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Antioxidant Activity and Anti-Nutritional Factors in *Acacia Nilotica* Gum

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Abstract: The rapidly growing civilization and economy need the judicious usage of floral and faunal heritage. *Acacia nilotica* (Family: Fabaceae) is a beneficial plant species and each part of it exhibits numerous biological and pharmacological effects. The gum exudates of *Acacia nilotica* trees are underutilized plant products that can be explored for their potential as a therapeutic and curative agent. Its nutritional composition, interaction with anti-nutritional factors and antioxidants can determine its utility as a food. The present investigation was aimed to estimate the anti-nutritional factors and antioxidant activity of *Acacia nilotica* gum. It also focused on establishing a relationship of the obtained data with the ethnomedicinal and pharmacological properties claimed in the literature. The analysis was carried out using standard methods and protocols involving spectrophotometric and gravimetric principles. The anti-nutritional components like tannin, trypsin inhibitor, phytate, oxalate, total saponin, steroidal saponins, alkaloid content of *Acacia nilotica* gum was found to be 0.2369±0.0354 g tannic acid equivalent/100 g, 0.0108±0.0037 TIU/mg, 156.3333±9.4516 mg/100 g, 132.5±28.3152mg/100 g, 12.7183±6.7788 mg diosgenin equivalent /g, 0.0705±0.01 mg diosgenin equivalent /g, 0.1333±0.0577 g/100 g respectively. The amounts of total phenols and flavonoids was determined to be 8.0033±2.7211 mg of gallic acid equivalent/g sample, 0.0458±0.0186 mg quercetin equivalent /g sample respectively with a significant DPPH Scavenging activity. The results indicate that the quantity of anti-nutritional factors in gum is within the range of the contents of anti-nutritional factors found in commonly consumed foods. The substantial presence of phenol, flavonoids and DPPH activity in the gum indicates the presence of high antioxidant properties. The presence of anti-nutritional factors and antioxidants in favourable levels can impart diverse physiological responses. The present study concluded that *Acacia nilotica* gum can be used as a nutritious food commodity with a plethora of recuperative activities.

Keywords: Antinutrients, Antioxidants, Gum, *Acacia nilotica*, nutrients.

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1. INTRODUCTION

Plants are the indispensable commodities that are needed for sustenance and survival. They develop into natural ecosystems that are habitats of all living organisms and also serve as a source of food, nutrients, medicines etc. throughout the globe for generations. This immensely vast heritage needs to be explored and if sustainably used, can cause benevolent effects not only for humans but for every creature on planet earth.[1] one amongst them is the *Acacia nilotica* tree, which is a treasure trove with each part exhibiting diverse functionalities. *Acacia nilotica (Babul tree)* is a multipurpose, evergreen, nitrogen-fixing leguminous tree which is widely found in several parts of India. It is also abundant in subtropical and tropical Africa from Egypt Mauritania southwards to South Africa and from Asia eastwards to Pakistan. Its leaves, bark, pods, roots and gum were used extensively as traditional medicines in ancient times in the treatment of several health ailments.[2-3] the gum exudates obtained from the stems and branches of *Acacia nilotica* tree have been claimed to possess a wide spectrum of potentialities due to the inherent bioactive components. It is a natural, water-soluble hydrocolloid that has been increasingly recognized as a prolific commodity having various pharmacological and industrial applications. [4-5] the analysis carried out on this gum suggests it to be a highly nutritious and wholesome food commodity. The composition of this gum varies with the geographical location and other environmental conditions[6]. An investigation of its nutritional aspect revealed notable observations which are presented below in Table1.[6]

Table 1: Nutritional and Chemical Analysis of *Acacia nilotica* Gum[6]

Nutritional Parameters	Quantity in *Acacia nilotica* Gum
Moisture (%)	6.494
Ash (%)	1.81
Nitrogen (%)	0.42
Protein (%)	2.25
Fat (%)	0.00
Total Carbohydrates (%)	87.88
Galactose (%)	26.44
Arabinose (%)	39.62
Rhamnose (%)	10.15
Uronic Acid (%)	12.782

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It is quite evident from Table no. 1 that the gum is high in carbohydrates, minerals (as indicated by its ash content which is directly proportional to the inorganic matter) and it contains negligible amounts of protein. Its low moisture content increases the shelf stability of the gum. The rheological and thermal properties of the gum make it suitable for numerous applications in the food and pharmaceutical industry[6]. As we know the utility of food in our body also depends upon the concentration of anti-nutritional components present in it. These phytochemicals or anti-nutritional factors are bioactive chemicals of plant origin and several studies have proved them to be clinically effective against several maladies.[7] some common anti-nutritional factors widely found in plants are mentioned in Table no.2.

