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Amino acid estimation and phytochemical screening of *Indigofera astragolina* leaves

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ABSTRACT

Fresh leaves of Indigofera astragalina were collected and pretreated for phytochemical screening. The n-hexane, methanol, and water extracts prepared from the leaves were found to contain alkaloids, saponins, tannins, steroids, cardiac glycosides, flavonoids and phlobatannins. The leaves contained seventeen amino acids of which nine are essential (predominantly Total Aromatic amino acids;6.92, Leucine;6.71 and Lysine;3.90 g/100g sample) while eight which are non essential amino acids include major once like Glutamic acid;9.70, Aspartic acid;6.78 and Arginine ;5.20 g/100g sample. The results of the analyses were compared with other green leaves consumed and investigated to fall within the limits prescribed as medicinal agents.

Key words: Phytochemical screening, *Indigofera Astragolina*, Leaves

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INTRODUCTION

Human beings and animals require food to carry out essential functions, which include growth, development and reproduction . 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. Over the last two decades, studies have revealed that wild or semi-wild plants are nutritionaly important because of high vitamins, minerials, essential fatty acids and fibre contents . Some of the plants also enhance taste and colour in diets [2]

Some of these wild plants contain chemical substances that produce a definite physiological action on the human being.About 25% of all prescribed medicines today are substances derived from plants [1]. Most of these plants are used traditionaly in the treatment of diseases. Among such plants are *Uvaria chamae* (used in the treatment of piles, menorrhegia,

epistaxis, haematuria and haemolysis), *Cnestic ferruginea* (used in the treatment of migraine and sinusitis and anaemia for woman with abortion and ovarian problems) and in the treatment of gonorrhea, joint and waist pains, arthritis, rheumatism, stroke and syphilis [3] and *Cussonia arborea* used for the treatment of sexually transmitted diseases, menstrual pains and severe rashes. Inspite the increasing research into nutrients and medicinal agents of wild edible plants not much has been done about *Indigofera astragalina*.

Plant Description: *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]. *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]. The plant is a herbaceous legume which is simply regarded as weed, it is distributed in southern Africa like in Zimbabwe [5], grow well in China and in west Africa; Nigeria and Niger [4]. The plant grow well on unfertilized land with annual rainfall of 650 – 800mm and mean annual temperatures of 21^oC-32^oC [5]

Economic importance of *Indigofera* species :The genus *Indigofera,* have about 700 species. They occur throughout the tropical and subtropical regions of the world, some of these species, *Indigofera tinctoria* and *Indigofera suffruticosa* are used to produced indigo dyes while some have medicinal values such as *Indigofera articulate* used for the treatment of toothache, *Indigofera oblongifolia*, *Indigofera suffruticosa*, and *Indigofera aspalthoides* are used as anti – inflammatories for treatment of insect stings, snake bites and swellings; and *indigofera arrecta* extract is used to relieve ulcer pain. Phytochemical investigation of *Indigofera* species shows that they are rich in organic and fatty acids, flavonoids such as rotenoids and coumarins[6] .Study conducted on *indigofera astragalina* shoot revealed the presence of polyphenol, lignin (insoluble fibre), phosphorus, nitrogen and potassium. Phytochemical screening and anti – microbial study of *indigofera astragalina* extracts revealed the presence of tannins, cardiac glycosides, saponins, resins and alkaloids, and also show activity against *S. aureus* and *E. Coli*, and a very good cholinesterase inhibition [4]. The importance of these nutrients, phytochemical compounds as well as anti – nutritive factors in nutrition are:

Amino acids

These are building blocks of proteins. They are bifunctional compounds containing both an amine group and a carboxylic acid group. In other words, they are derivatives of the carboxylic acids in which an amino group replaces a hydrogen atom in the carbon chain[7]

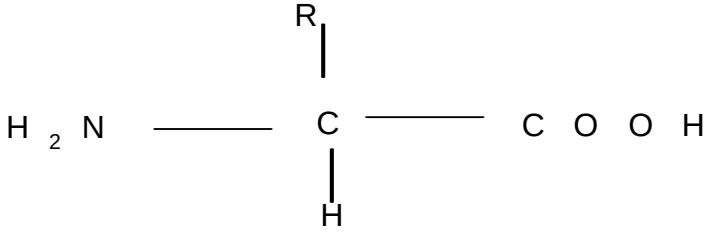

Figure 1.2; The general structure of amino acids

Most amino acids occuring naturally in proteins are of the α type, having the amino group attached to the carbon atom adjacent to the carboxyl group, just as in above structure, except

proline which has an imino (NH) group instead of an amino group. The nature of the 'R' group, which is referred to as the side-chain, varies in different amino acids. It may simply be a hydrogen atom, as in glycine, or it may be a more complex radical containing, for example, a phenyl group [8]

