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Quantitative Analysis of Nutrients in the Gum Exudates of *Acacia nilotica*

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ABSTRACT

Acacia nilotica is an evergreen tree found throughout Indiaand it belongs to the *leguminosae* family. The gum of this tree has been used in some parts of the country for its salutary therapeutic benefits. The gum was obtained from *Acacia nilotica* trees of West Bengal, India and was investigated for macronutrients and micro nutrients. The results of the present study showed that some essential minerals like calcium, iron, sodium, potassium, phosphorus were present in significant quantities which were 366.37, 25.41, 11.91, 124.87, 2.96 mg per 100 gram of pulverised gum respectively. The quantity of minerals like chromium, zinc, selenium, copper, magnesium, manganese was 0.67, 10.28, 0.54, 0.66, 22.61, 5.73 mg/kg gum respectively. It was found to be an energy dense gum containing 87.05 per cent of carbohydrate with substantially low fat, protein and vitamin content. The outcome of proximate analysis and high mineral content may account for the wide spectrum of medicinal and pharmaceutical properties of gum of *Acacia nilotica* which has been claimed in the pertinent literature.

Key Words: *Acacia nilotica*, Minerals, Nutrients, Vitamins, Medicinal, Properties

INTRODUCTION

Plants have been a major source of medicines in all cultures since the beginning of human civilisation. In the traditional system, various indigenous plants are used in the diagnosis, prevention and treatment of many ailments. In this modern era, there is a growing demand of plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. *Acacia nilotica* is a pioneer species, relatively high in bioactive secondary compounds and can exert a variety of functions [1,2].

Plant Details:

Table 1: Taxonomical classification[3]

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Subfamily	Mimosoideae
Genus	*Acacia*
Species	*Nilotica*

The plant parts of *Acacia nilotica* has been reported to have therapeutic uses arising from its wide spread folkloric and customary uses(3). Ethnobotanical studies suggested the usage of *Acacia nilotica* gum for the treatment of skin irritation and smoothening of the inflamed membranes of the pharynx, alimentary canal and genito-urinary tracts [4,5]. The bark or gum of the plant *Acacia nilotica* is used in West Africa to treat cancers and tumours of ear, eye or testicles[5]. The gum of the babul (*Acacia nilotica*) has also been used by the Bhils of Rajasthan to fill the dental cavities and to maintain oral health[6].The beneficial role *Acacia nilotica* Gum for promoting health of women after parturition has been revealed in a project titled Biomedical Studies and IPR (Intellectual Property Rights) where medicinal plants which were used in the

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treatment of Women Diseases in Sindh were also documented. In some regions of the Indian subcontinent, it furnishes the prime important ingredient of the nourishing food used for lactating mothers especially during the first three months of postnatal period[7].

Acacia nilotica (Babul tree) is one of the major gum-yielding acacia species found in India [8]. The composition of a gum varies with the plant species as well as geographical conditions and hence investigation of the indigenous *Acacia nilotica* gum can play an important role in exploring its potential for a wide range of applications [9].

In literature a very few reports are available on the gum exudate of *A. Nilotica* of West Bengal region in India. The present study aimed to quantify the various nutrients in this underutilised plant commodity that can facilitate us to comprehend its nutritive, curative and restorative properties.

MATERIALS AND METHODS

Sample collection

The gum of *Acacia nilotica* was collected from village Matchpota in Nadia district of West Bengal, India. They were authenticated for their unambiguous identity by Prof. N.D.Paria, Department of Botany, University of Calcutta and Botanical Society of India, Central National Herbarium, Howrah, West Bengal.

Preparation of Sample

The handpicked lumps of gum were cleaned to remove if any dirt present by wiping the surface of the nodule with water. It was then air dried and pulverized in a mechanical grinder. The powdered material was then stored in a clean and sterile bottle made up of glass. It was then kept in a dry and dark place at room temperature.

Analysis of Nutrients:

1. Moisture Content [10,11]

The porcelain crucibles were first washed and cleaned properly. It was then dried in a hot air oven at a temperature of 110 ºC for 10 minutes. The crucibles were then carefully transferred into a desiccator and then were kept in it for 30 minutes for cooling; it was then weighed again. This process was repeated until a constant weight was attained. The weight so obtained was labelled as W1.

2 grams of powdered sample was accurately weighed and put into previously weighed crucibles and then reweighed; and the weight thus obtained was labelled as W2.

