



Research Article

EVALUATION OF ANTIOXIDANT AND PROXIMATE COMPOSITIONS OF THE LEAF EXTRACT OF *GNETUM GNEMON* L.

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ABSTRACT

Gnetum gnemon is an important leafy vegetable among the tribal people of the state. The leafy vegetables consist a notable amount of proximate constituents as well as the mineral contents. Therefore the present study deals with the investigation of preliminary phytochemical investigation, antioxidant as well as the proximate composition of the leaf of *G. gnemon*. The study revealed that the leaf methanolic extract and the water extract shows the presence of significant amount of secondary metabolites. The methanol extract was recorded highest DPPH scavenging activity comparable to the standard ascorbic acid. The investigation of proximate and mineral content proves that the leaf of *G. gnemon* is a good source of nutrients.

Keywords: Proximate, Minerals, Secondary metabolites, *Nyishi*

INTRODUCTION

The lofty mountainous state of India, Arunachal Pradesh popularly called “land of rising sun” is exceedingly diverse in geology, topography, climate and flora and fauna often referred to as “Paradise of the Botanist”. The state exhibits a plethora of trees, shrubs, herbs, epiphytes, ferns, cryptogams and houses numerous rare, endangered, and endemic species. The state with 82% of forest cover (broadly tropical, subtropical, temperate, sub temperate, alpine) which have flavored luxuriant flora¹. The region falls within ten threatened biota “hot spots” of tropical forest in the world and also in the 25 biodiversity hotspots in the world².

The documentation of the medicinal plants has been a continuous process throughout the globe. Among the total recorded (2, 97,000 – 5, 10,000) plant species in the world³, about (10-20) % is used as medicine in treatment of various diseases⁴. Arunachal Pradesh, a botanical paradise is tucked in the Eastern Himalaya and well known for its rich bio resources and ethnocultural diversity. The use of medicinal plants in the treatment and ailment of various diseases in Arunachal Pradesh has been in practice since time immemorial. In the last few decades, there has been worldwide revival on the use of the medicinal or herbal plant drugs/phytochemicals in diverse areas including medicine, nutrition etc. As per as documentation of medicinal and aromatic flora of the state is concerned, scanty works have been done till date. Among them Joseph⁵ from Siang district, Tiwari *et al.*⁶ from Tirap district, Das⁷ from Siang valley, Haridashan⁸ from whole state, etc were the pioneers of ethnobotanical research in Arunachal Pradesh. Among the important medicinal plant species *G. gnemon* is an important one which is used as leafy vegetable among the tribal people of the state. The young twigs are also available in the local market. *Gnetum* is the lone genus in the

family Gnetaceae consisting about 30 species. The majority of the species of *Gnetum* is lianas. The plants are dioecious, with the male plants producing catkins of stamens and the female’s catkins of ovules barely protected by an envelope⁹. From an evolutionary perspective, *G. gnemon* is interesting taxa, whose origin and relationship to angiosperms are not completely understood. Chamberlien¹⁰ wrote that the Gnetales during the upper Cretaceous as a branch of the Coniferales, which in turns evolved from the Pteridophytes (fern). However, because *G. gnemon* and other members of the order (*Ephedra* and *Welwitschia*) have some characteristics also found in the angiosperms (for example, leaves that look like angiosperms leaves), some botanist believe that the Gnetales are the ancestors of the angiosperms¹⁰. The young leaves are used as a vegetable by the local people of the state.

Medicinal plants provide the primary health care service to the countryside people because they are having potential source of primary & secondary metabolites¹¹. The metabolites like alkaloids, phenols, terpenoids, flavonoids etc are the secondary metabolites which are not directly involved in the physiology of the plant¹² whereas the mineral contents (both micro and macro), vitamins, crude fiber etc are the primary metabolites which are directly involved in the physiology of the plant body. The analysis of the crude fat, crude fiber, crude protein, ash content etc. are called as the proximate composition of medicinal and edible plants play a crucial role in assessment of the nutritional significance¹³. Therefore the present study deals with the preliminary phytochemical investigation, nutritional and antioxidant activity of the leaf of *G. gnemon*.

MATERIALS AND METHODS

The plant material was collected from Rajiv Gandhi University Botanic Garden, Arunachal Pradesh. It was identified with the

help of the flora of Arunachal Pradesh. The herbarium was prepared following the methodology of Jain and Rao¹⁴ and the voucher specimen (Voucher No: PB/RGUH/2016/23) was deposited to Rajiv Gandhi University. Important ethnobotanical utilization was recorded with the consultation of local people.

Plant material

The leafy plant material were collected and washed thoroughly in running water to remove dust particles. Leaves were then cut into small pieces and dried in hot air oven at 35^oc for overnight. The dried samples were powdered and kept in moisture free container for further chemical analysis.

