International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.6, No.2, pp 1405-1412, April-June 2014

Qualitative Estimation of the Presence of Bioactive and Nutritional Compound in *Lasia Spinosa*: An Important Vegetable Plant used by the Bodos of Kokrajhar District.

Jahnovi Brahma[1]*, Sharmistha Chakravarty[2] & Dr. P Rethy[3]

[1]Department of Botany* ,Science College, Kokrajhar, Assam, India;
[2]Department of Zoology, Science College, Kokrajhar, Assam, India;
[3]Department of Forestry, NERIST, Itanagar. Arunachal Pradesh, India.

***Corres.author: jahnovibrahma6@gmail.com**

Abstract: Qualitative phytochemical analysis of this plant confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, terpenoids, saponin, steroids, proteins, reducing sugars, carbohydrates and trace elements. This paper deals with the significance of these phytochemicals with respect to the role of these plants in traditional medicinal system. Nutritive values, protein, moisture, fats, ash, total solids, carbohydrate was estimated. Nutritional analysis of *Lasia spinosa* showed that Protein 17.6 kcal/100g, moisture 83 kcal/100g, fats 1.16 kcal/100g, ash 34 kcal/100g, total solids 17 kcal/100g, carbohydrate 35.7 kcal/100g and nutritive value 224 kcal/100g were present. Micronutrients like Zinc 7.44 ppm, Magnesium 6.22 ppm, Molybdnum 1.18 ppm, Copper 0.31 ppm, Iron 17.06 ppm and Manganese 1.33 ppm were present.This preliminary study draws attention to the need for further studies of the active principles identified in the reported species for the treatment of many diseases in order to understand their mode of action in controlling diseases.
Keywords: Phytochemical, *Lasia Spinosa*, Nutritional analysis.

INTRODUCTION

Phytochemicals are the natural bioactive compounds found in plants. These phytochemicals work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions[1]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, steroid, terpenoid, carbohydrate and phenolic compounds[2]. *Lasia spinosa* commonly called as 'Sibru' by the Bodo tribes of Assam is a member of the family Araceae. The plant is a stout, marshy plant with a creeping spiny rhizome. It is a large herbaceous plant that can grow up to 2 m tall; leaves arise from the base and are arranged like a rosette. Each leaf is divided into several lobes and numerous small arms called the leaf stalk along the veins found at the back of leaves. Plants grow and spread via underground stems which are rich in starch. It is largely a plain green plant and is saved by its attractive, curious-looking inflorescence. Typical of an aroid, the real flowers are borne on a spike-shaped spadix. Unlike most other aroid species, the spathe is not sail-like and the tip of the spathe is long and extended, twists along its length and held erect above the plant. Fruits of this plant are each aggregated on a club-like structure. The tender leaves and rhizomes are used as vegetables and are recommended for a variety of diseases in Ayurvedic medicine.

The plant is grown or cultivated in marshy areas, muddy streams and swampy grounds[3]. In the indigenous system of medicine the plant is recommended for a variety of disorders such as cholic, rheumatism, intestinal disorders. In addition, stalk and leaves demonstrate profound anticestodal efficacy. Rhizome is used in the treatment of lung inflammation, bleeding cough and the whole plant is used for in uterine cancer[4].

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 *Lasia spinosa* as the cheapest source of multivitamins. This study therefore focuses on the phytochemical screening, mineral analysis and the proximate analysis of *Lasia spinosa* leaves with a view to assessing its nutritional potentials in relation to its ethno medicinal uses.

A. Scientific Classification

Kingdom : Plantae

Phylum : Tracheophyta

Class : Liliopsida

Order : Arales

Family : Araceae

Genus : Lasia

Species : Spinosa[5]

MATERIALS AND METHODS

1. Sample collection

Fresh plant samples were collected from the field; fresh tissues 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 ^{o}C± 2^{o}C 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[6].

2. 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[7]. 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 ^{o}C 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.

3. Proximate composition

a) Determination of crude protein

The crude protein was determined using micro Kjeldahl method[8]. 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 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-

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

Protein content will be estimated by conversion of nitrogen percentage to protein[9]. Thus,

Protein % = N% x conversion factor (6.25)

b) Estimation of Oils and Fats

Crude fat was determined by Mojonnier method[10].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,

% 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)

c) 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[11]. 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^{o}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[12]. Hence,

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

d) Determination of Total solids

Total solids were estimated by deducting moisture percent from hundred as described by James (1995)[10]. Therefore,

% of total solids = 100 - percentage of moisture

e) Determination of Ash content percentage

For determination of ash content, method of AOAC (1984)[13] was followed. According to this method, 10 gm of each sample will be weighted out in a silica crucible, this crucible was heated in muffle furnace at 300°C for one hour, and then it was 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-

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

f) Determination of Carbohydrates

Determination of available carbohydrates in the sample was calculated by difference method as described by James (1995)[10] based on Traditional Carbohydrate Determination. Thus,

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

g) Determination of nutritive value

The total energy value in kcal/100g was estimated by using the method described by FAO (2003)[14] as shown below:

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

4. 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 were 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[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 then atomised and the atoms then absorb radiation of a characteristic wavelength from a hallow-cathode lamp. The absorbance was measured, which was proportional to the amount of analyte 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].

