

Original Research Article

Analysis of Amino and Fatty Acids Composition of *Senna alata* Seed

Author: R.A. Adigun^{1*}, U.Z. Faruq¹, U.A. Birnin Yauri¹, Y.J. Oyeniyi²

¹ Department of Pure and Applied Chemistry, Usmanu Danfodiyo University,
Sokoto

² Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto
activatedrasheed@gmail.com

ABSTRACT

Aim: To investigate the amino acids and fatty acids contents of *Senna alata* seed.

Study Design: To Collect and analyze the seed sample of *Senna alata* from Osun State in South Western Nigeria.

Place and duration of study: Seed samples of *Senna alata* were collected from Irepodun Local Government, Osun state, Nigeria at the start of the raining season in March, 2013.

Methodology: The collected samples were identified at Taxonomy unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, as *Senna alata*. The determination of the amino acid profile in the sample was determined using methods described in literature—while the determination of the protein content was done ~~etermined~~ using micro Kjeldahl method. The amino acid was analyzed using a Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM). The fatty acids content was determined by converting the fatty acids to fatty acid methyl esters (FAMES), after which it was loaded to a gas chromatography coupled with mass spectrometer for analysis.

Results: The protein content of the seed was $12.75 \pm 0.05\%$ while the amino acids content revealed glutamic acid and aspartic acid as predominant non essential amino acids while leucine as the most predominant essential amino acid. The sample also contains appreciable amounts of other essential amino acids. The fatty acid content of *Senna alata* seed oil revealed unsaturated fatty acid; linoleic acid as the major fatty acid (34.26%), followed by saturated fatty acid; palmitic acid (13.73%). The oil was having more unsaturated fatty acid (51.90%) than the saturated fatty acid (43.34%).

Conclusion: *Senna alata* seed is a good source of valine, isoleucine, leucine, phenylalanine, tyrosine and threonine. The high amount of linoleic acid and palmitic acid also suggest the edibility of *Senna alata* seed oil.

Keywords: amino acids, fatty acids, essential amino acids, non essential amino acids, *Senna alata*

1. INTRODUCTION

Researchers have shifted their interest towards discovering new potential food sources because of growing demands for protein and other energy supplying foods like fats and oils to sustain the increasing world population [1]. ~~The most important sources of protein are~~ Grain, oilseeds and legumes are the most important sources of protein, followed by meat and milk [2], therefore plant seeds are potential sources of protein.

Amino acid analysis is the method used to determine the amino acid content of proteins. Proteins are polymeric molecules consisting of covalently bonded amino acids organized as a linear polymer. Protein can be quantified using amino acid analysis, to determine the identity of proteins based on their amino acid contents following protein hydrolysis [3]. Amino acids are the building block of protein joined together by peptide bond through condensation polymerization with elimination of water molecule between NH_2 and COOH groups [3], and so hydrolysis of proteins using strong inorganic acid and enzymes yields a mixture of amino acids. The amino acid content of protein is important for human nutrition; it affects the taste, aroma and colour [3]. Proteins also have their contributione ~~insignificantly to~~ the physical properties of food through their ability to build or stabilize gels, foams, emulsions and fibrillar structures. The nutritional energy value of proteins given as ~~(17 kJ/g or 4 kcal/g)~~ is as high as that of carbohydrates [32].

Oils are among the most important industrial and domestic materials for different applications [4]. Due to the different categories of biological mixtures such as fatty acids, there is need for their separation and quantification to be able to determine the appropriate applications to which the oil can be directed. This is usually achieved using gas capillary chromatography [5]. Vegetable oils are natural products of phyto origin which consist of ester mixtures that are formed from glycerols with chains of fatty acid containing about 14 to 20 carbon atoms with different degrees of un-saturation [6]. Vegetable oils have important functions in food products, and they act as medium for fat-soluble vitamins (A, D, E, and K) [7]. They also provide energy and essential linoleic and linolenic acids, responsible for growth [8].