Preparation of plant materials

Leaf powder was soaked in different solvents i.e., acetone, chloroform, ethanol and water with occasionally shaking at room temperature for 48 hours. Samples were then filtered and kept for evaporation to concentrate the extract following slow evaporation method¹⁵.

Phytochemical screening

Condensed leaf extracts were performed for preliminary phytochemical screening such as alkaloids (Wagner, Hager and Mayer's test), carbohydrates and glycosides (Fehling, Benedict and Molisch's test), phenols and tannin (Lead acetate and FeCl₃ test), saponin (Foam and Haemolysis tests), steroid (Salkowski test), fixed oils and fats (Spot test), flavonoid (Lead acetate test) and proteins (Biuret test) by following standard procedures¹⁵.

Quantitative determination of the secondary metabolites

The quantitative estimation of saponin, flavonoid, polyphenol, alkaloids and tannin etc are done by using a slight modification of the standard protocol^{16,17}.

Antioxidant Activity by DPPH method

The antioxidant activity of both plant extracts and the standard were assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method¹⁸.

The free-radical scavenging activity (RSA) of the extracts against DPPH was calculated according to the formula:

$$\% \text{ RSA} = 100 \times \left(1 - \frac{A_E}{A_D} \right)$$

where A_E is the absorbance of the standard antioxidant or extract, and A_D is the absorbance of the negative control

Proximate analysis

The analysis of moisture, crude fat, fiber, protein and ash were determined using the standard protocols from the manual of Methods in food Analysis, Academic Press, New York¹⁹. All values are presented as average of triplicate analysis.

Mineral analysis

The ash content was prepared with the help of muffle furnace in 600^o C and solution was prepared by dissolving ash with distilled water. Minerals were analyzed using the solutions and dissolving it in 10 % HCl (25 ml) and 5 % lanthanum chloride (2 ml), boiling, filtering and making up to standard volume with

deionized water. The mineral elements were determined in the solutions obtained above-Na and K by flame photometry, Model 405 (Corning, Halstead Essex, UK) using NaCl and KCl to prepare standards. Phosphorus was determined colorimetrically using a Spectronic 20 (Gallenkamp, London, UK) instrument, with KH₂PO₄ as a standard. All other elements (Ca, Mg, Zn, Fe, Mn, Cu and Cr) were determined by atomic absorption spectrophotometry, Model 403 (Perkin-Elmer, Norwalk, Connecticut, USA)²⁰.

RESULTS

G. gnemon grows at the hillsides in primary and secondary forests of the tropical rainforest climate with bimodal or uniform rainfall patterns. It does not tolerate salt spray and can't grow in the coastal areas. It is a small to medium-size tree (unlike most other *Gnetum* species, which are lianas), growing to 15–20 m tall. The leaves are evergreen, opposite, 8–20 cm long and 3–10 cm broad, entire, emerging bronze-coloured, maturing glossy dark green. The fruit-like strobilus consist of little but skin and a large nut-like seed 2–4 cm long inside. Leaves are dark green, shiny, smooth, acute at both the ends, opposite, and variable in size and shape. Typical size of leaves is 10-20 cm(4-8inches) long and 4-7cm (1.6-2.8inches) wide. Leaf shape is elliptic, lanceolate, and ovate oblong. Branches flush and flower throughout the year. Flowers monosexual; catkin-like formation; female flower 5-8 at each nodes have an ovulate strobilus 6-10cm (2.4-4 in) in length and bears the ovules or seed, it is also called as megasporangiate strobilus This axis bears a "pair of opposite sheathing bracts at the base, followed by five or six whorls of ovules, with five to seven ovules in whorls". Male flower consists of stamen and perianth, the staminate strobilus or male cone which bears microsporangia (pollen sac) is called a microsporangiate strobilus (Fig 1).

Fruits are yellow, turning purple-red or orange-red with maturity, and ovoid, 1-3.5cm in length (Fig 2). The skin is thin. In Indonesia this species fruits three times per year, March-April, June-July, and September-October. Fruits are large ovoid or ellipsoid seed per fruit.

The young leaves and tender strobili of *G. gnemon* are used as vegetables after boiling as well as salad. The fibers of the stems are used for making traditional rope by the *Nyshi* people. In addition to the young leaves, flowers and fruits are used as vegetables, eaten raw or boiled. The young inflorescences are also fried and taken as vegetable⁷.