Table 2: Some Anti-nutritional Factors in Plants [8]

Sl. no.	Anti-nutritional Factors
1	Tannin
2	Phytate
3	Oxalate
4	Saponin
5	Trypsin Inhibitors
6	Cyanogenic Glycosides
7	Alkaloids
8	α-Amylase Inhibitor
9	Chymotrypsin Inhibitor
10	Haemagglutinin/ Lectin
11	Gossypol
12	Glucosinolates

These anti-nutritional components are secondary metabolites that are produced by plants as a defence mechanism but it retards the digestibility of some nutrients as well. It also exhibits several positive effects on our metabolism. These anti-nutritional components can cause adverse effects when their level increases beyond a certain level. So, judicious consumption of nutrients in combination with anti-nutritional components can be highly beneficial.[9] Along with the nutritional and anti-nutritional profile, the antioxidant potential is also a factor that enhances the significance of a plant product as a healthy and nourishing food. In this present era, the prevalence of degenerative diseases has increased tremendously and is a concern worldwide. Diseases like cancer, cardiovascular diseases, diabetes mellitus, arthritis and Alzheimer's disease have been associated with oxidative stress and generation of free radicals which involves Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS).[10] these free radicals are scavenged by the antioxidants.

Antioxidants are capable of delaying or preventing oxidation of oxidizable substrates when present at lower concentrations than the substrate[11]. A variety of antioxidants of both intrinsic and extrinsic nature are mentioned in Table 3. So, supplementation with exogenous antioxidants and boosting up of our endogenous antioxidant defences in the body can be helpful to combat the undesirable effects of oxidative stress and thus can reduce the incidence of several life-threatening diseases.[12]

Table 3: Classification of Antioxidants[11,13-14]

Endogenous Antioxidants: ***Intrinsic Antioxidant Defence System within the Body***	**Exogenous Antioxidants:** ***Extrinsic Antioxidants derived from plants or foods.***
Catalase	Ascorbic Acid
Superoxide Dismutase	Tocopherol
Glutathione Peroxidase	Carotenoids (β-Carotene)
Glutathione Reductase	Phenols
Glutathione Transferase	Retinol
Ceruloplasmin	Flavonoids
Ubiquinones	Zinc
Glutathione	Copper
Uric acid	Iron
Lipoic Acid	Selenium
Bilirubin	Lycopene

So, it can be comprehended from the antecedent literature that the anti-nutritional composition and antioxidant activity of the gum exudate obtained from *Acacia nilotica* can be associated with its hidden prospects as a nutritive ingredient or food. The analysis can help to understand its therapeutic activity and health claims as mentioned in traditional and ethnic medicine. The outcome of the study can impart ideas of using this underutilized and unused gum in newer ways and newer approaches. The aim and objective of our study are to quantify the anti-nutritional and antioxidant components in the gum which can help us to understand its digestibility and effectiveness as a food commodity. The study also aims to relate the data obtained from the present investigation on *Acacia nilotica* gum exudates with the traditional claims of gum of other *Acacia* species.

2. MATERIALS AND METHODS

2.2.1. *Sample Collection*

The gum exudate of *Acacia nilotica* was collected from a village named Matchpota in Nadia district of West Bengal, India. It was authenticated for its unambiguous identity by K. Karthigeyan, Scientist, Central National Herbarium, Botanical Society of India, (Under the Ministry of Environment, Forest & Climate Change, Government of India) Howrah, West Bengal and the voucher no. of the specimen was TR-5, 160572 dated 7.02.2019.

2.2.2. *Preparation of Sample*

The handpicked lumps of gum were cleaned to remove 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 mass obtained after grinding was stored in a clean sterile bottle in a dark place at room temperature.

2.2.3. *Chemicals and Reagents*

All chemicals and reagents used in this study were of Analytical grade from E.Merck, India and Sigma Aldrich, Germany

