for the formation of a yellow precipitate. Occurance of a yellow colour indicated the presence of alkaloids.

2. 1 gm of powdered sample was boiled with water and to this 10 ml of dilute HCL was dissolved in it. Then a very small quantity of picric acid was mixed. The occurance of a 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 until a pinkish colour appers. 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 then filtered while hot. Few drops of 20% NaOH solution was added to this 1 ml of the 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 the filtrate was taken, then freshly prepared 1% ferric chloride and 1 ml of Potassium ferrocyanide was added to the filterate. Formation of bluish green colour indicated the presence of phenol.

Test for Terpenoids

1. Salkowski test: - The aqueous extract of the sample solution was mixed with 2ml of chloroform and then a few drops of concentrated Sulphuric acid (H_2SO_4) is carefully added to drop by drop to form a layer. It is then shaken and allowed to stand for some time. Formation of yellow coloured upper layer indicated the presence of terpenoids.

2. 1 ml of the filtrate was dissolved in 1 ml of acetic acid to which is added a few drops of concentrated Sulphuric acid allowing to run down the side of the test tube. The appearance of pink or pinkish brown ring or pinkish colour indicated the presence of terpenoids.

Test for Steroids

1. 2ml of acetic anhydride was added to the extract. To this extract 2ml of concentrated sulphuric acid was added. The change of colour from violet to blue or green colour indicated the presence of steroids.

2. Salkowski test: - The aqueous extract was first mixed with 2ml of chloroform and then a few drops of concentrated Sulphuric acid (H_2SO_4) is carefully added drop wise to form a layer, then shaken well and allowed to stand for some time. Appearance of red colour indicated the presence of steroids.

Test for reducing sugars

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

2. A small fraction of the extract were added vigorously with 5ml of distilled water and then filtered. To this filterate is added an equal volume of Fehling‟s solution A and B and then shaken vigorously. Occurance of a brick red precipitation indicated the presence of reducing sugars.

Test for cardiac glycoside (Killer-killani test)

5 ml of each extract was treated with 2 ml of glacial acetic acid, to this is added one drop of ferric chloride solution to which was added 1 ml of concentrated tetraoxosulphate acid. Appearance of a brown ring at the interface indicates a de-oxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just gradually throughout the thin layer. Occurance of a greenish ring indicated the presence of cardic glycoside.

Determination of mineral composition

The mineral elements are suitably estimated by Atomic Absorption Spectrophotometer (AAS) since their atoms do not get easily excited under ordinary flames[15]. The principle of the method is based on nebulising a sample solution into an air acetylene flame where it is vaporized. Elemental ions were 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[16].

RESULTS AND DISCUSSIONS

The present study carried out in the plant samples reveals the presence of many bioactive compounds. Curative properties of these wild edible plants are perhaps due to the presence of flavonoids, phenols, sterols, terpenoids etc. The results of phytochemical analysis (Table 1) reveals that the species *Hibiscus sabdariffa, Lippia alba, Blumea lanceolaria, Cayratia trifolia, Hibiscus cannabinus* and *Lasia spinosa* showed the presence of high amount of alkaloids, saponins, steroids, flavonoids, phenolic compounds and tannins. Reducing sugars is found to be present in trace amounts in the species of *Gonostegia hirta, Lippia alba* and *Ipomoea aquatica.* Analysis of phytochemical constituents of the selected plant specimens reveals the presence of alkaloids, tannins, terpenoids, phenols, saponins, steroids, flavonoids, small amounts of reducing sugars and cardic glucosides. All these compounds are natural disease preventing, health promoting, antioxidant substances that serve the task of reducing oxidative damage in humans induced by free radicals and oxidative stress conditions. The importance of this phytochemicals in various antibiotics used in treating common pathogenic strains has recently been reported by many[17, 18]. It was earlier recorded that bitter leaf contains an alkaloid which is capable of reducing headaches associated with hypertension[19, 20]. Similarly medicinal importance of tannins and saponins which are components of traditional herbal preparations are used in managing various common ailments[21, 22]. Similarly, steroidal compounds are of utmost importance due to their relationship with compounds such as sex hormones[23]. Thus, phytochemical analysis of these wild edible plant species showed the presence of essential substances which can compete favorably well with other conventional edible leaves that could fulfill the growing demands of plant based foods for human nutrition.