The crucibles containing the samples were placed in an oven maintained at 103ºC for 14 hours. They were removed and transferred to desiccators to cool and again weighed. The process was repeated for several times until the constant weight was reached to get final weight (W3). The percentage of moisture content was calculated.

$$\% \text{ Moisture} = \frac{\text{Initial Weight (W2)- Final Weight (W3)}}{\text{Weight of the sample}} \times 100$$

2. Ash Content [10,11]

Porcelain crucibles were washed and dried in an oven to a constant weight at 100ºC for 10 minutes. They were allowed to cool in a desiccator and then weighed (W1). 2.0 grams of each sample were weighed into each of the previously weighed porcelain crucibles and reweighed (W 2). The crucibles containing the samples were transferred into a Muffle furnace, which was set at 550ºC for 8 hours to make it into ash. They were then removed and allowed to cool in the desiccators then finally weighed (W 3). The percentage of ash content was calculated.

$$\% \text{ of Ash} = \frac{\text{Weight of Ash (W3-W1)}}{\text{Weight of the Sample (W2-W1)}} \times 100$$

3. Estimation of Carbohydrates [12]

100 mg of the sample was weighed into a boiling tube. It was then hydrolysed in a boiling water bath for 3 hours with 5 ml of 2.5N HCl and cooled to room temperature. It was then neutralised with sodium carbonate until the effervescence ceases and the volume was made up to 100 ml. It was centrifuged, supernatant was collected and 1ml aliquot was taken for analysis. Standard glucose Stock was prepared by dissolving 100mg in 100 ml water. Working standard was prepared by mixing 10 ml of stock solution with 100 ml of distilled water. So, the concentration of working standard was 10mg/ 100ml. A series of standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard solution containing 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg of glucose respectively. 0.1 ml of sample or test solution (aliquot) was also taken in test tube. The volumes of all the test tubes were made up to 1 ml by adding distilled water. Then 4ml of anthrone reagent (200mg anthrone was dissolved in 100 mL of ice-cold 95% H_2SO_4) was added to all the test tubes which were followed by heating for 8 minutes in a boiling water bath. It was then cooled rapidly and the green to dark green colour was read at 630 nm.

4. Fat Estimation[10,11]

Fat was estimated as crude ether extract of the dry material. 10 grams of dried sample wasweighed accurately into a thimble and plugged with cotton. The thimble was then placed in a Soxhlet apparatus and extracted with anhydrous ether for about 16 hours. The ether extract was filtered into a weighed conical flask. The flask containing the ether extract was washed 4 to 5 times with small quantities of ether

and the washings were also transferred. The ether was then removed by evaporation and the flask with the residue dried in an oven at 80-100°C, cooled in a desiccator and weighed.

Fat content (g/100g sample) = $(w_e \times 100) / w_s$

Where w_e isthe weight of ether extract and w_s is the weight of the sample.

5. Protein Content[10, 13]

2 grams of sample was weighed into a dry Kjeldahl flask. About 5g of digestionmixture (4.9 grams K_2SO_4 + 0.1 gram of $CuSO_4$) and 20ml of pure concentrated H_2SO_4 were added to the same sample and themixture digested by heating for 4-5 hours. Glass beads were added to preventbumping. After the contents of the flask became clear, the process of digestion in the Kjeldahl flask was continued for atleast 1 hour more. The contents of the Kjeldahl flask were cooled, diluted withdistilled water and the mixture made alkaline by adding an excess of 40% NaOH(about 75ml). A small quantity of pumice powder was added to prevent bumpingduring distillation. The ammonia liberated was distilled into a receiver containing25ml of N/10 H_2SO_4. The excess of acid in the receiver was back titrated againstN/10 NaOH using 3 drops of methyl red indicator (0.1 gram of Methyl Red Indicator was dissolved in 60 ml alcohol and water was added to make the volume to 100 ml) . A reagent blank was similarlydigested and distilled. This titre value of Blank was subtracted from the value obtained for thesample to get the true titre value 'b'.

