Amino and Fatty acids profile of Merremia Hederacea seed oil

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ABSTRACT

Background: *Merremia hederacea known as ivywood belongs to class of underutilized plant. Vegetable oils are utilized 90% of the time for dietary purposes. The need to harness underutilised plant as alternative for the production vegetable oil with less competitiveness has become imperative.*

Objectives: *The objective of this research was to determine the amino and fatty acid profile of M. hederacea seed oil.*

Methods: *Oil was extracted from pulverized seed using petroleum ether. The extract was subjected to column chromatography in the order of n-hexane, ethyl acetate and methanol. The amino and fatty acid compositions of the n-hexane fraction were analyzed using gas chromatography mass spectrometry. Data were analysed using ANOVA with the statistical package Mini tab version 14.0.*

Results: *The amino acids detected were Asp (2.18±0.012), Thr (0.96 ±0.01), Ser (4.04±0.023), Gly (1.51 ±0.017), Val (4.63 ± 0.015), Phe (1.45 ±0.012), Pro (2.98±0.011), Leu (2.30±0.00), Met (0.89±0.008), Ile (0.85±0.014), Tyr (4.62±0.014), Trp (3.31±0.006), Glu (3.74±0.014), His (1.44±0.017) and Arg (1.78±0.012), with Val been most abundant. The essential and non-essential amino acids were 46.3 and 53.7% respectively. Eleven fatty acids detected were octanoic, decanoic, hexadecanoic, 9-octadecenoic (oleic), pentadecanoic, palmitoleic, 9,12-octadecadienoic, 9,12,15-octadecatrienoic, octadecanoic, eicosanoic and tetracosanoic acids, with oleic acid (22.84%) being most ample. Linoleic and linolenic acids were 8.23 and 4.61%, respectively.*

Conclusion: *The oil is relatively abundant in saturated fatty acid (60.77 %) and could be a better replacement for the available vegetable oils in the market. The oil can have a positive impact on nutritional and health status due to the presence of essential amino and fatty acids.*

Keywords: *Merremia hederacea,* valine, oleic acid, gas chromatography, essential fatty acids

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INTRODUCTION

Oil seeds serve as major sources of edible oil as the demand for vegetable oil increases (1). The need to explore underutilized plant species especially those not cultivated for food become an option to address the challenges associated with continuous increase in the demand for oil both domestically and industrially. *Merremia hederacea* seed oil belongs to underutilized oil.

Oil is basically triacylglycerol of plant origin (2), having varying nutritional composition in terms of amino acids, fatty acids and some trace minerals and vitamins. Amino acids are building blocks of proteins that are linked together in different orders to generate variety of protein critical to life and are also important precursors of many biosynthetic pathways (3). Some of these amino acids can be synthesized by humans within the biological systems while others cannot. The amino acids that can be synthesized by the biological system are referred to as non-essential amino acids while those that cannot be synthesized are referred to as essential amino acids. The essential ones thus need to be provided in the diet (4). The proteins in which these amino acids are used to build have wide range of functions. The function ranges from catalytic activity (enzymes), transport (haemoglobin, myoglobin and albumin) and structural (actin and myosin) functions, growth, to repair of worn-out tissues. *Merremia hederacea* herb is climber belonging to the family of Convolvulaceae (5). It is an indigenous plant commonly found in tropical Africa and well distributed in south Asia, Australia, India, China and Bangladeshi (6). It grows mostly in riverine forest and grass lands. The fruits are globose capsules obscurely 4-anguled, valved wrinkled (7). Before now, no nutritional value has been attributed to *M. hederacea* seed oil. Hence, the need to evaluate the amino acids and fatty acids constituents of the plant seed oil in order to ascertain its nutritional and health benefits

MATERIALS AND METHODS

Materials

The plant was collected in its natural habitant at Wuya village specifically at Latitude 9^{0}10' 0 "N.^{0} and Longitude 5^0.49' 60 ^{0} E using Global Positioning System (GPS) in Lavun local government area of Niger State. The plant was identified at National Institute for Pharmaceutical Research and Development (NIPID), Idu-Abuja, where voucher specimen was deposited (NIPRD/H/6754). The whole plant sample was washed under running tape water and air dried at room temperature. The seeds were harvested from dried whole plant by hand picking and thereafter the pulverized using mortar and pestle, and kept in an air tight container for further analysis.