There are nine essential (indispensable) amino acids, which cannot be synthesised in the body, and if one of these is provided in inadequate amounts regardless of the total protein intake, it will not be possible to maintain nitrogen balance. Two amino acids, cysteine and tyrosine, can be synthesised in the body only from essential amino acids precursors – cysteine from methionine and tyrosine from phenylalanine. The non-essential (dispensable) amino acids are the ones that can be synthesised from metabolic intermediates, as long as there is enough total protein in the diet. Even if any one of the amino acids is omitted from a diet, the nitrogen balance can still be maintained[9]

The importance of amino acids cannot be over emphasized as they form the basement or building block of the molecular structure of the important and very complex class of compounds known as the protein . They are also required for synthesis of metabolic products, including: purines and pyrimidines for nucleic acid synthesis, heam, thyroid hormones, and as well as melanin .The chemical structures of the 20 amino acids commonly found in natural proteins are shown in appendix [8].

EXPERIMENTAL SECTION

Fresh Plants and Leaves 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 amino acids: The defatted sample (2.0g) was dried and milled into a fine powder. The powder (3.0g) was weighed into a glass ampoule to which $5cm^3$ of 6MHCl and 5 moles norleucine (2-aminohexanoic acid) internal standard were added. The ampoule was evacuated by passing nitrogen gas to prevent oxidation of some amino acids during hydrolysis. The ampoule was then sealed using a Bunsen burner flame and stored in an oven thermostated at 110^oC for 24hours. After hydrolysis, the ampoule was cooled, broken at the tip and the content was filtered.

The filtrate was then evaporated to dryness at 40^oC under vacuum in a rotary evaporator. The residue was dissolved to 5µl (for acid and neutral amino acids) or 10µl (for basic amino acids) with acetate buffer, pH 2.2. The aliquot was then loaded into cartridge of amino acid analyser. The chromatograms obtained along with automatic pen records indicate amino acid peaks corresponding to the magnitude of their respective concentrations. Quantification was performed by comparing the peak area of each amino acid in the sample to the area of the corresponding amino acid standard of the protein hydrolysate (0.02µmole).

Calculation: Total height (TH) of each peak produced by the chart record of the analyzer was measured. The half height (TH/2) of each peak on the chart was determined and the width (W) of the peaks at the half – height was measured and recorded.

Total height was multiplied by the width to obtain the area of each peak.
The norluecine (Neu) equivalence (NE) for each amino acid in the standard mixture (Aastd) was calculated using equation xix below.

$$NE = \frac{\text{Area of neu peak}}{\text{Area of each Aastd}} \quad \text{(i)}$$

Amino acids standard constant for each type was evaluated using the equation.
Sstd = Nestd X Molecular weight of each Aastd x Nmole Aastd (ii)

The amount of each amino acid in the sample (Aasmp) in g/100g protein was calculated using the equation:

$$(\text{Aasmp}) = \frac{\text{Area of each Aasmp}}{\text{Area of Neu in the sample}} \times \frac{\text{Sstd x dilution x 16}}{\text{W(g) x N(\%) x 10 x V}} \quad \text{(iii)}$$

Where V is the Vol of buffer solution loaded and W(g) is the sample weight

Phytochemical screening
Extraction: The powdered sample (50g) was weighed into a conical flask and 250 cm^3 of n – hexane was added. The mixture was stirred and covered. It was allowed to stand for 36 hours and filtered using muslin cloth. The filtrate was concentrated using the rotary evaporator to about 50cm^3. The procedure was repeated with methanol and distilled water as solvents using fresh 50g of the ground plant sample for each extraction. All extracts were cooled and stored in the refrigerator [10]

Phytochemical analysis: The extracts were analysed for the presence of alkaloids, saponins, tannins, flavonoids, cardiac glycoside, phlobatannins and steroids.

Tests for alkaloids : The acidified solutions of the extracts were used for the test. HCl (1cm^3, 1%) was added to 3cm^3 of each extract separately in six different test tubes (three for each extract) and then filtered, to the filterate [11]

(i). The Mayer's reagent (5 drops) was added to each filtrate separately in test tubes. A light brown precipitate indicated the presence of alkaloids.

(ii). Wagner's reagent (5 drops) was added to each filtrate separately. A reddish brown precipitate indicated the presence of alkaloids.

(iii). Dragendorff's reagents (5 drops) was added to each filtrate separately. An orange brown precipitate formed which indicated the presence of alkaloids.