Calculation

If 'a' g of the sample was taken and if 'b' and 'c' ml of alkali of normality 'd' wererequired for back titration and to neutralise 25ml of N/10 H_2SO_4 respectively then

Protein content (g/100g of sample)= [(c-b) * (14 * d) * 6.25 * 100] / a * 1000

6. Crude Fibre[10,11]

About 2g of the moisture free sample was weighed into a 500ml beaker and 200 ml of boiling 0.255 N (1.25% W/V) sulphuric acid was added. The mixture was boiled for 30 minutes with bumping chips keeping the volume constant by addition of water at frequent intervals. A glass rod was placed in the beaker to ensure smooth boiling. At the end of this period, the mixture was filtered through a muslin cloth and the residue washed with hot water till free from acid. It was then boiled with 200ml of 1.25 % sodium hydroxide solution for 30 minutes. It was filtered through muslin cloth again and washed with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portions of water and 25ml of alcohol. The residue was removed and transferred to ash dish (pre weighed dish W_1). The residue was dried for 2 hours at 130±2°C. The dish was cooled in a desiccator and weighed (W_2). It was then ignited for 30 minutes at 600±15°C. Cooled in a desiccator and reweighed (W_3).

% crude fibre in ground sample= [Loss in weight in ignition(W_2-W_1)-(W_3-W_1)/ Weight of the sample] × 100

7. Energy Value [14]

The energy value of the 100g of food sample was calculated by using the followingformula.

Energy content = $4\times w_c + 4\times w_p + 9\times w_f$

Where w_c is the carbohydrate content (g/100g),

w_p is the protein content (g/100g) and

w_f is the fat content (g/100g).

8. Mineral Estimation

The working solution was prepared for measuring the minerals like Ca, Fe, Na, K, Cr, Se, Cu, Co, Mg, Mn, Pb, Zn, P, Mo using AOAC (Association of Official Analytical Chemists)method. The instrument used for mineral estimation was ICP (OES) or Inductively Coupled Plasma / Optical Emission Spectrometry, model ICAP6800, serial number ICP 20073108, calibrated with NIST (National Institute of Standards and Technology) certified multi standards[15].

9. β-Carotene Estimation[16]

β-Carotene content of sample was determined by using the colorimetric method of Srivastava and Kumar (2002). Five grams of the sample were crushed in 10–15 ml acetone by adding a few crystals of anhydrous Na_2SO_4. The supernatant was decanted into a beaker and the process was repeated twice. The combined supernatant was transferred to a separating funnel and 10–15 ml of petroleum ether were added and mixed thoroughly. After discarding the lower layer, the upper layer was collected in a 100 ml volumetric flask and the volume was made up with petroleum ether. The OD (Optical Density) was recorded at 452 nm and β-carotene was expressed as mg/100 g by the following formula.

$$\beta\text{-Carotene (mg/100g)} = \frac{\text{Optical Density} \times 13.9 \times 10^4 \times 100}{\text{Weight of the Sample} \times 560 \times 1000}$$

10. Vitamin-C Content[17]

The blue colour produced by the reduction of 2,6-dichlorophenol indophenol by ascorbic acid was estimated colorimetrically.2 gram of sample was weighed and blended with convenient volume of 6% HPO_3 to make 50 ml. The mixture was filtered and 5 ml of filtrate is placed in a 50 ml separating funnel. The same amount of extractant (6% HPO_3) was taken in 2 more separating funnels, B and C. Funnel B served as the Dye Blank and to funnel C which served as Standard was added 0.1 ml (equivalent to 0.1 mg ascorbic acid) of the ascorbic acid standard solution. An amount of acetate buffer

(pH 4) equal to the volume of the extract taken was then added to all the three funnels, followed by 2ml of the dye solution (25 mg of the sodium salt of 2,6-dichlorophenol was dissolved in distilled water and made up to 200 ml). 10 ml of Xylene was then added quickly and the contents shaken for 6-10 sec. After the layer separation, the lower water layer was removed and the colour in the Xylene extract was measured in a colorimeter at 500 nm.

11. Estimation of B-Vitamins

Vitamin B1 and Vitamin B2 were estimated by Fluorometric method [18,19] and Vitamin B3 was estimated by Colorimetric Method [20].

RESULTS

1. Estimation of Macronutrient contents and proximate principles of *Acacia nilotica*gum.

Sr. no.	Nutrients	Quantity (per 100g)
1	Moisture	14.5 g
2	Energy Value	351.37 Kcal
3	Carbohydrate	87.05 g
4	Protein	0.50 g
5	Fat	0.13 g
6	Crude Fibre	0.15 g