Reagents

All reagents used were of standard and analytical grade.

Oil extraction procedure

Five hundred grams of the pulverized seed was extracted under reflux with petroleum ether. The marc was filtered and concentrated on waterbath. The extract was subjected to column chromatograph using n-hexane, ethylacetate and methanol. Two grams of the extract was dissolved in petroleum ether and mixed silica gel (60-120 mesh) sufficient to saturate the solution. The mixture was air dried to eliminate the solvent. The mixture was loaded on to a packed column and n- hexane was used to run the column first. Each fraction was monitored using UV lamp on the spotted TLC plates from each fraction. Ethylacetate was introduced in to the column, when there was no component detected in the elute using n-hexane. The oily n-hexane fractions were pooled together. The oil was stored in a clean, air-tight dark bottle and placed in wooden box protected from direct sunlight at a temperature between 27-30 ^{0}C The n-hexane fraction was further subjected to amino and fatty acid analysis.

Amino acid analysis

Gas chromatography mass spectrometry was used for the identification and quantification of amino acid constituents of the oil. The amino acid peaks were identified by comparison of the retention time of individual peaks of different amino acids with the retention times of amino acid standard from Sigma Aldrich (Sigma-Aldric, St Loius, USA)

Defatting sample

The sample was defatted using chloroform/methanol mixture of ratio 2:1. 1.0 g of the sample was put in extraction thimble and extracted for 1 hour in soxhlet extraction apparatus.

Extraction procedure and derivatization of amino acids

100.0 mg of the defatted sample with 1 g of fine quartz sand were thoroughly ground in a thick ceramic dish and homogenized with 5 ml distilled water. After centrifugation for 5 min, 0.5 ml of the supernatant was passed slowly through a separating funnel filled with wools and eluted with 4M NH_4OH.
The derivatization method included esterification of the carboxylic function step, by adding 200 ml butanol=HCl 3M, for 1h at 110^{0}C, followed by acetylation of the amine functional group using 100 ml trifluoroacetic anhydride, for 20min at 60^{0}C. After the esterification and acetylation reactions, the excess solvents were removed by nitrogen. The dried derivatives were dissolved in 500 ml ethyl acetate and analyzed by GC–MS.

Analysis of amino acid

The GC–MS analysis was performed using a Varian 3800/4000 gas chromatograph / mass spectrometer. The samples were injected into the gas chromatograph on Agilent capillary column, 30m x 0.25mm, 0.25μm film thickness using a temperature program from 70^{0}C, 2min, 5^{0}C/min to 110^{0}C, 10^{0}C/min to 290^{0}C, and 16^{0}C/min to 300^{0}C. The flow rate of nitrogen, the carrier gas, was 1mL/min. In total, 1 μL of each sample was injected in a split mode utilizing a TriPlus autosampler. The following conditions were followed: transfer line temperature 250^{0}C, injector temperature 200^{0}C; ion source temperature 250^{0}C, splitter: 10:1. The electron energy was 70eV and the emission current, 100mA. The 15N-methionine was used as the internal standard for quantitative determination.

Amino acid standards were used for the validation of the method. This involved injecting standard solutions of amino acids, following the above-presented extraction and derivatization procedure. A linear regression curve was produced using amino acid standards with known concentrations (0–100 µg/mL) and 50 µg/mL of an internal standard, from the curve the concentration of each amino was determined.

Fatty acid analysis

The oil was hydrolyzed and the fatty acids were converted to their fatty acid methyl derivatives. The constituent fatty acids and their concentrations were determined using gas chromatography mass spectrometer.

Data analysis

Data were analysed using Analysis of variance (ANOVA) of the statistical package Mini tab version 14.0. Results were expressed as mean ± standard error of mean of triplicate determinations.

RESULTS

A yellow edible vegetable oil extracted from the seed of *M. hederacea* had a yield of 36g per 100g of the sample.