2.3. *Analysis of Anti-nutritional Components in Acacia Nilotica Gum*

2.3.1. *Tannin*

The tannin content in the gum was estimated by the Folin Denis Method. Folin Denis Reagent was prepared by refluxing 100 g of sodium tungstate, 20 g of phosphomolybdic acid, 50 ml phosphoric acid and 750 ml distilled water in a suitable flask for 2 hours and the volume was made up to 1litre with water. Sodium Carbonate Solution was also prepared by dissolving 350 g sodium carbonate in 1 litre of water at 70–80° C which was then filtered through glass wool after allowing it to stand overnight. Standard tannic acid solution was made by mixing 25 mg tannic acid in 50 ml of distilled water (Concentration of Stock Solution: 0.5 mg/ ml). Standard Curve was obtained by preparing "working tannic acid solution" of varying concentrations (5-50 μg/ ml) and then adding 5 ml of Folin Denis Reagent, 10 ml of $NaCO_3$ and the volume was made up to 100 ml with distilled water; all the volumetric flasks were mixed thoroughly, and the absorbance was read at 700 nm after 30 minutes. To extract tannin from the sample, 0.5 g of the powdered gum was weighed and transferred to a 250 ml conical flask and then 75 ml of water was added into it. The flask was gently heated and boiled for 30 minutes. It was then centrifuged at 2000 rpm (rotation per minute) for 20 minutes and the supernatant was collected in a 100 ml volumetric flask and the volume was made up to 100 ml with distilled water. 1 ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml water. 5 ml of Folin-Denis reagent, 10 ml of Sodium carbonate solution was added and was diluted to 100 ml with water. It was then shaken well, and the absorbance was read at 700 nm after 30 minutes[15].

Calculation

The tannin content of the samples was calculated as tannic acid equivalents from the Standard graph having the following equation.

$$\boxed{[Y = 0.0015\,X, Intercept = 0, R2 = 0.9309]}$$

2.3.2. *Trypsin Inhibitor*

The activity of the enzyme trypsin was assayed using casein as substrate. Inhibition of this activity was measured in the extracts. 4 grams of the pulverized defatted plant material was treated with 40 ml of 0.005 M sodium phosphate buffer (pH 7.5) and 40 ml of distilled water. The samples were shaken for 3hours and then centrifuged at 700 g (here, g is relative centrifugal force) for 30 minutes at 15 ℃. The incubation mixture consisted of 0.5 ml of trypsin solution, 2 ml of 2% casein, 1ml of sodium phosphate buffer (pH 7.5, 0.1M), 0.4 ml of HCl (0.001 M) and 0.1 ml of the extract. In all cases, the total volume of the incubation mixture was kept 4ml. Incubation was carried out at 37°C for 20 minutes after which 6 ml of 5% trichloroacetic acid was added to stop the reaction and corresponding blanks were run concurrently. Within 20 minutes, absorbance was checked at 280 nm in the spectrophotometer. In this method, one Trypsin Unit (IU) was arbitrarily defined as an increase of 0.01 absorbance unit at 280 nm in 20 minutes for 10 ml reaction mixture under the conditions described and the Trypsin Inhibitory Activity as the number of Trypsin Units inhibited (TUI).[16]

Formula: Distilled water was taken as 'Blank'

Control- test=TIU (Absorbance)
0.01 Absorbance=1 TIU (Trypsin Inhibitory Unit)

TIU was expressed per mg protein in the sample and also as TIU/mg sample.

2.3.3. *Oxalic Acid*

The oxalic acid was extracted from food stuffs and precipitated as calcium oxalate which was titrated against standard potassium permanganate. 5 grams of well-grounded sample was taken and then 100 ml of 2(N) HCl was added to it and the mixture was shaken well for about 2 hours in a mechanical shaker. It was then centrifuged and filtered. The mixture was transferred to the same beaker and weighed. It was then boiled for about 15 minutes and cooled. The mixture was adjusted to the previous weight with distilled water, made up to 100ml with 2 (N) HCl, shaken well and filtered. 5 ml of phosphoric-tungstate reagent (24 grams of tungstate was dissolved in some water and then 40 ml of phosphoric acid was added to it; then the solution was diluted to 1 litre with water) was mixed with 25 ml of the filtrate, stirred well and kept overnight. The next day, it was centrifuged and filtered. 2 drops of methyl red (0.1% w/v) was added to 20 ml of the filtrate and then neutralized with ammonia. 5ml of Calcium Chloride Buffer (25 grams calcium chloride dissolved in 500ml of 50 % glacial acetic acid and 330 gram of sodium acetate dissolved in 500 ml of water; then two solutions were then mixed well) buffer was added and stirred well. The mixture was allowed to stand overnight; at the end of which it was filtered through Whatman No. 40 filter paper and was washed free from chloride using distilled water (silver nitrate test was used to detect its presence). The precipitate along with the filter paper was transferred to the same beaker and some distilled water was added followed by 5ml of 2(N) H_2SO_4. The mixture was heated to 80℃, over a burner and titrated against N/100 potassium permanganate solution.[17]

Calculation:

1ml of N/100 $KMnO_4$= 0.45 mg of Oxalic acid.

