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Investigating the foliage of *Indigofera astragalina* for their nutritive and antinutritive composition

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ABSTRACT

The fresh foliage of *Indigofera astragalina* collected from Gwiwa and Arkilla low cost areas of Wamakko local government area of Sokoto state, Nigeria were dried, powdered and subjected to nutritive and anti – nutritive investigation. The *n*-hexane, methanol, and water extracts prepared from the leaves were found to contain oxalates, phytate, tannins and cardiac glycosides, The proximate composition revealed the presence of moisture (51.00 ± 0.50 % fresh weight), ash (8.17 ± 0.58 % dry weight, DW), crude lipid (5.0 ± 0.5 % DW), crude fibre (2.67 ± 0.29 % DW), crude protein (8.23 ± 0.11 % DW) and carbohydrate (75.94 ± 0.64 %). The Anti – nutritive compositions which are oxalate (2.26 ± 0.00 mg/100g), phytate (12.26 ± 0.01 mg/100g), cyanogenic glycoside (0.24 ± 0.00 mg/100g) and tannins (3.05 ± 0.02 mg/100g). They also contained vitamin C (21.13 mg/100g). The results of the analyses were compared with other green leaves consumed. These results revealed that the leaves of *Indigofera astragalina* contained medicinal agents.

Key words : Foliage, *Indigofera Astragalina*, Nutritive, Antinutritive.

INTRODUCTION

Plants are the ultimate source of food and also provide shelter and medicinal agents [1]. The conventional food plants provide most nutrients needed for energy, body building, maintenance and regulation of body processes. Due to increasing population, economic crises in most developing nations especially in Nigeria, food insecurity have posed a serious threat to growth, development and survival [2]. Most people are now incorporating the non-conventional (wild) food plants in their diets, to provide not only nutrients but also traditional treatment for various ailments [2]. Over the last two decades, studies have revealed that wild or semi-wild plants are nutritionally important because of high vitamins, minerals, essential fatty acids and fibre contents [3]. Some of the plants also enhance taste and colour in diets.

Taxonomy of the Plant, *Indigofera astragalina*

Division: Magnoliophyta
 Class: Magnoliopsida
 Order: *Fabales*
 Family: *Fabaceae*
 Subfamily: *Faboideae*
 Tribe: *Indigofereae*
 Genus: *Indigofera*
 Species: *Indigofera astragalina*

Indigofera astragalina is commonly known in English as Hairy indigo. In the northern part of the country among the Hausa, it is called “Kaikai koma kan mashekiya”, and in the south-west among the Yoruba, it is known as Elu-aja [4]

Botanical description *Indigofera astragalina* is an erect hairy plant of about 40 – 70cm in height, with soft stem and green leaves. The leaves are pinnate with 5 – 13 leaflets; leaf size varies from 2 – 5cm long. The flowers are small, reddish – purple in colour and produced racemes of 2 – 10cm long[4]

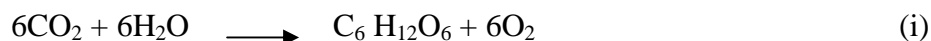
Distribution and habitat

The plant is a herbaceous legume which is simply regarded as weed, it is distributed in southern Africa like in Zimbabwe ,grow well in China and in west Africa; Nigeria and Niger Republic [4]. The plant grow well on unfertilized land with annual rainfall of 650 – 800mm and mean annual temperatures of 21°C-32°C.

Common food components of the leaves

Carbohydrates: The name carbohydrate is derived from the french words *hydrate de carbone* and was originally applied to neutral chemical compounds containing carbon, hydrogen and oxygen, with the last two elements present in the same proportions as in water [5]. This definition is not strictly correct since some compounds with general properties of the carbohydrates contain phosphorus, nitrogen or sulphur in addition to the carbon, hydrogen and oxygen. Also some compounds, e.g. deoxyribose (C₅H₁₀O₄) do not have hydrogen and oxygen in the same ratio as that in water [5]. In modern science, carbohydrates are defined as polyhydroxyl aldehydes, ketones, alcohols or acids, their simple derivatives, and any compound that may be hydrolysed to these [5].