Amino acid profile

The results for amino acid composition of *Merremia hederacea* seed oil is presented in Table 1. The peak from the gas chromatogram detected 16 amino acids with different retention time. The predominant amino acids in *M. hederacea* were valine (4.63 ± 0.015) and serine (4.04±0.023) while methionine (0.89±0.008) and isoleucine (0.85±0.014) had the least concentrations. The total essential amino acids which include threonine, valine, phenylalanine, leucine, methionine, isoleucine, tryptophan and histidine was 46.28% (17.61 mg) while non-essential amino acids comprised 53.72% (20.44 mg).

Fatty acid Profile

Table 2 represent the fatty acid composition of *M. hederacea* seed oil. Twelve fatty acids were detected in the oil, compounds 4 (9-Octadecenoic acid) and 10 (Oleic Acid) are isomers of the same compound. The fatty acid most abundant in the oil is oleic acid (10.21 and 12.63 for Z and E isomers respectively) while eicosanoic acid (1.90) has the least concentration. The non-essential fatty acids predominant (87.02 %) in *M. hederacea* seed oil while esstential fatty acid contributed 12.84 %.

DISCUSSION

The seed of *M. hederacea* gave an appreciable percentage of oil yields. Seeds are good source of edible oils, having both nutritive and calorific value, which make them vital in diet (8). Oils from seeds can be used in the production of margarine, cooking oil, ice-cream and dietary additive in infant food.

The main purpose of consuming diet is to nourish the body with vital nutrients. Protein containing diets provide amino acids required for growth and repair of the body's worn-out tissues (9). Amino acids are substrates for protein synthesis. Nine amino acids cannot be synthesized de novo but must be supplied via dietary intake (10). *M. hederacea* seed oil contain all essential amino acids (except lysine that was not tested) with valine being the most abundant. Additionally, the non-essential amino acids become essential during certain physiological condition like stress and illness. So there is an additional requirement even for the non-essential amino acids in these conditions (11).

M. hederacea oil is also rich in sulphur containing amino acids. Sulphur containing amino acids are normally scarce in most protein of plant origin (9). *M. hederacea* oil could serve as a better substitute to all available vegetable oils in use today.

Twelve fatty acids were detected and quantified but compound 4 (9-Octadecenoic acid, (E)-) and compound 10 (Oleic Acid, (Z)) are isomers of the same compound, oleic acid. So the numbers of fatty acids detected are eleven. Apart from the fact that these eleven fatty acids present in *M. hederacea* seed oil can generate energy via Β –oxidation, act as storage molecules in the form of triacylglycerol, they can also elicit some pharmacological and physiological functions. Fatty acids are known to alter immune cell functions as they change the makeup of cell membrane phospholipids to membrane lipid fluidity, signal transcription factors, and bioactive synthesis of lipid mediators (12).

Oleic acid which is the most abundant fatty acid in *M. hederacea* seed oil is a potent immune-stimulant according to the works of Jebali *et al*., (13) and Ishak (14). Oleic acid act by activating various immuno-competent cellular pathways and also modulate wound inflammation by inducing wound healing. Other fatty acids known for strengthening immunity and improving growth include oleic, palmitoleic, 6,9-octadecenoic, 8,11-eicosadenoic, docosahexanoic acid (DHA) and eicosapentaenoic acids (EPA) (15). The abundance of oleic oil in this plant could be responsible for the immune-modulatory potential ascribed to this particular in previous research work (16). This oil can serve as alternative source of edible oil, as the demand for vegetable oil increases. The oil is good for human consumption because it is also rich in essential fatty acids (linoleic and lenolenic acid). Linolenic acid is an omega-3 fatty acid from which other omega-3 fatty acids (EPA and DHA) are synthesized (17). Linoleic acid is an omega-6 fatty acid from which other omega-6 fatty acids (gamma linolenic and arachidonic acids) can be synthesized (18). The

Table 1: Amino acid composition of *Merremia hederacea* seed oil.