Formulae:

mg of Oxalic Acid/100 Gram Sample=Titre Value × N of $KMNO_4$× (0.45/0.01) ×Dilution Factor× (100/ Weight of Sample)

2.3.4. Alkaloid

The alkaloid content was determined by the method of Harborne (1973). About 5.0 g of sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added to it; then it was allowed to stand for 4 hours. This was filtered using Whatman No.42 filter paper and then concentrated in a water bath to ¼ of the original volume i.e. 50 ml. Then, concentrated NH_4OH was added dropwise to each extract until the precipitation was complete. The suspension was allowed to settle, and the precipitate was collected, washed with NH_4OH and then filtered. The residue was dried and weighed. The percentage of alkaloids was then calculated.[18]

2.3.5. Phytate

The phytate was extracted with trichloroacetic acid (TCA) & precipitated as ferric salt. The Fe (Iron) content of the precipitate was determined calorimetrically and the phytate phosphorus (P) content was calculated assuming a constant 4Fe:6P molecular ratio in the precipitate. 10 grams of powdered sample was weighed in a 125 ml Erlenmeyer flask.50 ml of 3% TCA was added to it and was kept in a mechanical shaker for 30 minutes. The suspension was then centrifuged at 3000 g (here, g is relative centrifugal force) for 10 minutes and the supernatant was collected.10 ml of the collected supernatant was poured into a 50 ml conical flask. 4 ml of $FeCl_3$ solution was added to it. The conical flask was heated in a boiling water bath for 45 minutes. If the supernatant was not clear within 30 minutes, then 1-2 drops of sodium sulphate in 3% TCA was added and heated continuously for another 15 minutes. It was again centrifuged at 3000 rpm for 10-15 minutes and then the clear supernatant obtained was decanted. The precipitate was washed twice by dispersing in 20 ml of 3% TCA and then heated in boiling water bath for 5-10 minutes & then centrifuged again. The washing of precipitate was repeated with distilled water. The precipitate was dispersed in a few ml of water and 3 ml of 1.5 (N) NaOH was added with mixing. The volume was made up to 30 ml with water and heated in a boiling water bath for 30 minutes. It was then filtered hot through a moderately retentive paper Whatman no. 2. The precipitate was washed with 60-70 ml hot water and the filtrate was discarded. The precipitate on the paper was dissolved with 40 ml of hot 3.2 (N) HNO_3 into a 100 ml volumetric flask. The flask was cooled to room temperature and diluted by making the volume up to 100 ml with distilled water. 5 ml of aliquot was transferred to 25 ml volumetric flask, and it was diluted to 15 ml with water. 5 ml of 1.5 (M) KSCN was added into the volumetric flask and then the volume was made up to 25 ml with water. The colour was read immediately within 1 minute at 480 nm. A reagent 'Blank' was run with each set of samples.

Preparation of Standard Fe $(NO_3)_3$Solution:

Working Standard Solution: 433 mg Fe $(NO_3)_3$ was dissolved in 100ml distilled water. 25 times dilution was done by taking 2ml of this standard stock solution and adding it in 50 ml of volumetric flask and the volume was made up to 50 ml with water. *To prepare the Standard Curve the solutions were mixed in a manner written below.* 2.5 ml, 5 ml, 7.5 ml, 10 ml & 12.5 ml of working standard solution was pipetted out in a series of 25 ml volumetric flasks. It was diluted to about 15 ml with water. 5 ml of 1.5 M KSCN was added, and the volume was made up to 25 ml with water & read immediately (within 1 minute) at 480 nm. A reagent Blank was run with each set.[19]*Calculation*: μg of iron present in the test was determined from the standard curve

$$[Y = 0.0003\,X, Intercept = 0, R2 = 0.9756]$$

Phytate Phosphorus was calculated by the equation given below.

Phytate P mg/100 g sample= (μg Fe× 15)/ Weight of Sample (g)

2.3.6. *Total Saponin*

Standard Saponin solution was prepared by dissolving 10 mg of diosgenin, 16 ml of methanol and 4 ml of distilled water. To the 250 μl of the aliquots (containing saponin concentrations ranging from 0.0125 to 0.125 μg of diosgenin) for each tube; 0.25 ml of 8 % vanillin reagent was added followed by the addition of 2.5 ml of sulphuric acid (72% v/v) slowly on the inner side of the wall. The solutions were mixed well, and the tubes were transferred to a 60 °C water bath. After 10 minutes of incubation, the tubes were cooled in an ice-cold water bath for 3-4 minutes. The absorbance was measured at 544 nm against the reagent blank.