Carbohydrates are an important class of naturally occurring organic compounds. They are often referred to as saccharides (latin, saccharum = sugar) because of the sweet taste of the simpler members [6]. They are found abundantly in nature, especially from plant sources. In the leaf of a plant, the simple compounds carbon dioxide and water are combined in the presence of sunlight and chlorophyll to form the simple sugar (+)-glucose [7] by the process of photosynthesis.



Proteins: Proteins are complex organic compounds of high molecular weight. In common with carbohydrates and fats, they contain carbon, hydrogen and oxygen, but in addition they all contain nitrogen and generally sulphur [5]. They are one of the primary constituents of living matter.

Chemically, proteins are high polymers whose structure can be arranged in four different ways [5] described below

i. Primary structure: Proteins are built up from amino acids by means of a linkage between the α – carboxyl of one amino acid and the α – amino group of another acid.

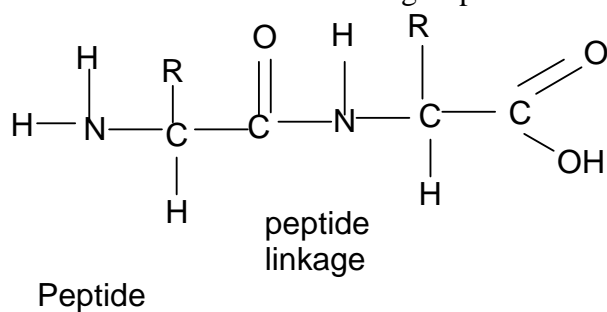


Figure 1: The structure of a peptide

This type of linkage is known as peptide linkage, and the structure is a dipeptide.

ii. Secondary structure: In this structure proteins are conformation of chains of amino acids resulting from the formation of hydrogen bonds between the imino and carboxyl groups of adjacent amino acids. This could be in form of regular structure (where the polypeptide chains exist in form of an α -helix or β -pleated sheet), or it may be irregular and exist in a random coil.

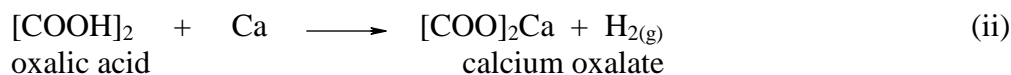
iii. Tertiary structure: This structure describes the interaction between the secondary structure and the R groups of the amino acids residues. This interaction causes folding and blending of the polypeptide chain, the specific manner of the folding gives protein its characteristic biological activity.

iv. Quaternary structure: Proteins possess quaternary structure if they contain more than one polypeptide chain. This structure is usually stabilised by forces such as hydrogen bonds and electrostatic or salt bonds formed between residues on the surfaces of the polypeptide chains. proteins are classified either as simple proteins or conjugated proteins. The simple proteins, examples are fibrous and globular proteins and conjugated proteins examples are glycoproteins, phosphoproteins, lipoproteins & chromoproteins[5]

Antinutritional factors

These are compounds found existing together with others (nutritional compounds) that are characterised as being toxic/poisonous as they hinder the bioavailability of some important mineral elements. Therefore, intake of plant food devoid of these substances in amount compatible with the optimal metabolic activities of the body for good health is of great importance [8]. The antinutritional compounds oxalates, phytates, cyanogenic glycosides, tannins and saponins are discussed in the follow sub-sections, but tannins and saponins, although anti-nutritive factors, also have therapeutic values.