Mineral composition of the above selected 8 different wild species (Table 2) showed that they contained zinc in the range

of 0.359 to 10.96 ppm, magnesium in the range of 1.1 to 6.43 ppm, molybdenum in the range of 1.18 to 6.57 ppm, iron in the range of 0.286 to 0.551 ppm, copper in the range of 15.97 to 27.44 ppm and manganese in the range of 0.42 to 3.447 ppm.

The magnesium content was found to be highest in *Hibiscus sabdariffa* (6.433 ppm) followed by *Lasia spinosa* (6.228 ppm) and *Cayratia trifolia* (4.1 ppm). The copper content was found to be highest in *Lippia alba* (0.551 ppm), *Gonostegia hirta* (0.486 ppm) and *Hibiscus sabdariffa* (0.43 ppm). Highest amount of molybdenum was found in *Hibiscus cannabinus* (6.58 ppm) followed by *Gonostegia hirta* (5.4 ppm). Manganese content was found highest in *Ipomoea aquatica* (3.44 ppm) followed by *Lippia alba* (3.34 ppm). Iron content was found to be high in *Lippia alba* (27.4 ppm), *Hibiscus cannabinus* (22.99 ppm) followed by *Hibiscus sabdariffa* (22.47 ppm).

Presence of high iron content in this wild food plants indicates that its daily intake in our diet could help in boosting the blood level especially in anaemic conditions.

CONCLUSION

The following conclusions are drawn from the present studies:

1. The wild edible plants serve as an important source of non-conventional food sources for the indigenous Bodo communities of Kokrajhar district.

2. The district is found to be a homeland to a large number of wild edible plants that are distributed in agricultural lands, marginal lands, waste lands and forest areas of the district which are selected and utilized with rich traditional knowledge systems by the Bodo communities.

3. Phytochemical analysis have shown that the species such as *Hibiscus sabdariffa*, *Cayratia trifolia*, and *Lasia spinosa* showed high amounts of secondary metabolites. Similarly the content of trace elements are found to be highly variable in all the selected wild edible plants. *Hibiscus sabdariffa, Lasia spinosa* and *Cayratia trifolia* showed high magnesium content, *Lippia alba*, *Gonostegia hirta* and *Hibiscus sabdariffa* showed high copper content. Molybdenum was found to be high in *Hibiscus cannabinus,* similarly *Ipomoea aquatica* and *Lippia alba* showed high manganese content and iron content was found to be preferably high in *Lippia alba.*

Thus from the following studies it can be specified that conservation and promotion of rich traditional knowledge systems of the tribal communities should be preserved and extensive quantitative phytochemical and pharmaceutical studies on selected nutritionally and medicinally valuable species should be done.

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Table 1. Secondary metabolites and reducing sugars in leaves of selected wild edible plants.

SL. no	Scientific name	PHYTOCHEMICALS								
		Tannin	Saponin	Alkaloid	Flavonoid	Phenol	Steroid	Terpenoid	Sugars	Glucoside
1.	*Lippia alba*	++	++	-	-	++	++	+	+	+
2.	*Lasia spinosa*	+	++	++	++	++	++	++	-	-
3.	*Gonostegia hirta*	++	++	-	-	++	-	+	++	+
4.	*Blumea lanceolaria*	+	++	+	++	++	-	++	-	-
5.	*Hibiscus sabdariffa*	++	++	+	++	++	++	++	-	+
6.	*Hibiscus cannabinus*	++	++	++	-	++	-	++	-	-
7.	*Cayratia trifolia*	++	+	++	+	++	-	++	-	-
8.	*Ipomoea aquatica*	++	+	++	-	++	-	++	+	++

Table 2. Determination of various amounts of micronutrients by Atomic absorption Spectrometer (ASS).