Test for saponins
i Frothing test
Each extract (2cm^3) in test tube was vigorously shaken and placed in a water bath for 2 minutes. Frothing was observed which indicated the presence of saponins.

ii Emulsion Test
Olive oil (5drops) was added to 3cm^3 of each extract in a test tube and the mixture was vigorously shaken. A stabe emulsion indicated the presence of saponins [12]
Test for cardiac glycosides : In clean test tubes, 2cm^3 of each extract was added separately with 3cm^3 of 3% ferric chloride followed by 3cm^3 of acetic acid. This gave a green precipitate and a dark brown coloured solution respectively. Finally 1cm^3 of concentrated sulphuric acid was carefully poured down the side of the test tube. Formation of a brownish – red layer after effervenscence at the interface was due to aglycone and the development of a greenish blue colour in the upper layer (acetic acid) layer was due to sugar [12]

Test for tannins : Two drops of 5% $FeCl_3$ were added to1cm^3 of each extract in a test tube. A dirty green precipitate indicated the presence of tannins [12]

Test for flavonoids: In this Sodium hydroxide test,Each extract (2cm^3) was acidified with 1% HCl and dissolved in 20% NaOH. A canary yellow colour indicated the presence of flavonoids.

i. Lead acetate test
Each extract (2cm^3) was treated with 10% lead acetate solution. A light yellow – milky precipitate indicated the presence of flavonoids [11]

Test for phlobatannins : Each extract (2cm^3) was acidified with 5cm^3 of 1% HCl. The formation of a red precipitate indicated the presence of phlobatannins [11]

Test for steroids :In this Liebermann's Test, Acetic anhydride (2cm^3) was added to 2cm^3 of each extract. The content was then cooled in ice for about 5 minutes. Concentrated sulphuric acid (1cm^3) was added along the wall of the test tube. The change in colour from violet to blue and then to green indicated the presence of steroidal nucleus (i.e aglycones component of cardiac glycosides).

ii. Salkowski Test: Concentrated sulphuric acid (2cm^3) was added to 2cm^3 of each extract. Effervescence followed by the appearance of a clear reddish – brown colour appreared at the interface confirmed the presence of a steroidal ring (i.e the aglycone component of the cardiac glycoside) [11]

RESULTS AND DISCUSSION

Amino acids: Table 1 revealed the results of estimated amino acids. Leucine, isoleucine and valine values were higher than the contents in *Cassia siamea* leaves [1] but the valine content is lower than the reference value, [13] Lysine and threonine values were found to be lower, when compared with [13], reference values. The lysine content is higher when compared with the lysine content in *Cassia siamea* leaves [1] and threonine content is lower than that of *Cassia siamea*. The total sulphur amino acids (cysteine and methionine) content was lower when compared with the content of Africa locust beans (*Parkia biglobosa L.*) [13,14] reference values. The total aromatic amino acids (phenylanine and tyrosine) content were found to be low than the content in African locust beans (*Parkia biglobosa L.*) [14] but higher than the FAO/WHO/UNU reference values [13]

Table 1: Amino acids composition of *I. astragalina* leaves

Amino acids	**Concentration** (g/100g sample)
Essential amino acids	
Cysteine	1.06
Isoleucine	3.44
Leucine	6.71
Lysine	3.90
Methionine	0.77
Phenylalanine	3.68
Threonine	2.77
Tyrosine	3.24
Valine	3.78
Total Sulphur amino acids	1.83
Total Aromatic amino acids	6.92
Non – essential amino acids	
Alanine	3.32
Arginine	5.20
Aspartic acid	6.78
Glutamic acid	9.70
Glycine	3.69
Histidine	2.23
Proline	2.09
Serine	2.31

Table 2: Phytochemical screening on the n- hexane, methanol and distilled water extracts of *I. astragalina* leaves

S/No	**Phytochemical test**	**n – hexane**	**Methanol**	**Distilled H_2O**
1	Alkaloids			
a	Mayer	-	+	+
b	Dragendorff	-	+	+
c	Wagner	-	+	+
2	Saponins			
a	Frothing	+	+	+
b	Emulsion	+	+	+
3	Tannins	+	+	+
4	Flavonoids			
a	NaOH	+	+	+
b	Lead acetate	+	+	+
5	Cardiac glycosides	+	+	+
6	Steroids	-	+	+
7	Phlobatannins	+	+	+

Keys: + Positive (present); - Negative (absent)

From the values, it can be seen that *I. astragalina* leaves contain a reasonable amount of essential amino acids, with methionine being the most limiting amino acid. The non – essential amino acids also play a vital role in human nutrition and metabolism [14], especially arginine and histidine which are referred to as semi – essential amino acid, which do not actually play a role in nitrogen balance but needed for growth and development in infants [15].