5. Test for qualitative estimation of bioactive compounds[17- 21]

After shade drying the dried plant samples were powdered in a mixer grinder and the dried powder was 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.

a. 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.

b. 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.

c. 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.

d. 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.

e. Test for Phenol

2 ml of filtrate was taken, and 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.

f. Test for Steroids and Terpenoids

1. Salkowski test: 1 ml of filtrate was mixed with chloroform and few drops of concentrated Sulphuric acid was 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 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.

g. Test for reducing sugars

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

RESULT AND DISCUSSION

The result of qualitative analysis of *Lasia spinosa* 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 35.74% 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[22].

Table 1: Qualitative Analysis of the Phytochemical Analysis of Lasia Spinosa,

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

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

An appropriate amount of protein in *Lasia spinosa* plant products were observed as 17.6%, ash percent 34%, moisture percent 83%, total solids 17% and fat percent of 1.16% which is showed in Table 2. FAO/WHO (1974)[22] 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[23,24]. Similarly total solids were measured as the amount of material dissolved in water such as carbonate, bicarbonate, chloride, sulphate, phosphate, nitrate, calcium, magnesium, sodium, organic ions and other ions[25]. The estimated total energy value in the leaves of *Lasia spinosa* per 100g was 224.04 kcal/100g. This high calorific value is an indication that it can be recommended to individuals suffering from overweight and obesity.

The inorganic mineral analysis of the leaves showed that it contained magnesium, iron, zinc, manganese, copper and molybdenum. These minerals were found in the order Iron>Zinc > Magnesium > Molybdenum > Manganese > Copper which is showed in Table 3. The iron content of *Lasia spinosa* leaves is 17.06±0.87ppm which is quite high than some cultivated vegetables such as spinach (1.6mg/100g)[26]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 7.442±0.01ppm and 1.334±0.08ppm which is adequate when compared with the recommended dietary allowances[27]. Manganese acts as activator of many enzymes while zinc is involved in normal functioning of immune system[28]. 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 (6.228±0.11ppm) is therefore adequate and hence could be of advantage to the improvement of healthy conditions of an individual[29] .The content of copper and molybdenum in *Lasia spinosa* leaves is found to be 0.316±0.02ppm and 1.180±0.06ppm. 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 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[30]. From the above study it is clear that consumption of the plant *Lasia spinosa* is adequate to supply the daily nutrient requirements for children and lactating mothers.

Table 2: Proximate Composition and Nutritive Quantity alongwith Trace Elements (K.Cal/100g) is given as Follows.

Sl.No.	Proximate composition	Result (kcal/100g)
1.	Protein	17.6
2.	Moisture	83
3.	Fat	1.16
4.	Ash	34
5.	Total solids	17
6.	Carbohydrates	35.74
7.	Nutritive value	224.04

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.	Lasia spinosa	7.442±0.01	6.228±0.11	1.180±0.06	0.316±0.02	17.06±0.87	1.334±0.08

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 *Lasia spinosa* that would be obtained with a view to obtain useful chemotherapeutic agent.

ACKNOWLEDGEMENT

The authors are grateful to the guidance of Dr. P Rethy, NERIST, Nirjuli, Itanagar for providing their support in doing studies.