The bioactive chemical compounds have great role in the curing of diseases. The bioactivity evaluation of the plant groups is like antimicrobial, antioxidant, anticancer, antilarval etc. The ethnic people use various types of plants in their daily life. They also use various plant products as biopesticides, insecticides, larvicides etc. in the raw form. The preliminary phytochemical screening reveals the presence of the secondary metabolites like alkaloids, phenols, saponins, terpenoids, flavonoids etc. which helps for future research. The result of the phytochemical test for various extract of the plant is summarized in the table 1. The quantitative determination of the secondary metabolites is shown in the table 2. Antioxidant activity of the leaf extract was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical scavenging activity. The methanol extract shows the lowest IC₅₀ value of 17.46% which is comparable with the standard ascorbic acid (12.71%) (Table no. 3). The man-made or natural substances which can prevent or delay any type of cell damage are the basic antioxidant substances. Vegetables and fruits are the main sources of antioxidants like vitamin C and E, selenium and among carotenoids like beta-carotene, lycopene, lutein and zeaxanthin etc. Leaf of *G. gnemon* contains the polyphenols as well as

flavonoid compounds which act as a free radical scavenging activity.

Determination of dry matter, moisture, crude fat, crude fiber, crude protein, ash content etc. are the main foundation of the biochemical profiling and nutritive value of the leaf plant samples. The proximate compositions including moisture content, total ash, crude protein, crude fiber, crude fat and free amino acid of leaf are shown in the table 4. Minerals are the essential nutrients, which are said to be present in small amounts in the body or in several parts per million (ppm)²¹. They are essential because each element plays an important role in metabolic processes of the human body and their absence can cause deficiency symptoms in animals²². The macronutrients as well as the micronutrients are recorded in the table 5 & 6 respectively.

DISCUSSION

Ethnobotanical investigation of *G. gnemon* indicates that the leaves have a good market demand as a leafy vegetable among the tribal society. The outer flash of the seed can be fried to make a chewy snake or added to other dishes²³. Preliminary phytochemical investigation indicated the presence of the secondary metabolites like alkaloids, carbohydrates and glycosides, phenolic compounds and a small amount of saponin, flavonoids, gum and mucilage, (Table 1). Among the four different solvents methanol and water are more active than the non polar solvent chloroform and petroleum ether. The quantitative investigation indicated that the leaf extract contains a significant number of secondary metabolites. Tannin content is reported as the highest amount (7.32±1.08), saponin (5.32±2.17), flavonoid (0.92±0.04), phenol (0.89±0.01) which indicated that the leaf of *G. gnemon* is highly active against different diseases. Due to presence of the secondary metabolites like polyphenols, flavonoid and tannic acid in notable amount in the leaf extract, the antioxidant activity showed a comparable result with the

standard antioxidant agent ascorbic acid. Antioxidant compounds present in the plant material play an important role as a health protecting factor. Scientific research on the antioxidant activity suggests that it reduces the risk for chronic diseases including cancer, heart related diseases, decline in immune system etc.

Ash is the remaining residue after the removal of moisture as well as the organic materials (protein, fat, carbohydrates, vitamins, organic acids etc) by burning at 500°C which indicates the amount of mineral content in the plant materials²⁴. The leaf of *G. gnemon* contains a good quantity of ash contents showing in the figure 3, which indicated the presence of notable amount of both micro and macro nutrients. Crude fibers, which is the non-digestible carbohydrate and lignin which also enhances the digestibility, its presence in high level can cause intestinal irritation and lower digestibility and decreased nutrient usage²⁵.

The micronutrients also called as the trace elements which are the most essential nutrients for human health. Trace elements take part in the function of different enzymes and are required in a number of metabolic processes in the body. Copper (Cu) plays a vital role in the enzyme metabolism²⁶. Among the mineral contents Fe, Ca, P, K, Na, Zn, Mn etc are most important ones and they constitute an essential part of any balance diet. Iron (Fe) and copper (Cu) both are especially involved in the synthesis of cytochrome oxidase. But excess amount of copper can lead to jaundice (Wilson’s disease)²⁷. Zinc (Zn) has a vital role in the activation of certain enzymes like dehydrogenase, alkaline phosphatase and carboxypeptidase. The leaf of *G. gnemon* is a good source of N (1.29±0.09%) as well as the other macronutrients like Mg (0.27±0.07) and K (0.44±0.61%) (Fig 4). The micronutrients are also detected in a good quantity in the young leaf of *G. gnemon* (Fig 5). So from the above study it can be inferred that the investigated plant sample is a good source of medicines for the treatment of various diseases as well as a nutritious leafy vegetable.