Phytochemicals

The results of Phytochemical analysis (Table 2) from the three extracts (methanol, n- hexane and distilled water), indicate that *I. astragalina* leaves contain alkaloids saponins, tannins, flavonoids, cardiac glycoside, steroids and phlobatannins. All these compounds have physiological action on human systems. The alkaloids are used as analgesics, stimulants, anaesthetic, hallucinogens and antibacteria agents [16]. The glycosides are reported to posses strong anti – bacteria activities . They exist as antibiotics like streptomycin, neomycin, kanamycin, paromomycin, gentimycin and tobromycin [17]. Steroids are also another important class of plant constituents. Due to their relationship with sex hormone [1], vegetable containing steroids are given to expecting or breast feeding mothers to enhance hormonal balance [1]. Tannins are active detoxifying agents by precipitating the protein component and hence inhibiting their growth. This effect agrees with the mode of action of aminoglycoside antibiotic (e.g streptomycin in which they first latch onto surface receptor on the bacteria before exerting their action) . Other uses of tannins are to stop haemorrhage, to treat diarrhea, as well as local burns treatment where they precipitate proteins in the burned area, thus forming a protective layer [18]

Saponins which are highly toxic when injected into the blood stream, cause haemolysis of the red blood cells and destroy them . They are also used to reduce body cholesterol by preventing its re – absorption and suppressing rumen protozoa by reacting with cholesterol in the protozoan cell membrane thereby causing it to lyse . Saponins also find applications in foaming fire extinguishers, emulsifiers, insecticides, etc. [16]. Flavonoids are another plant constituents with antibacterial and antifungal properties. They are used in treating stomach ulcer and inhibit HIV – 1 integrase and HIV – 1 protease enzymes which are responsible for the HIV replication [1]

CONCLUSION

Out of twenty six amino acids known, *I. astragalina* leaves contains seventeen of which nine among are essential and eight are non-essential amino acids. Phytochemical screening revealed the presence of alkaloids, saponins, cardiac glycosides, flavonoids, and steroids. All these phytochemicals have potential therapeutic or physiological actions on human system, for that the leaves can stand as a potential source of some vital drugs.

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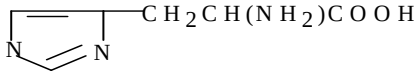
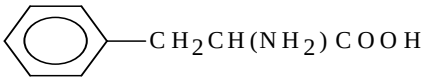
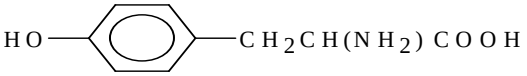
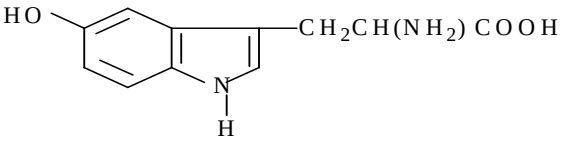
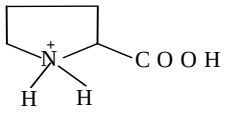
Appendix 1: Table showing common Amino acids commonly found in proteins

Amino acid/ Abbreviation	Structure
Glycine (Gly)	$H_2C(NH_2)COOH$
Alanine (Ala)	$CH_3CH(NH_2)\ COOH$
Valine (Val) *	$(CH_3)_2CH\ CH(NH_2)\ COOH$
Leucine (Leu) *	$(CH_3)_2CH\ CH_2CH(NH_2)\ COOH$
Isoleucine (Ile) *	$CH_3CH_2CH(CH_3)CH(NH_2)COOH$
Serine (Ser)	$HOCH(NH_2)COOH$
Threonine (Thr) *	$CH_3CH(OH)CH(NH_2)COOH$
Cysteine (Cys) *	$HSCH_2CH(NH_2)COOH$
Methionine (Met) *	$CH_3S(CH_2)_2CH(NH_2)COOH$
Aspartic acid (Asp)	$HOOCCH_2CH(NH_2)COOH$
Asparagine	$H_2NCO(CH_2)CH(NH_2)COOH$
Glutamic acid (Glu)	$HOOC(CH_2)_2CH(NH_2)COOH$
Glutamine (Glu-Q)	$H_2NCO(CH_2)_2CH(NH_2)COOH$
Arginine (Arg)	$H_2NC(=NH)NHCH_2CH_2CH_2CH(NH_2)COOH$
Lysine (Lys) *	$H_2N(CH_2)_4CH(NH_2)COOH$
Histidine (His)*	
Phenylalanine (Phe) *	
Tyrosine (Tyr) *	
Tryptophan (Try) *	
Proline (Pro)	

** Essential amino acids.*