Senna alata is an example of those plants whose seeds have not been fully exploited for nutritional purposes. *Senna alata* is a flowering ornamental shrub (angiosperm) belonging to

the family ~~fabaceae~~fabaceae. It grows in many tropical countries including Nigeria. It grows up to 2-3 m high with bright yellow flowers. It is known as Asuwon oyinbo in Yoruba, Ilisko in Hausa, Nelkhi in Igbo, Ekunu kparagi in Nupe, Adedenghyi in Epira and ringworm tree or candle tree in English [9, 10]. Most of the previous studies on *Senna alata* have concentrated on the plant's leaves. Therefore, the aim of this study is to determine the amino acids and fatty acids composition of *Senna alata* seed.

2. MATERIAL AND METHODS

2.1 Sample collection

Dried seeds of *Senna alata* were collected from Irepodun Local Government Area, Osun State, Nigeria. The collection time was March, 2013. The plant was identified at the Taxonomy unit of Biological Sciences Department, Usmanu Danfodiyo University, Sokoto as *Senna alata*. The plant's parts used for identification were leaves, fruit and flower. The fruits were cut open to expose the seeds, after which the seeds were removed. The seeds of the plant were dried at ambient temperature (average 33°C), and then pulverized with the aid of pestle and mortar. The powder was sieved and stored in air tight polythene bag until it was required for use [11].

2.2 Protein Content and Analysis of Amino Acids

The protein content of the seed was determined ~~by using~~ micro-Kjeldahl method. The amino acid profile in the sample was determined using methods described by [12]. The sample was dried to constant weight, defatted ~~after that,~~ hydrolyzed, ~~and then~~ evaporated in a rotary evaporator. ~~The residue was~~ and loaded into the Technicon Sequential Multi-Sample Amino Acid Analyzer (TSM).

2.2.1 Defatting Sample:

The sample was defatted using chloroform/methanol mixture of ratio 2:1. 4.0 g of the sample was put in extraction thimble and extracted for 8 hours in soxhlet extraction apparatus [12].

2.2.2 Hydrolysis of the Sample

2.0 g of the defatted sample was ~~put~~weighed into glass ampoule. 7.0 cm³ of 6M HCl was ~~introduced into it while expelling~~added and oxygen was expelled by passing nitrogen into the ampoule ~~(this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine).~~ The glass ampoule was ~~then~~ sealed ~~using~~ with Bunsen burner flame and ~~then placed~~ut in an oven, set at 105°C for 22 hours. The ampoule was ~~allowed to~~

79 cooled before broken-open at the tip for opening and the constituent~~tent~~ was filtered to
80 remove the ~~humin~~humins [13].

81 The filtrate ~~gotten~~ was ~~then~~ evaporated to dryness at 40°C under vacuum ~~using in~~ a rotary
82 evaporator. The residue was dissolved with 5.0 cm³ of acetate buffer (pH = 2) and
83 ~~kept~~stored in plastic specimen bottles, which were ~~placed~~kept in the freezer. The
84 hydrolysate was then loaded into TSM analyzer [14].

85 2.2.3 Loading of the Hydrolysate into TSM Analyzer

86 The ~~total~~ amount of the hydrolysate ~~used in the loading~~loaded was 10 µL. This was
87 ~~discharged~~pended into the cartridge of the analyzer. The TSM analyzer is designed ~~forte~~ the
88 separation~~one~~ and analysis~~size of~~ free acidic, neutral and basic amino acids of the hydrolysate
89 [13].

90 2.2.4 Method of Calculating Amino Acid Values from the Chromatogram Peaks.

91 The net height ~~of-of~~ each peak produced by the chart recorder of TSM was ~~taken~~measured.
92 The half-height of the peak on the chart was ~~located~~found and width of the peak on the half
93 height was ~~precisely~~accurately measured and recorded. Approximate area of each peak
94 was ~~calculated~~then obtained ~~from the product of~~by multiplying the height ~~and~~with the width
95 at half-height [15].

96 The norleucine equivalent (NE) for each amino acid ~~present in~~the standard mixture was
97 calculated using the formula [15].