Serial No	AMINO ACID	CONCENTRATION (mg/100g)
1	Aspartate	2.18±0.012
2	Threonine*	0.96 ±0.01
3	Serine	4.04±0.023
4	Glycine	1.51 ±0.017
5	Valine*	4.63 ± 0.015
6	Phenyl alanine*	1.45 ±0.012
7	Proline	2.98 ±0.011
8	Leucine*	2.30±0.00
9	Methionine*	0.89±0.008
10	Isoleucine*	0.85±0.014
11	Tyrosine	4.62±0.014
12	Tryptophan*	3.31±0.006
13	Glutamate	3.74±0.014
14	Histidine*	1.44±0.017
15	Arginine*	1.78±0.012
16	Alanine	1.37±0.006
17	TEAA	17.61 (46.28%)
18	TNEAA	20.44 (53.72%)
18	TAA	38.05

Values represent the mean± standard error of mean, essential amino acids are indicated by asterisk (*). TEAAS=total essential amino acids TNEAA =total non-essential amino acid, TAA= total amino acids

Table 2: Fatty acid composition of *Merremia hederacea* seed oil

Peak	Name of compound	Molecular Formula	Molecular Mass	Composition (%w)
1	Octanoic acid	$C_8H_{16}O_2$	144	4.31
2	n-Decanoic acid	$C_{10}H_{20}O_2$	172	4.92
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	18.61
4	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	10.21
5	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	8.10
6	Palmitoleic acid	$C_{16}H_{30}O_2$	254	3.41
7	9,12-Octadecadienoic acid (Z,Z)-*	$C_{18}H_{32}O_2$	280	8.23
8	9,12,15-Octadecatrienoicacid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	4.61
9	Octadecanoic acid	$C_{18}H_{36}O_2$	284	18.95
10	Oleic Acid, (Z)	$C_{18}H_{34}O_2$	282	12.63
11	Eicosanoic acid	$C_{20}H_{40}O_2$	312	1.90
12	Tetracosanoic acid	$C_{24}H_{48}O_2$	368	3.98

* indicates the essential fatty acid

importance of omega-3 and omega-6 fatty acid is in the ability of EPA in them to prevent blood clot, reduce pain and swelling associated with inflammation while DHA is required for growth and development of functional brain in infant and maintenance of normal brain function in adults (17, 19). Arachidonic acid is an inflammatory mediator within biological system (20).

The *M. hederacea* seed oil is composed of 60.77% saturated fatty acids and 39.3% unsaturated fatty acids. Unsaturated fatty acids could be mono or poly unsaturated fatty acid. Oleic and palmitoleic acids belong to the class of mono unsaturated fatty acid. Palmitoleic acid is a lipokine that is capable of regulating different metabolic processes such as increasing insulin sensitivity in muscle, B- cell proliferation, prevention of endoplasmic reticulum stress and lipogenic activity in white adipocytes (21).

Poly unsaturated fatty acids can reduce inflammation and increase longevity (Canicylio *et al*., 2023-). Polyunsaturated fatty acid lower plasma cholesterol, triacylglycerol levels, LDL concentrations, inhibit monocyte formation, inflammation, adhesion and reduce atherosclerosis and other cardiovascular diseases (23, 24). Polyunsaturated fatty acids, especially omega-3-fatty acids have been associated with 8% reduction of chronic diseases such as diabetes, cancer, Alzheimer and cardiovascular disease risks. Its consumption is also known to decrease anxiety and depression (25).

This oil could be use as cooking oil, production of margarine and food/feed supplements. It can also have application in confectionaries (bread, crisps, cookies, sandwiches) due to its organoleptic properties (26, 27). The oil can also supply essential amino acids and fatty acids needed by the body via exogenous sources. The oil could be useful to manufacturers in industries that require alternative sources of oil to fortify food/feed, pharmaceutical industries in drug production.

In addition to the presence of some essential amino acids in Mh seed oil, the presence of omega 3 and omega 6 fatty acids (linoleic and linolenic acids) are added advantages since these fatty acids are not synthesized by the human body. The oil from this underutilized plant is rich in both essential amino acids and fatty acids and can serve as a source of essential nutrients in human and animal diet. The oil could also be used to supplement the diet of malnourished children, convalescents and augment animal feed.

CONCLUSION

The oil from this underutilized seed contains in valine, linoleic and linolenic acids and could provide some quantity of the essential amino and fatty acids required for growth and development. The oil can also serve as alternative source of vegetable oil, to reduce competitiveness among available ones. The oil has some nutritional value due to the presence of these important amino and fatty acids.

RECOMMENDATION

The oil could serve an alternative source of oil to the available ones and further study should be conducted to monitor if any effect will manifest after a long-term usage.

CONFLICT OF INTEREST

There was no conflict of interest.

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