To extract saponin from the sample, 0.5 gram of defatted ground sample was weighed into a capped 25 ml tube. 10 ml of 80% methanol was added to the sample. It was then placed in a shaker overnight. The mixture was then centrifuged at 3500 rpm for 10 minutes. The supernatant was collected in a 25 ml volumetric flask. The resultant residues were re-extracted 3 times with 5 ml of 80% methanol by Centrifugation at 35000 rpm for 10 minutes & the supernatant was pooled in the 25 ml volumetric flask. The final volume was made up to 25 ml with 80% aqueous methanol. The absorbance was again read at 544 nm against the reagent blank. Total Saponin in the gum exudates was calculated by the calibration curve given below.[20]

$$[Y = 24.801\,X, Intercept = 0;\; R2 = 0.9552]$$

2.3.7. *Steroidal Saponin*

At first Reagent A was prepared by adding 0.5 ml of anisaldehyde to 99.5 ml of ethyl acetate and was mixed thoroughly. Reagent B was prepared by adding 50 ml of concentrated sulphuric acid to 50 ml of ethyl acetate. Standard saponin solution was prepared by dissolving 10 mg of diosgenin in 100 ml of 99.5 % ethyl acetate (0.1 mg/ml). The Sample Extraction method was the same as Total Saponin as described above. Calibration Curve was prepared by taking 0, 20, 40, 60, 80, and 100 μl of the diosgenin standard solution (containing 0, 2, 4, 6, 8, and 10 μg of diosgenin) in test tubes and the volume was made up to 2 ml with ethyl acetate. Then, 1 ml of reagent A and 1 ml of reagent B was added to it. After stirring, the test tubes were kept at room temperature for 30 minutes. Absorbance was measured at 430 nm against the reagent blank (0 μl of the diosgenin standard solution).[21]*Regression Line Obtained on Experimentation:*

$$[Y = 316.75\,X, Intercept = 0;\; R2 = 0.922]$$

2.4. *Estimation of Antioxidant Activity in the Gum Exudates of Acacia nilotica*

2.4.1. *Total Phenol*

Commercially available Folin Ciocalteu reagent (2N) was diluted with an equal volume of distilled water. The resultant solution was kept in a brown-coloured bottle and stored in a refrigerator at 4° C. Standard Solution was prepared by dissolving 10 mg of Gallic acid in 200 ml of methanol to achieve a concentration of 0.05 mg/ml. 400 mg of ground sample was taken in a conical flask and then 40 ml of diethyl ether containing 1% acetic acid was added. These were mixed well to remove the pigments. After 5 minutes, the supernatant was carefully discarded. Then, 20 ml of 70% aqueous acetone was added & the flask was sealed with a cotton plug, covered with aluminium foil. It was kept in an electrical shaker for 2 hours for extraction. It was then filtered through Whatman Filter Paper no. 1 & the sample can be kept at 4°C until analysis. To get the Standard Curve , 1000 μl of standard Gallic acid solution (containing 0.5μg-5μg gallic acid/ ml) was added to 0.5 ml Folin Ciocalteu Reagent and 2.5 ml of 20 % $NaCO_3$. 50μl of the sample was taken and its volume was made up to 1 ml by adding 950μl of Distilled water followed by the addition of 0.5 ml of Folin reagent and 2.5 ml of 20 % $NaCO_3$.The whole content was mixed properly and kept for 40 minutes and then Optical Density was measured at 725 nm.[22] *Regression Line obtained on Experimentation:*

$$\boxed{[Y = 46.196\,X, Intercept = 0;\ R2 = 0.9415]}$$

2.4.2. Flavonoids

The sample extract was prepared by macerating 25 grams of gum powder into 250 ml methanol for 48 hours. The Calibration Curve was prepared by using quercetin as Standard.25 mg of quercetin was dissolved in 50 ml methanol (0.5 mg/ml). The concentration of Quercetin in the standard curve ranged from 0.025 to 0.5 mg/ml. Then, 0.2 ml of K-Acetate (1M) and 0.2 ml of $AlCl_3$ (10%) was poured into it followed by the addition of 5.6 ml of distilled water. 1 ml of sample extract was taken and then again 0.2 ml of K- Acetate (1M) and 0.2 ml of $AlCl_3$ (10%) was added to it followed by the addition of 5.6 ml of distilled water. It was then left at room temperature for 30 minutes and the absorbance was read at 415 nm.[23] *Regression Line obtained on Experimentation:*