Oxalates: Oxalic acid (ethanedioic acid) is one of the most important dicarboxylic acids. It occurs in rhubarb, sorrel, tomatoes and other plants. Oxalates are formed when oxalic acid reacts with metal ions especially calcium [6]



The presence of oxalic acid in food reduces the bioavailability of calcium, magnesium, etc. This acid is an active poison which depresses the central nervous system [6]. When oxalates are formed, the crystals (oxalates) can precipitate around the renal tubules causing renal stones which may lead to renal damage known as kidney stones. Soluble oxalates are toxic to the body and at a dose of 2-5g and above, oxalic acid is an active poison [6]

Cyanogenic glycosides: Plants such as legumes, tubers, cereals and cassava, release hydrocyanic acid upon enzymatic hydrolysis of plant compounds like Amygdalin, linamarin and lotaustralin [9]

They have the ability to form a stable complex with Fe^{3+} cytochrome oxidase system of aerobic organisms leading to death by cellular anoxia [10]

Total phytates: Phytic acid is an organic acid found in plant material especially in seeds in a form of a phosphorus storage compound. The most prevalent effect of phytic acid in nutrition is the chelating of certain essential mineral elements such as Ca, Mg, Fe, P and Zn to form insoluble salts phytates, thus altering their absorption. Phytates also inhibit the functions of some digestive enzymes such as pepsin, pancreatin and α – amylase. Never the less phytate also find useful application as anti – carcinogens that protect against colon cancer. It is also known to be a potent antioxidant [11]

Tannins: Tannins are compounds characterised as being non – crystallisable complex compounds, which normally form colloidal solutions in water. They are usually of high molecular weight (1000 – 5000). Tannins are complex phenolic compounds which are usually found in vegetables [9]. They form complexes with protein by precipitating the protein. They also combine with digestive enzymes thereby making them unavailable for digestion . Industrially, tannins are defined as substances of plant origin, which have the ability to cross link with protein and capable of transforming raw animal skin into leather [12] .Tannins are classified into two, namely:

i. hydrolysable tannins. These are esters of gallic acid and glycosides of these esters. They hydrolyse upon heating with dilute acids, giving glucose and either gallic acid (gallitanins) or ellagic (ellagitannins),[12] .Examples of some of these tannins are given in Figure 2.

ii. condensed tannins. They are polymers derived from various flavonoids. They do not hydrolyse upon heating with dilute acids but oxidise and polymerise giving insoluble red amorphous precipitates (phlobaphenes) e.g tea [9].

Tannins are also found to play an active anti nutritional role in the body where they affect the gastrointestinal tract, interfere with the absorption of iron and with a possible carcinogenic effect [9] .They also have therapeutic value as astringents, since they are able to precipitate proteins. Through this effect, they can be used to stop haemorrhage and to treat diarrhea as well as burns [9].

The objectives of this research is to analyses the leaves of *Indigofera astragalina* for their nutritional and antinutritional factors in the leaves and to compared the results with those reported for other green plants in literatures so as to make recommendations on the importance or otherwise of the leaves in terms of human nutrition and medicinal use.