REFERENCES

1. D Koche, R Shirsat, S Imran. Phytochemical screening of eight traditionally used ethnomedicinal plants from Akola district (ms) India. Int J Pharma Bio Sci. 2010; 1(4):.
2. J Pascaline, M Charles, Lukhoba C. Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district Kenya. J Animal & Plant Sci, 2011; 9(3): 1201-1210.
3. Jayaweera DMA. Medicinal plants (indigenous and exotic) used in Ceylon. Part I. Colombo: National Science Council of Sri Lanka,1981, 134-135.
4. The Wealth of India, An Encyclopedia of India's Raw Material Resources, 1992, ISBN:81-85038-00-7(SET).
5. Gupta, A.K. 2011. *Lasia spinosa*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2.
6. D.K. Bhandari, J.C. Sharma, S.S. Dahiya and M.L. Batra, Research Methods in Plant Sciences:Allelopathy. Volume4. Plant Analysis, Scientific Publishers (India),Jodhpur(2007) (pp.39-48).
7. O.P.Sangwan, Deepak Khatak and D.S. Dahiya, Research Methods in Plant Sciences: Allelopathy.Volume 4. Plant Analysis, Scientific Publishers (India),Jodhpur(2007) (pp.49-62).
8. Matissek, R., F.M. Schnepel and G. Steiner, 1989. Lebensmittelanalytik. Springer Verlag, Berlin,Heidelberg, New York, London, Paris, Tokyo, pp: 440.
9. AOAC. 1984. "Official Method of Analysis".14th ed. Assoc. Off. Anal. Chem. Washington, D.C.James CS (1995). Analytical chemistry of food. Seale-Hayne Faculty of Agriculture, Food and Land use,Dept. Agric. Food stud. Uni. Polymouth, UK. 1: 96-97.
10. James, C.S., 1995. Analytical Chemistry of Foods. 1st Edn. Chapman and Hall, New York.
11. Wildey, S.A.; Corey, R.B.; Iyers, T.G. and Voigt, G.K. (1979). Soil and Plant analysis for tree culture, Oxford and IBH Publishing Co., New Delhi.
12. AOAC (2000). Official Methods of Analysis 17th Edition. Association of the Analytical Chemists. Inc.Virginia, USA.Edition. Association of the Analytical Chemists. Inc. Virginia, USA.
13. AOAC, 1984. Official Methods of Analysis. 14th Edn., Association of Official Analytical Chemists,Washington, DC., USA., pp: 522-533.
14. FAO, 2003. Food energy-methods of analysis and conversion factors. Food and Nutrition Paper 77, Food and Agriculture Organization of the United Nations, Rome, Italy, pp: 1-93.
15. Ojeka, E. O. and Ayodele, J. T. Determination of chromium, copper, lead and nickel in some Nigerian vegetables oils. Spectrum 1995; 2(1&2): 75 – 78.
16. S.S. Narwal, and O.P. Sangwan. Research Methods in Plant Science: Allelopathy.Plant Analysis 2007; 4.
17. Ajayi IA, Ajibade O, Oderinde RA. Preliminary phytochemical analysis of some plant seeds. Res.J.Chem.Sci. 2011;1(3):58-62.
18. De S, Dey YN. Phytochemical investigation and chromatographic evaluation of the different extract of tuber of *Amorphaphallus paeonifolius*. Int J Pharm Biomed Res 2010; 1(5): 150-157.
19. Soni H, Sharma S, Patel SS, Mishra K, Singhai AK. Preliminary phytochemical screening and HPLC analysis of flavonoid from methanolic extract of leaves of *Annona squamosa.* Int Res J Pharm 2011; 2(5): 242-246.

20. Bekele T. Antidiabetic activity and phytochemical screening of crude extract of *Stevia redaudiana* Bertoni and *Ajuga remota* Benth grown in Ethiopia on alloxan induced diabetic mice. Thesis submited on 2008. Dept of pharmaceutical chemistry, School of Pharmacy, Addis aba ba University.
21. Kantamreddi VSSN, Lakshmi YN, Kasapu VVVS. Preliminary phytochemical analysis of some important indian plant species. Int J Pharma Bio Sci 2010; 1(4): 351-357.
22. FAO/WHO, 1974. Hand book on Human Nutritional Requirements, FAO Nutritional Studies, 28: 63-64.
23. Jain , N., R.K. Shahid and S.M. Sondhi. Analysis for mineral elements of some medicinal plants.Indian Drugs 1992; 29: 187-190.
24. Nielsen, S.S., 1998. Introduction to Food Analysis techniques. Text Book. Aspen Publishers, USA.
25. American Public Health Association, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., 127-131.
26. Turan, M., S.Kordali, H.Zengin, A. Dursun and Y.Sezen. Macro and micro mineral content of some wild edible leaves consumed in Eastern Antolia. Acta Agric. Scand. Section B Plant Soil Sci. 2003; 53: 129-137.
27. World Health Organization (1999): Management of severe malnutrition: A manual for physicians and other senior health workers. Geneva, p4.
28. McDonald, P.,Edwards, R.A., Greenhill, F.D. and Morgan, C.A.(1995): Animal nutrition . Prentices Hall, London.
29. Maynard, L.A., Loosil, J.K. Hintx, H.F. and Warner, R.G.(1979): Animal nutrition. 7th edition. Tata McGraw-Hill publishing company limited, New Delhi. Pp240-242.
30. Kisker, C., Schindelin, H., Baas, D., Retey, J., Meckenstock, R.U. and Kroneck. P.M.H. (1999). Structural comparison of molybdenum cofactor-containing enzymes. FEMS Microbiol Rev 1999; 22 (5): 503-521.

*****