$$\boxed{[Y = 25.012\,X, Intercept = 0;\ R2 = 0.9547]}$$

2.4.3. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The capacity of the extract to scavenge the stable 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical was measured. To prepare sample extract, 2.5 grams of powdered gum was macerated with 25 ml of 80% methanol for 48 hours; the resulting dark brown extract was filtered using Whatman No. 1 filter paper and used for the analysis. To a set of clean and dried test tubes, 3 ml of methanol and 150µl of 0.1 % DPPH reagent was added and mixed thoroughly. To these test tubes, 1 ml of the sample extract was added in increasing concentration of 5-25µg/ml.1 ml solution of Ascorbic acid in varying concentration (5-25µg/ml) was taken as Standard. It was also added to 150 µl of 0.1 % DPPH and 3 ml methanol. A set of Blanks were prepared which contained 150 µl of 0.1 % DPPH, 4 ml of methanol. The solutions were mixed properly and allowed to stand for half an hour at room temperature and final absorbance was measured at 517 nm.[24]

$$\boxed{\textbf{\% DPPH Scavenging Activity} = [(A_0-A)/A_0] \times 100}$$

A_0=Absorbance of Control A=Absorbance of test Sample
IC_{50} of both Ascorbic acid (Standard) and the sample was calculated and compared (*IC_{50}* value is the concentration of the sample/Standard required to inhibit 50% of the DPPH radical).

3. STATISTICAL ANALYSIS

Data are presented as Mean ± Standard Deviation (n=3). The Line of Regression equations were obtained using MS Excel 2007.

4. RESULT

Table 4: Quantitative Analysis of Anti-nutritional Factors in *Acacia nilotica* Gum

Sl. No.	Anti-nutritional Factors	Quantity (Mean±S.D.)
1	Tannin	0.2369±0.0354 g Tannic acid equivalent/100 g sample
2	Trypsin Inhibitor	0.0108±0.0037 TIU/mg sample
		21.6667±7.3427 TIU/mg protein
3	Phytate	156.3333±9.4516 mg/100 g sample
4	Oxalate	132.5±28.3152mg/100 g sample
5	Total Saponin	12.7183±6.7788mg diosgenin equivalent /g sample
6	Steroidal Saponin	0.0705±0.01 mg diosgenin equivalent /g sample
7	Alkaloid	0.1333±0.0577 g/100 g

Table no. 4 depicts the quantitative analysis of the gum exudates of *Acacia nilotica* which indicates that it contains numerous anti-nutritional components in varying amounts. The content of these chemicals can directly influence their biochemical role in the body which can be studied further. As the amounts of these anti-nutritional factors are relatively low, it can be used as an edible food commodity having multifarious benefits. The quantity of tannin, phytate , total saponin , steroidal saponins was determined using calibration curve or Regression equation. The estimation of alkaloid and oxalate involved gravimetric and titrimetric principles respectively.

Table 5: Antioxidant Potential of *Acacia nilotica* Gum

Sl. No.	Parameters of Antioxidant Assay	Quantity (Mean±S.D.)
1	Total Phenol	8.0033±2.7211mg of Gallic Acid Equivalent/g sample
2	Flavonoids	0.0458±0.0185 mg quercetin equivalent /g sample
3	DPPH Scavenging Activity	IC_{50} of sample=39.111µg of sample/ml IC_{50} of Ascorbic Acid=32.514µg of Ascorbic Acid/ml

**** All the experiments were performed in triplicate and the data are reported as Mean ± Standard Deviation (SD).***

Table no. 5 indicates that the *Acacia nilotica* gum possesses the considerable antioxidant potential and thus it can be used as a functional food having many physiological benefits. The total phenol, flavonoids were determined using the Straight-Line Curve (mentioned in the method section) obtained using gallic acid and quercetin as standards.

Table 6: DPPH Activity of *Acacia nilotica* in comparison to Ascorbic Acid

Concentration(µg/ml)	% Inhibition of Methanolic Sample Extract	% Inhibition of ascorbic acid
25	31.9603	38.4461
50	63.9206	76.8923

The percentage of Inhibition of DPPH free radical by the methanolic extract of the sample is slightly lower than that of standard Ascorbic acid. It indicates that *Acacia nilotica* can serve as a potent free radical scavenger and thus possess antioxidant properties (Table no.6).