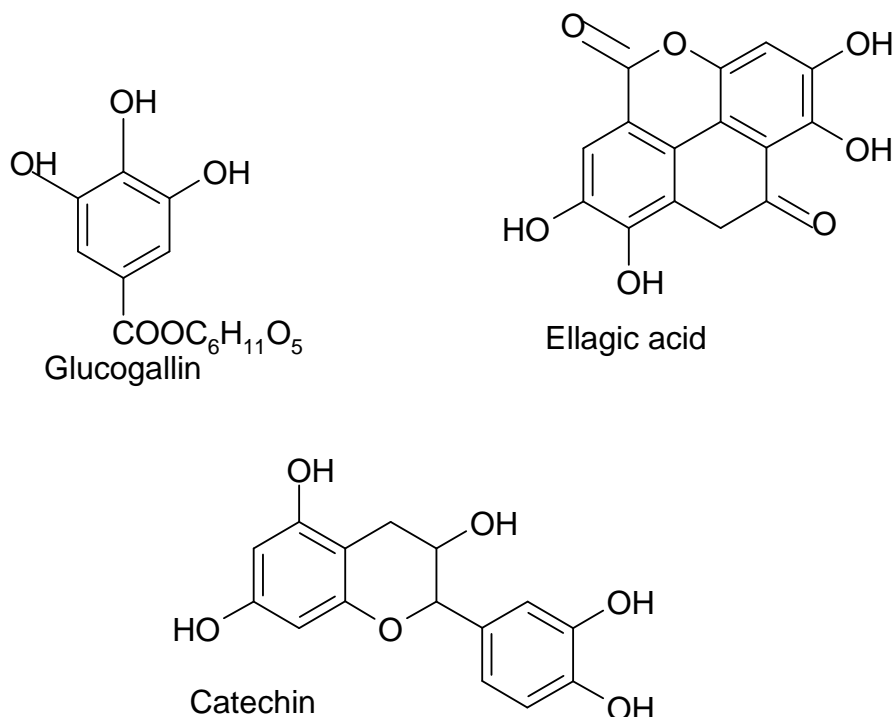


Figure 2 Structures of some tannins

EXPERIMENTAL SECTION

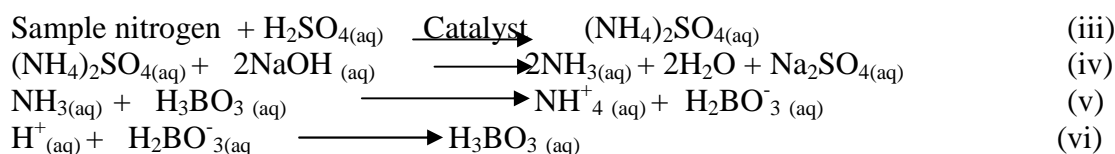
Fresh and tender plants of *Indigofera astragalina* were collected from Gwiwa low-cost and Arkilla Federal low-cost areas in Wamakko Local Government Area of Sokoto State. It was identified by a taxonomist in the Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto.

All reagents were of analytical reagent grade unless otherwise stated. Distilled water was used in the preparation of solutions and dilution unless otherwise stated.

Sample treatment and extraction: The leaves were separated from the stalks, washed and sun dried for three (3) days, blended into fine powder using a blender machine, sieved and stored in a covered plastic container for further use. All determinations unless otherwise stated were carried out in triplicates.

Determination of crude protein content

The crude protein of the sample was determined using the micro – Kjeldahl method described by AOAC [13]. The principle of this method is based on the transformation of protein and that of the other nitrogen containing organic compounds, other than nitriles and nitrates into ammonium sulphate by acid digestion.



The sample (2g) was weighed along with 20cm³ of distilled water into a micro – Kjeldahl digestion flask. It was shaken and allowed to stand for sometime. One tablet of selenium catalyst was added followed by the addition of 20cm³ concentrated sulphuric acid.

The flask was heated on the digestion block at 100⁰C for 4 hours until the digest became clear. The flask was removed from the block and allowed to cool. The content was transferred into 50cm³ volumetric flask and diluted to the mark with water.

An aliquot of the digest (10cm³) was transferred into another micro-Kjeldahl flask along with 20cm³ of distilled water, and placed in the distilling outlet of the micro – Kjeldahl distillation unit. A conical flask containing 20cm³ of boric acid indicator was placed under the condenser outlet. Sodium hydroxide solution (20cm³, 40%) was added to the content in the Kjeldahl flask by opening the funnel stopcock. The distillation start and the heat supplied was regulated to avoid sucking back. When all the available distillate was collected in 20cm³ of boric acid, the distillation was stopped. The nitrogen in the distillate was determined by titrating with 0.01M of H₂SO₄; the end point was obtained when the colour of the distillate changed from green to pink.