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$$\text{NE} = \frac{\text{Area of Norleucine Peak}}{\text{Area of each amino acid}} \dots\dots\dots (1)$$

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101 ~~The a~~ constant S_{std} ~~was calculated~~ for each amino acid in the standard mixture ~~was calculated~~
102 ~~as~~:

103 ~~Where~~ $S_{\text{std}} = \text{NE}_{\text{std}} \times \text{Molecular weight} \times \mu\text{MAA}_{\text{std}} \dots\dots\dots (2)$

104 ~~Finally~~, The amount of each amino acid present in the sample was ~~finally~~ calculated in
105 g/16gN or g/100g protein using the following formula:

106 $\text{Concentration (g/100g protein)} = \text{NH} \times \text{W@NH/2} \times S_{\text{std}} \times \text{C} \dots\dots\dots (3)$

$$\text{where } C = \frac{\text{Dilution} \times 16}{\text{Sample Wt (g)} \times \text{N\%} \times 10 \times \text{Vol. loaded} \times \text{NH} \times \text{W (nleu)}} \dots\dots\dots (4)$$

Where: NH = Net height; W = Width @ half height; nleu = Norleucine.

2.3 Analysis of Fatty Acids

Fatty acids composition of the oil was ~~analyzed~~^{determined} by converting the extracted oil using n-hexane to fatty acid methyl esters (FAMES) as described by [4]. The fatty acid methyl esters (FAMES) were prepared as follows: 1 cm³ of n-hexane was put into 0.1 cm³ vegetable oil and 1 cm³ sodium methoxide (1.55 g of NaOH in 50 cm³ of methanol) solution was added in the oil solution. The solution was stirred vigorously using magnetic stirrer for 10 s at 50⁰C. The solution was removed from the heater and 2 cm³ of 2M HCl was then added to prevent hydrolysis. The solution was left for 10 min to separate out the clear solution of fatty acid methyl esters from the cloudy aqueous layer. The upper layer was collected carefully which was injected into gas-chromatography system (Agilent network GC system, model 6890N) coupled with Agilent Technologies 5973 Network Mass Selective Detector with 7683B Series Injector. The spectra of the separated compounds were compared with the database of the spectra of known compounds saved in the NIST02 Reference Spectra Library. Data analysis and peak area measurement were carried out using Agilent Chemstation Software.

3. RESULTS AND DISCUSSION

3.1 Amino Acids Profile of *Senna alata* Seed

The protein content of the seed was found to be 12.75 ± 0.05 and the level of amino acids content in the seed is presented in Table 1. The result revealed the glutamic acid and aspartic acid as predominant non essential amino acids while leucine as the most predominant essential amino acid. The sample also contains appreciable amounts of other essential amino acids. The seed contained high amounts of glutamic acid, aspartic acid, and leucine, and low amount of the sulfur-containing amino acid, methionine. The seed contains a relatively high amount of essential amino acids.

In Table 2, the result of the comparison of the essential amino acids content of the *Senna alata* seed with the reference standard [163] was shown, from which valine has a chemical

score above the standard value of 100 while isoleucine, leucine, phenylalanine+tyrosine and threonine are very close to the standard value. This implies that *Senna alata* seed is a good source of valine, isoleucine, leucine, phenylalanine, tyrosine and threonine.

In spite of the presence of good quantities of essential amino acids as components of the proteins, the seed sample cannot be counted as good source of these amino acids as the overall protein content of the *Senna alata* seeds is low.

Table 1: Amino Acids Content of *Senna alata* Seed

(protein content: 12.75 ± 0.05)

Amino acid (abbreviation)	Composition (g/100 g protein)
Lysine (Lys)*	3.24
Histidine (His)*	2.16
Arginine (Arg)	4.51
Aspartic acid (Asp)	7.64
Threonine (Thr)*	2.78
Serine (Ser)	3.11
Glutamic acid (Glu)	8.67
Proline (Pro)	2.20
Glycine (Gly)	3.61
Alanine (Ala)	4.01
Cysteine (Cys)	0.62
Valine (Val)*	3.83
Methionine (Met)*	0.56
Isoleucine (Ile)*	2.35
Leucine (Leu)*	6.35
Tyrosine (Tyr)*	2.31
Phenylalanine (Phe)*	3.78