5. DISCUSSION

The current study implied to evaluate the anti-nutritional and antioxidant activity of *Acacia nilotica* gum. The analytical investigation propounded it as a natural product containing many bioactive phytochemicals which can be associated with the curative and restorative properties asserted in the available literature. Anti-nutritional components are the chemicals that are produced by plants as a defence mechanism but they can influence the digestibility and utility of food in a mischievous manner. Anti-nutritional Factors (ANFs) can act as a double-edged sword because they can exert adverse effects when consumed in excess and can also have a wide range of beneficial biological effects when used wisely.[7, 25] Almost all the non-communicable diseases associated with metabolism are the results of damage created by free radicals that can affect cellular components including lipids, protein and nucleic acid such as DNA leading to subsequent cellular death through necrosis or apoptosis.[26]Thus, consumption of a diet rich in antioxidant can keep many debilitating diseases at bay. Research studies corroborate that the presence of nutrients, ANFs and Antioxidants in food can enhance its therapeutic, nutritive and remedial ability. Tannin reduces nutrient utilization but at the same time, they have been reported to furnish antiviral, antibacterial, antiparasitic and antioxidant effects. [27-30]Alkaloids are also widely found in plant products with diverse ameliorative potentials. Certain alkaloids are highly toxic and can cause symptoms like rapid heart rate, paralysis, diarrhoea, acute necrosis of the liver and even death. At the same time, certain alkaloids can stimulate the nervous system and can also act as an analgesic, pain reliever, diuretic, antihypertensive, antitumor, antimicrobial agents.[8] Excessive amounts of Phytic acid or Phytate intake can cause it to form chelates or insoluble complexes with minerals like Cu, Zn, Ca, Fe etc. resulting in deficient absorption of dietary minerals leading to nutritional deficiency disorders. In vitro and in vivo studies have evaluated the effects of phytic acid on various types of cancers and have been proved to have anticarcinogenic activity. Its ability to prevent and inhibit tumour development has been attributed to its effect on modulating the differentiation, proliferation and apoptosis of neoplastic cells.[31]Saponins can reduce food intake by affecting palatability and also affects digestibility by inhibiting various digestive enzymes. Saponins are terpene glycosides that occur in abundance in various plant species. Research studies unveiled that saponins can exert several physiological and protective functions like anti-inflammatory, anti-diabetic, anti-HIV (Anti-Human Immunodeficiency Viruses), anti-atherosclerotic, and gastro-protective, hepatoprotective and hypolipidemic activity[32]. Due to these properties, saponins are employed commercially in manufacturing drugs, medicines, emulsifiers, adjuvant, taste modifiers, sweeteners and precursors of hormone synthesis etc.[32] Oxalate functions as chelating agents and may bind many metals especially toxic metals such as mercury and lead. It has been estimated that 80% of kidney stones are formed from calcium oxalate. People suffering from kidney disorders, gout, and rheumatoid arthritis are advised to avoid foods with high oxalate content. [33-34]Trypsin Inhibitors are small protein molecules that can inhibit the action of proteolytic enzymes thus hampering protein digestion and absorption. When consumed in excess amounts they can cause growth retardation and pancreatic hypertrophy. But it exhibits numerous beneficial roles by acting as anticarcinogenic, antifungal, antidiabetic agents.[35] Flavonoid is a broad group comprising many secondary metabolites found in plants that can exert salutary effects like anti-inflammatory, anti-allergic, antioxidant, anti-microbial, antidiarrheal and anti-carcinogenic properties. Flavonoids when consumed in high concentrations of chelate metals such as iron zinc and reduce the absorption of these nutrients. They can inhibit enzymes within the gut and may also precipitate proteins. [32, 36]Phenols or polyphenols are the plant metabolites that occur ubiquitously in nature. They have an ability to conjugate with other molecules and thus can scavenge free radicals responsible for oxidative stress. Experiments have shown phenols to possess antitumor, antiviral, antimicrobial, hypotensive and antioxidant properties. [32, 37] DPPH as we know is a stable free radical and any compound or food that scavenges these radicals is indicative of its ability as an antioxidant. [38] If we ponder into the table given below and relate the quantity of anti-nutritional factors in commonly consumed foods, we will be able to deduce several findings.