Crude protein is a measure of nitrogen in the sample. It was calculated by multiplying the total nitrogen content by a constant, 6.60. This is based on the assumption that, proteins contain about 16%N which includes both true protein and non – protein N and does not make a distinction between available or unavailable protein. The crude protein was calculated using equation presented elsewhere [14]

Determination of carbohydrates

The method of James [15] was adopted where the total proportion of carbohydrate in the leaves sample was obtained by calculation using the percentage weight method. That is by subtracting the % sum of food nutrients: % protein, % crude lipids, % crude fibre and % ash from 100%. This is done by using the equation below.

$$\% \text{ CHO} = 100\% - (\% \text{ cr. protein} + \% \text{ cr. lipid} + \% \text{ cr. fibre} + \% \text{ ash}) \quad (\text{vii})$$

Antinutritive analysis

Determination of total oxalates: Total oxalate was determined. To 1g of the ground powder, 75cm³ of 1.5M H₂SO₄ was added. The solution was carefully shaken on a mechanical shaker for 1 hour and then filtered using Whatman No.1 filter paper. The filtrate (25cm³) was then collected and titrated against 1.0M KMnO₄ solution till a faint pink colour that persisted for 30 seconds appeared. 1cm³ of 0.1M KMnO₄ = 0.00450g oxalic acid

Determination of total phytates

The powdered sample (4g) was soaked in 100cm³ of 2% HCl for 3 hours and filtered. The filtrate (25cm³), 5cm³ of 0.3% NH₄SCN and 53.5cm³ of water were mixed together and titrated against standard FeCl₃ solution (containing 0.00195g Fe/cm³) until a brownish yellow colour which persisted for 5 minutes appeared. Phytin – phosphorus (cm³ Fe = 1.19 mg phytin phosphorus) was determined and phytate content was calculated by multiplying the value of phytin – phosphorous by 3.55 [16]

Determination of tannins

The sample (500g) was weighed into a 100cm³ volumetric flask, 50cm³ of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50cm³ volumetric flask and made up to the mark with water. The 5cm³ of the filtrate was pipetted

out into a test tube and mixed with 3cm³ of 0.1M FeCl₃ in 0.1M HCl and 3cm³ of 0.008M potassium ferrocyanide. The absorbance was measured using a spectrophotometer at 520nm wavelength, within 10min. A blank sample was prepared the colour was developed as for the sample and absorbance read at the same wavelength. A standard was prepared using tannic acid. Tannin concentration was calculated in (mg/dl) using the equation xviii [9]

$$\text{Tannin} = \frac{\text{Abs of test sample}}{(\text{mg}/100\text{g}) \text{ Abs of standard}} \times \text{conc. of standard} \quad (\text{viii})$$

Determination of cyanogenic glycosides

This is based on the reaction between alkaline picrate and hydrogen cyanide (HCN) resulting in an orange colour which is measured at 490nm. The lipid free extract (2.0g) was dissolved in 10cm³ of water allowed to stand for 24hours, it was then filtered and 1.0cm³ of filtrate was pipetted into a test tube, 4cm³ of alkaline picrate solution was added and incubated for 5 minutes in a water bath at 90°C. The test tube was cooled to room temperature and absorbance of the solution was recorded at 490nm [13]. The concentration of cyanide in the extract was determine from the table of standards for cyanide and their absorbance below.

Table 1: standards for cyanide and their absorbance

Conc(mg%)	1.0	0.2	0.3	0.4	0.5	0.6	0.7
Abs at 490nm	0.132	0.270	0.412	0.540	0.660	0.800	0.920

RESULTS AND DISCUSSION

The results of the various analyses conducted on the sample are presented in Tables 2-3.