2. Analysis of minerals in the gum exudates of *Acacia nilotica*

Sr. No	Name of the Minerals	Unit	Quantity
1	Ash	g/100 g	2.98
2	Calcium as Ca	mg/100g	366.37
3	Iron as Fe	mg/ 100g	25.41
4	Sodium as Na	mg/ 100g	11.91
5	Potassium as K	mg/ 100 g	124.87
6	Chromium as Cr	mg/kg	0.67
7	Selenium as Se	mg /kg	0.54
8	Copper as Cu	mg /kg	0.66
9	Cobalt as Co	mg/ kg	<0.1
10	Magnesium as Mg	mg /kg	22.61
11	Manganese as Mn	mg /kg	5.73
12	Lead as Pb	mg /kg	0.26
13	Zinc as Zn	mg /kg	10.28
14	Molybdenum as Mo	mg /kg	BDL (DL: 1.0)
15	Phosphorus as P	mg /100 g	2.96

BDL: Below Detection Limit; **DL:** Detection Limit

3. Vitamin Content of the gum of *Acacia nilotica*

Sr. no.	Vitamin	Unit	Quantity
1	β- Carotene	µg/ 100 g	114.675
2	Vitamin-C	mg/100 g	13.18663
3	Vitamin B1 (Thiamine)	mg /kg	BDL (DL: 10)
4	Vitamin B2 (Riboflavin)	mg /kg	BDL (DL: 10)
5	Vitamin B3 (Niacin)	mg /kg	BDL (DL: 10)

BDL: Below Detection Limit; **DL:** Detection Limit

DISCUSSION

In a developing country like India, food scarcity is a colossal problem and has been plaguing the growth and development of precious human resource adversely. The rich heritage of flora and fauna which nature has bestowed us need to be explored and properly utilised as a source of nutrients. Plants and plant products have been widely used since time immemorial. Variety of gum exudates are used for their nutritional, culinary and therapeutic values throughout the world.

Natural gums are promising biodegradable polymeric materials which can serve as attractive alternatives to synthetic products because of their biocompatibility, low toxicity, environmental friendliness and low price[21].

The study undertaken here suggests that the Gum exudate obtained from *Acacia nilotica* is a very useful commodity which is not only energy dense but also an excellent reservoir of many minerals. Protein Energy Malnutrition is a very common phenomenon in our nation affecting children of rural and slum areas and thus weakening the backbone of the society. This gum can be utilised for producing edible products and can also be incorporated with other food ingredients to enhance its nutritive value. Literature studies indicated its utility and benefits in physiological conditions like pregnancy and lactation. Inadequate nutrient intake is a major cause of maternal mortality and Intrauterine Growth Retardation in foetuses. Thus, these traditional food products need to be advertised more and people should be encouraged to use this underutilised gum. The high carbohydrate content can exert hepatoprotective activity and stimulating property thus can be used in medicinal and pharmaceutical formulations.

Diarrhoea is a leading cause of childhood mortality in India and literature studies suggested it to play a protective role in its treatment. *Acacia nilotica* gum due to its high carbohydrate and mineral content can be beneficial in this condition by enhancing availability of glucose and absorption of solutes.

Its low protein and low fat content makes it useful in conditions associated with renal and cardiac functionalities. Its

high mineral content makes it a magical ingredient. Minerals are indispensable in our diet because they serve as co-factors for many physiological and metabolic functions. It plays pivotal role in human life provides healthy growth and developments. High calcium and iron content of this gum exudate is can combat deficiency diseases associated with it. Presence of high amount of minerals like magnesium, manganese, chromium, Zinc etc. can help in the prevention of many degenerative diseases.

It contains appreciable amount of β-carotene, Vitamin C but negligible amount of thiamine, riboflavin and niacin.

Its low moisture content influences its perishability and thus can be stored for a long time period. It's lower water activity hinder microbial activity and enhances shelf life.

CONCLUSION

A majority of the plant products remain virtually unexplored and thus new insight has to be developed into their potential use as therapeutic agents. It has high calorific value and the substantial mineral content can make it a viable supplement and immense source of dietary mineral in human food to fight various diseases. This natural gum can be used for encapsulation and production of several synthetic drugs with decreased side-effects. Its nutrient composition shows that it can have several physiological and nutritional benefits which need to be investigated. The ethno medicinal and traditional claims need reconnaissance and if properly employed can impart positive affect on nation's economy.

Declaration

Competing interests: The authors declare no potential conflicts of interest.

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List of Abbreviation:

Sl. no.	Abbreviation	Full form
1.	BDL	Below Detectable Level
2.	AOAC	Association of Official Analytical Chemists
3.	ICP (OES)	Inductively Coupled Plasma / Optical Emission Spectrometry
4.	NIST	National Institute of Standards and Technology
5.	O.D.	Optical Density

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