Table 1: Preliminary phytochemical investigation of the leaf extract of *Gnetum gnemon*

Constituents	Chemical test	Extracts			
		Chloroform	Petroleum ether	Ethanol	Water
Alkaloids	Hager’s test	-	-	+	+
	Myer’s test	-	-	+	+
	Wagner’s test	+	+	+	+
Carbohydrates and Glycosides	Fehling’s test	+	+	+	+
	Benedict’s test	-	-	+	+
	Molisch’s test	-	+	-	+
	Barfoed test	-	-	+	-
Steroid	Salkowski’s test	+	-	+	-
Saponin	Foam test	-	-	+	+
Phenol	FeCl ₃ Sol. test	-	+	-	+
	Lead acetate test	-	-	+	+
Flavonoid	Lead acetate test	-	+	+	+
Protein	Biuret test	-	-	+	+
Gum and Mucilage	Alcohol 95% test	+	-	-	+
Tannins	FeCl ₃ Sol. test	-	-	+	+

Table 2: Quantitative analysis of the secondary metabolites

Plant species	Alkaloid	Total phenol	Flavonoid	Tannin	Saponin
<i>Gnetum gnemon</i> (leaf)	1.07±1.04	0.89±0.01	0.92±0.04	7.32±1.08	5.32±2.17

Table 3: Antioxidant activity of the leaf extract of *Gnetum gnemon*

Plant Parts	Extract	µg/ml concentration							IC ₅₀
		5.0	10.0	20.0	40.0	60.0	80.0	100.0	
Leaf	Acetone	22.21	36.57	42.74	60.28	63.57	68.97	71.28	22.34
	Methanol	25.29	40.96	55.02	63.09	68.21	70.25	75.24	17.46
	Water	23.54	31.34	38.32	51.58	60.47	64.35	67.23	29.02
Ascorbic acid		29.93	47.73	59.27	65.21	71.46	76.32	78.12	12.71



Fig 1: *Gnetum gnemon* plant in natural habitat



Fig 2: Mature Seed of *Gnetum gnemo*

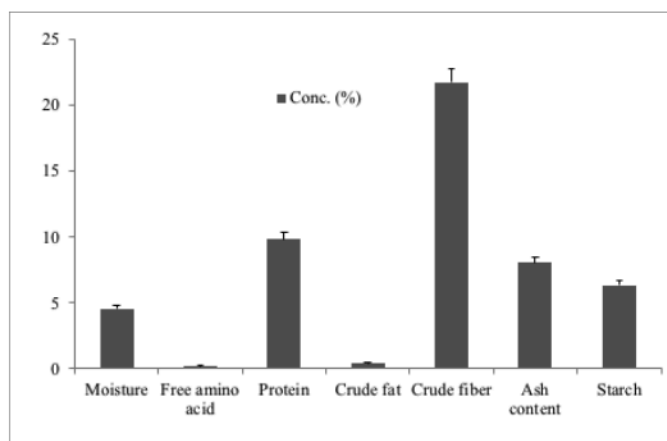


Fig 3: Proximate composition of the leaf of *Gnetum gnemon*

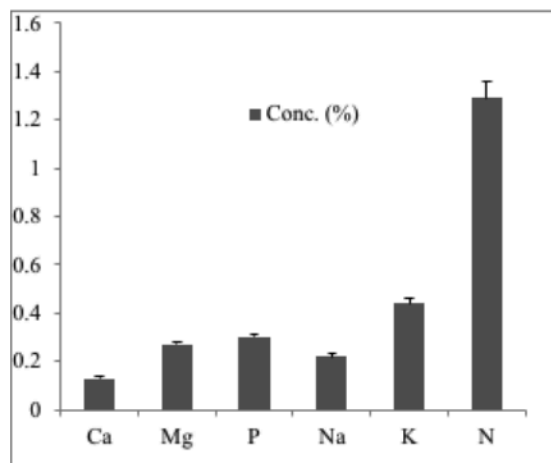


Fig 4: Macronutrient composition of the leaf of *Gnetum gnemon*

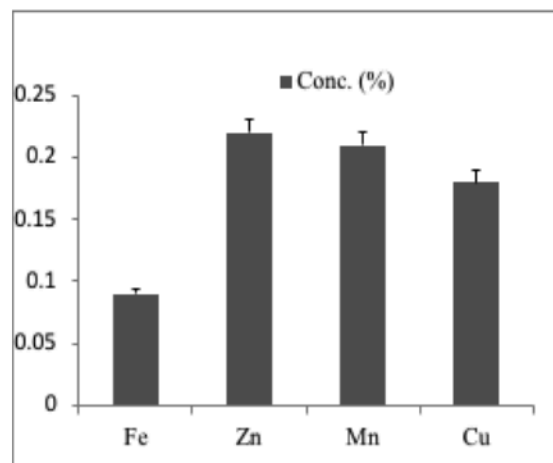


Fig 5: Micronutrient composition of the leaf of *Gnetum gnemon*

CONCLUSION

The present study highlighted the ethnobotanical important of the leaf of *G. gnemon* and also enlisted the composition of the secondary metabolites as well as the mineral constituents. The leaf contains a good amount of micro as well as macronutrients which takes a decisive role in the human diet. So there is a need of the conservation of the species to check its exploitation as the species is concerned.

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