Table 7: Anti-nutritional Factors (ANFs) in Different Foods [39]

Source	Type (ANFs)	Amount
Legumes (Soya, Lentils, chickpeas, peanuts, beans)	Phytic Acid/ Phytate	386-714 mg/100g
	Saponins	106-170 mg/100g
	Cyanide	2-200 mg/100g
	Tannins	1.8-18 mg/g
	Trypsin inhibitor	6.7 mg/100g
	Oxalates	8 mg/kg
Grains (wheat, barley, rye, oat, millet, corn, spelt, Kamut, sorghum)	Phytic acid	50-74 mg/g
	Oxalates	35-270 mg/100g
Nuts: almonds, hazelnut, cashew, pignoli , pistachio, brazil nuts, walnuts, macadamia, *etc.*	Phytic acid	150-9400 mg/100g
	Lectins	37-144 μg/g
	Oxalates	40-490 mg/100g
Seeds: sesame, flaxseed, poppy seed, sunflower, pumpkin	Phytic acid	1-10.7 g/100g
	Alpha-amylase inhibitor	0.251 mg/mL
	Cyanide	140-370 ppm
Tubers: carrot, sweet potato, Jerusalem artichoke, manioc (or tapioca), yam	Oxalates	0.4-2.3 mg/100g
	Tannins	4.18-6.72 mg/100g
	Phytates	0.06-0.08 mg/100g
Nightshades: potato, tomato, eggplant , pepper	Phytic acid	0.82-4.48 mg/100g
	Tannins	0.19 mg/100g
	Saponins	0.16-0.25 mg/100g
	Cyanide	1.6-10.5 mg/100g

Table 8: Trypsin Inhibitor Content of some Seeds [40]

Food Stuff	Protein Content (mg/g)	Specific Activity (TIU/mg protein)
Soybean -*Glycine max*	113.66±0.66	14.61±1.41
Horse gram –*Macrotyloma uniflorum*	53.68±0.87	97.15±3.05
Babul - *Acacia nilotica* (Seeds/ pods)	88.11±0.59	98.63± 0.98

As shown in table no. 7 & 8, we can elucidate that the amount of ANFs in *Acacia Nilotica* falls within the range of ANFs found in the other food commodities usually consumed. The phytic acid content in commonly used legumes and nuts ranged from 386-714mg/100g, 150-9400mg/100g respectively (Table no.7).[39] However, the quantity of phytate in *Acacia nilotica* gum was found to be 156.3333±9.4516 mg/100 g sample, which is quite similar to the amounts found in nuts.The amount of tannin content in legumes, tubers were found to range from 0.18-1.8 g/100g, 4.18-6.72 mg/100g respectively.[39] In the current study, the tannin content of the gum exudates was found to be 0.2369±0.0354 g tannic acid equivalent/100 g sample, which seems closer to the amount of tannin found in legumes. The trypsin inhibitor content of Horse gram seed was found to be 97.15±3.05 TIU/mg protein.[39] the trypsin inhibitor content of *Acacia nilotica* gum was observed to be 21.6667±7.3427 TIU/mg protein, which is about 4.5 times lesser than the amount found in Horse gram seeds. As the protein content in this gum is too low, the Trypsin Inhibitor (TI) present in it will not cause any significant effect on its nutritional aspect as we know that the gum is high in carbohydrates containing approximately 87.88 %.[6]The oxalate content of grains and nuts were found to be 35-270mg /100 g, 40-490 mg /100 g respectively.[39] The oxalate content of the gum was 132.5±28.3152mg/100 g. It indicates that the oxalate content of the gum falls within the range of oxalate content found in widely used grains and nuts.The saponin content of legumes, nightshades were found to be 106-170mg/100g and 0.16-10.5 mg/100 g respectively.[39]The total saponin in the gum exudates was found to be 12.7183±6.7788 mg diosgenin equivalent /g, which is considerably low.So, the ANFs present in *Acacia nilotica* gum may not affect the digestibility of nutrients significantly. On the flip side, we can see that it can also serve as a fair source of antioxidants. The presence of these secondary metabolites and antioxidants are suggestive of a positive effect on health and well-being.

6. CONCLUSION

The present study deals with the enquiry of antioxidants and ANFs in *Acacia nilotica* gum. It is an underused plant product that can be used as merchandise if properly analyzed. Its nutritional composition and content of other phytochemicals signify it to have multifarious benefits. Its high carbohydrate and mineral content with low vitamin, protein and fat content as established from the literature survey; and its synergism with ANFs and antioxidants can make it a commodity of high importance. ANFs have adverse as well as favourable influences. There is a lack of knowledge about the tolerance levels of these compounds on the human and individual variation that can arise when consumed. So, further research is needed to comprehend its usage as a component of Food Sustainability.

7. AUTHOR CONTRIBUTION STATEMENT

Ms Rukshana Irani conceptualized the study and obtained the data upon experimentation. Dr Kazi Layla Khaled assisted in designing and analyzing the data and also provided the necessary guidance for the interpretation of the result. The authors discussed the methods and reviewed the result and approved the final version of the manuscript.

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9. CONFLICT OF INTEREST

Conflict of interest declared none.

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