Botanical name	E L E M E N T S					
	Zn	Mg	Mo	Cu	Fe	Mn
Lippia alba	0.783	1.10	ND	0.551	27.44	3.348
Lasia spinosa	7.433	6.221	1.178	0.3156	17.0405	1.332
Gonostegia hirta	9.56	3.26	5.393	0.486	21.96	2.996
Blumea lanceolaria	10.96	2.571	3.034	0.301	15.97	2.432
Hibiscus sabdariffa	8.827	6.43	ND	0.4305	22.474	1.983
Hibiscus cannabinus	6.377	3.0345	6.572	0.286	22.99	0.4235
Cayratia trifolia	5.107	4.1023	ND	0.2906	21.56	1.64
Ipomoea aquatica	0.359	1.1287	ND	0.1678	22.39	3.447
Mean	6.2	3.5	2.022	0.354	21.47	2.2
Standard deviation	3.7	1.9	2.1	0.117	3.4	1.0

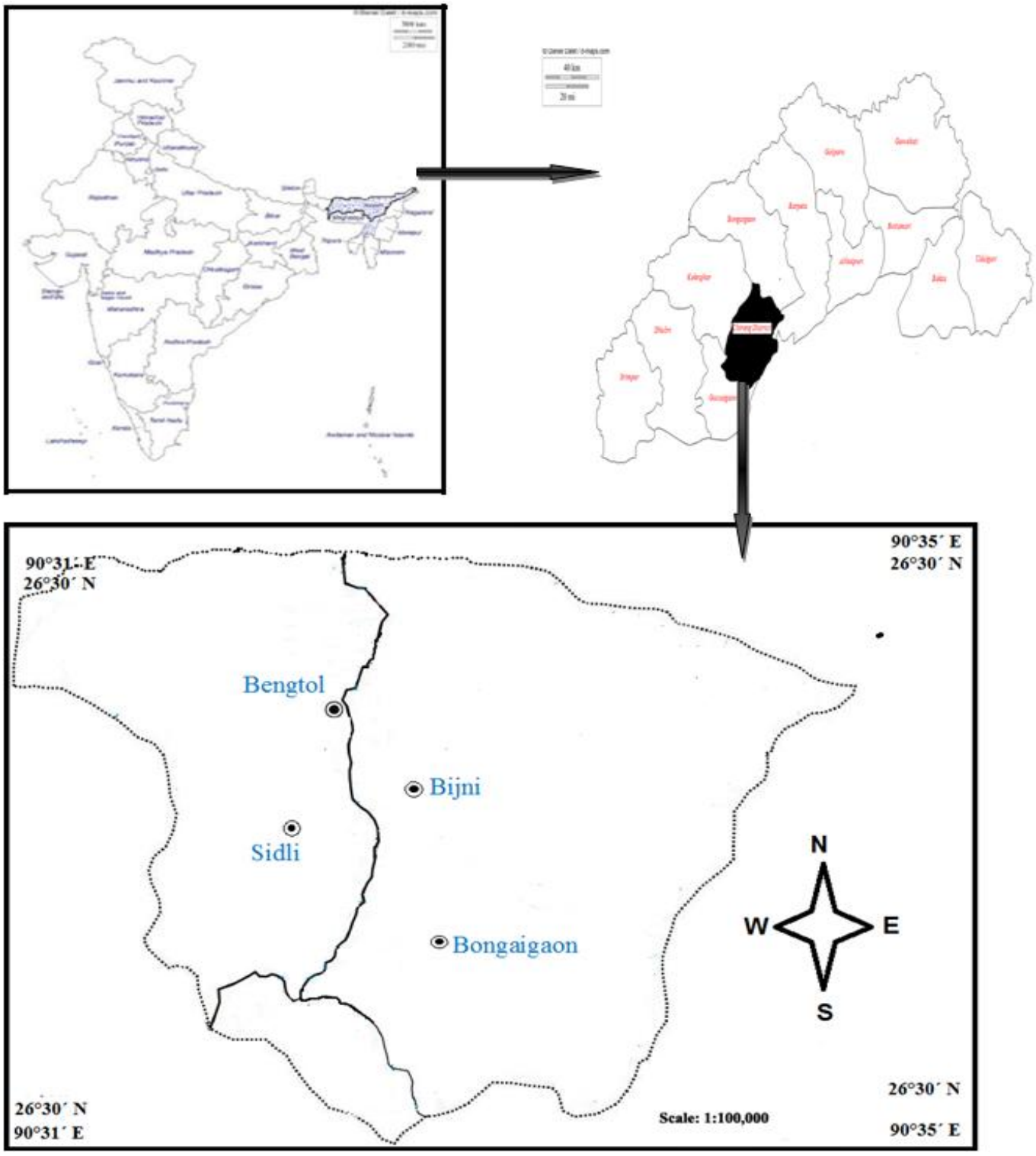

Fig. 1: Map of the study site