* Essential amino acid

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155 **Table 2: Essential Amino Acids Content of *S. alata* seed (g/100 g protein compared**
156 **with reference standard)**

Amino acid	Composition (g/100 g protein)	FAO/WHO/UNU (g/100 g protein)	Chemical score (%)
Ile	2.35	2.8	84
Leu	6.35	6.6	96
Lys	3.24	5.8	56
Met + Cys	1.18	2.8	42
Phe + Tyr	6.09	6.3	97
Thr	2.78	3.4	82
Val	3.83	3.5	109

157 Chemical score = %amino acid/reference standard x 100 result = 1/7

158 **3.2 Fatty Acids Profile of *Senna alata* Seed Oil**

159 The crude lipid content of the seed was found to be 4.79 ± 0.05 . The fatty acid content of
160 *Senna alata* seed oil was shown in Table 3, from which the unsaturated fatty acid; linoleic
161 acid formed the major constituent (34.26%), followed by saturated fatty acid; palmitic acid
162 (13.73%).

163 The oil was having more unsaturated fatty acid (51.90%) than the saturated fatty acid
164 (43.34%). The linoleic acid content is lower than the report of [174] as (45.5%) while the oleic
165 acid content was reported as 25.6%. The value of palmitic acid in this research was 13.74%
166 which is lower than 18.7% reported by [174]. The difference in the values could be as a
167 result of ~~due to~~ the different methods of analysis used. Gas Liquid Chromatography was
168 used by [174], using five fatty acid standards which then expressed the amounts as

percentage. But in this research, the Gas Column Chromatography used was coupled with Mass Spectrometer which was able to analyze all the compounds present in the oil with their respective amounts in percentage. Twenty six compounds were identified here compared to five fatty acids analyzed by [174].

The high linoleic acid present means the oil could be used in the prevention of distinct heart vascular diseases [185]. Linoleic acid is the main fatty acid present in vegetable oils like soybean oil and corn oil, while the highest saturated fatty acid in *Senna alata* seed oil; palmitic acid is the main fatty acid in palm oil [185]. This could suggest the edibility of *Senna alata* seed oil.

Table 3: Fatty Acids Content of *Senna alata* Seed Oil (%)

Fatty Acid	Composition (%)
12-methyltridecanoic acid	0.61
9-Hexadecenoic acid (Palmitoleic acid)	1.33
Hexadecanoic acid (methyl ester) (Palmitic acid)	11.27
n-Hexadecanoic acid (Palmitic acid)	2.47
Cis-10-Heptadecenoic acid	0.59
Heptadecanoic acid	0.62
9,12-Octadecadienoic acid (Linoleic acid)	34.26
Octadecanoic acid	7.29
9-octadecenoic acid (Oleic acid)	6.61
9,12-epithio-9,11-octadecanoic acid	0.99
11-eicosenoic acid	1.72
Eicosanoic acid (Arachidic acid)	4.49
15-Hydroxyl-9,12-octadecadienoic acid	1.78
9,10-dihydroxyoctadecanoic acid	1.53
6,9,12-Octadecatrienoic acid (γ-linolenic acid)	0.72
Heneicosanoic acid	0.70
9,10-Methylene-octadec-9-enoic acid (Sterculic acid)	2.26
Octadecanoic acid (Stearic acid)	0.99
20-methylheneicosanoic acid	3.96
Tricosanoic acid	1.59

Tetracosanoic acid	4.45
Pentacosanoic acid	1.14
Hexacosanoic acid	1.24
Others	7.42

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180 **4. CONCLUSION**

181 Although the seed has low protein content of 12.75%, *Senna alata* seed is a good source of
 182 valine, isoleucine, leucine, phenylalanine, threonine and threonine. This implies that the seed
 183 must be supplemented with other protein rich foods if it is to be taken in diet. The higher
 184 amount of unsaturated fatty acids confirmed the liquid state of the oil. The high amount of
 185 linoleic acid and palmitic acid also suggests the edibility of *Senna alata* seed oil.

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