Table 2: Proximate composition of *Indigofera astragalina* leaves (% dry matter \pm SD)

Parameter	Level (%)
Moisture (% wet weight)	51.00 \pm 0.05
Ash	8.17 \pm 0.58
Crude Lipid	5.0 \pm 0.50
Crude Protein	8.23 \pm 0.11
Crude Fiber	2.67 \pm 0.29
Carbohydrate	75.94 \pm 0.64

Values expressed as: Mean \pm SD; The calorific value is 578.87 kcal/100g

Table 3: Anti – Nutritive factors in *I. astragalina* leaves

Parameter	Concentration mg/100g
Oxalate	2.26 \pm 0.00
Phytate	12.26 \pm 0.01
Cynogenic glycoside	0.24 \pm 0.00
Tannins	3.05 \pm 0.02
[Oxalate] / [Ca] ratio	0.20
[Oxalate]/[Ca + Mg] ratio	0.10
*[Ca] [Phytate] / [Zn] ratio	0.06

Values are expressed as: Mean \pm SD; *Molar ratio (mol/kg)

Proximate analysis: The result revealed that the moisture content, (51.00 \pm 0.50%) is higher than those of some common Nigerian leafy vegetables such as *Xanthosem sagittifolium* (14.7%), *Gnetum buchholsianum* (33.8%), *Vernonia amygdaline* (27.4%), *Adansonia*

digitata (9.5%) [17], but lower compared to moisture content within the range of 58.0 – 90.64% reported in some other Nigerian green leafy vegetables [18]. *I. astragalina* leaves has relatively average moisture content but that can also show some possible microbial activities during storage [10]. The ash content $8.17 \pm 0.58\%$ indicates that the leaves are rich in mineral elements. The value obtained is lower compared to 18.00% for Balsam apple leaves [10]

The leaves contained $5.0 \pm 0.5\%$ crude lipid, which is lower than 11% in water spinach leaves [19], but higher when compared to spinach leaves (0.3%) and Chaya leaves (0.4%) and 1.60% in *Amaranthus hybridus* leaves [20]

The crude protein content of $8.23 \pm 0.11\%$ obtained in present study is higher compared to 6.30% in water Spinach [19], 4.6% in *Momordica foecide* leaves consumed in Swaziland, but lower compared with 11.29% in balsam apple leaves [21], 24.85% in sweet potatoes leaves, and *Piper guineeses* and *Talinum triangulare* with values of 29.78% and 31.00% respectively. The recommended dietary allowance (RDA) for children, adult males, adult females, pregnant women, and lactating mothers are 28,63,50,60,65g of protein daily [22]. For 100g of *Indigofera astragalina* leaves to provide 8.23g of proteins, then it indicates that the leaves are a poor source of daily proteins.

The crude fibre content of 2.67% is low compared to 7.20% in sweet potatoes leaves 13% in *Tribulus terrestris* (Tsaída) leaves, [10]. 29.00% in balsam apple leaves [21], but the value is within the range of 0.70 – 12.0% for most leafy vegetables. Dietary fibre helps to reduce serum cholesterol level, risk of coronary heart disease, colon and breast cancer and hypertension [22]. The recommended daily allowance (RDA) for fibre is 18 – 35g that means 100g of *I. astragalina* cannot provide the daily fibre requirements of the body.

The carbohydrate content of the leaves is considerably high $75.94 \pm 0.64\%$ compared to some other leafy vegetables like *Tribulus terrestris* (“Tsaída”), 55.67%, 54.20% in water spinach leaves [19] and within the range with 75% in sweet potato leaves [23] but lower than 82.8% in *Corchorus tridens* leaves [23]. Carbohydrate and lipid are the principal sources of energy, the carbohydrate content per 100g of *I. astragalina* provide 578.87 kcal of energy, this indicates that the leaves of this plant can contribute meaningfully to the daily energy requirement for an adult which is 3000kcal/day [2]

The calorific value of *I. astragalina* leaves is 578.87kcal/100g on dry weight which is high compared to 248.8 – 307.1kcal/100g reported in some Nigeria leafy vegetables [24]. This is expected because of the high carbohydrate content of 75.94%. For that, *Indigofera astragalina* can serve as a good source of energy for the body.

Antinutritive factors:

The oxalate content of *I. astragalina* leaves was found to $2.26 \pm 0.00\text{mg}/100\text{g}$ dry matter. This value was higher when compared to $02.20 \pm 0.07\text{mg}/100\text{g}$ in *Borassus aethiopum* but lower compared to 28.89mg/100g in *Cassia siamae* leaves [1] and $3.15 \pm 0.07\text{mg}/100\text{g}$ in *Tribulus terrestris* leaves [25]. According to Ladeji *et al.*, (2004) oxalate can bind to calcium present in food thereby rendering calcium unavailable for normal physiological and biochemical roles. Oxalates present in food is insoluble, it may also precipitate around soft tissues such as the kidney, causing kidney stones. The low level of oxalate in the leaves, make the [oxalate] / [Ca] and [Oxalate/ [Ca +Mg] ratios to be 0.20 and 0.10 respectively. These values are below the critical level of 2.5 which is known to impair calcium

bioavailability [5]. This implied that *Indigofera astragalina* leaves have good calcium bioavailability.

The phytate contents of the leaves is 12.26 ± 0.01 mg/100g which is higher compared to 8.24mg/100g in *Cassia siamea* leaves [1] and 2.13 ± 0.51 mg/100g in *Parkia biglobosa* [5] but lower than 392.23mg/100g in egg plant and 1214 ± 15.56 mg/100g in *T. terrestris* [25]. Phytic acid can bind to mineral elements such as calcium, zinc, manganese, iron and magnesium to form complexes that are indigestible, thereby decreasing the bioavailability of these elements for absorption. The low phytate content in the leaves indicate that the consumption of the leaves will not affect the bioavailability of minerals especially Ca and Zn for absorption, and the [Ca] [Phytate/ [Zn] molar ratio which is 0.06mol/kg is below the critical level of 0.5mol/kg [8]. The leaves thus have good calcium bioavailability.

The cyanogenic glycoside (hydrocyanic acid) content in the leaves is 0.24 ± 0.00 mg which is low when compared with 4.79 ± 0.24 mg/100g in *T. terrestris* leaves [25] and 20mg/100g in the raw leaves of *Celosia argentea*. Consumption of high levels of cyanide is associated with serious health problems, spastic paraparesis known as Konzo lethal dose range for human of cyanide taken by mouth is estimated to be only 0.5 – 3.5mg/kg body weight [25]. With respect to cyanide content the leaves of *I. astragalina* are safe for consumption.

The tannins content in the leaves is 3.05 ± 0.20 mg/100g, this value is low when compared to 7.40 ± 0.14 mg/100g, in *Balanite aegyptiaca* (Desert date), and 4.83 ± 0.15 mg/100g in *Vitex donianan* but higher than 0.93 ± 0.11 mg/100g in *Parkia biglobosa* [8]. Tannins in food impose an astringent taste affecting palatability, reduce the intake of the food and consequently body growth. Tannins can bind to both exogenous and endogenous proteins including enzymes of the digestive tract, thereby affecting the utilisation of protein [19]

Vitamin: Vitamin C is an excellent antioxidant and free radical scavenging nutrients protecting cells from damage by oxidants. The vitamin C content is 21.13mg/100g in the leaves is higher when compared with 10mg/100g in *Diospyrus mespiliformis*, but lower when compared with 54mg/100g in orange juices [25]. The RDA of vitamin C is 60mg/day for a male adult. Ascorbic acid in the body increase iron absorption from the intestine and it is also required for connective tissue metabolism especially the scar tissues, bone and teeth [25]. The leaves of *I. astragalina* can actually provide the daily needs of vitamin C.

CONCLUSION

The *Indigofera astragalina* leaves have high calorific value. *I. astragalina* leaves are quite safe for consumption, since they contain low anti-nutritive agents such as the oxalate, phytate, cyanogenic glycoside, and tannins content. It could have potential therapeutic or physiological actions on human system. Other anti-nutritive agents such as aflatoxins, trypsin inhibitor, α – amylase inhibitor, etc. which could hinder availability of mineral nutrients should be investigated.

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