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Physico-chemical study of Adenanthera pavonina seed oil growing in Democratic Republic of Congo

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Abstract : The chemical analysis of the seed oil of *Adenanthera pavonina* growing in Democratic Republic of Congo revealed that this oil contained 16.20% of saturated fatty acids and 83.50% of unsaturated fatty acids. Linoleic and oleic acids make up 69.3% of the total fatty acids and were abundant unsaturated fatty acids accounting for 52.6 and 18.7% respectively, while palmitic acid was the abundant saturated fatty acid accounting for 7.5.%. The oil also contained appreciable amount of proteins (31.04 ± 2.30 g/100 g), essential amino acids (45.92%), non essential amino acids (53.83%), carbohydrates (53.05 ± 1.06 mg/100mg, crude fat (18.03 ± 1.41 mg/100 mg), starch (44.75 ± 1.04 mg/100 mg) and total amino acids (99.30%), phospholipids (37.3 ± 1.70%) and triglycerides (34.64 ± 1.22% as the abundant lipid classes. In addition, the level value of saponification (166.31 ± 0.04 mg KOH/g) and peroxide (30.12 ± 1.62 mEq/kg) indices indicated a resemblance of the oil to those used for food. Mineral elements composition showed sufficient amount of Na, P, Fe, Zn, Cu, K and Al. among which K (1284.22 mg/100 g) and Na (842.35 mg/100 g) were the most abundant. The extracted oil is characterized by its white translucent colour, odourless and becomes solid at room temperature (30°C).
Keywords: *Adenathera pa*vonina, Fabaceae, seed oil, physico-chemical, characterization.

1. Introduction

Adenathera pavonina L. **(**Fabaceae) (Synonym: *Adenanthera gersenii* Scheff, *A. polita* Miq, *Corallaria parvifolia* Rumpf.) is a tree species used for its timber in several countries in the word. The plant is useful for nitrogen fixation and it is often cultivated as a forage, a medicinal plant and urban tree. The beauty of the its seeds, their uses as beads and for necklace and their nourishing qualities have been combined to scatter the plant (1).

A. panonia is known as a food tree because its seeds and young leaves are cooked and eaten by people (2). Also, in some countries, the seeds are roasted, elsewhere boiled, roasted and shelled before eaten with rice by children and adults alike (3). In Democratic Republic of Congo (D.R.Congo), the plant is not known as medicinal plant, but the oil from the seeds is used as food for humans and animals. To our best knowledge, there is very little studies previously reported on the chemical composition of this seed oil. (4). In the present study, an extensive analysis was undertaken aiming the determination of physical characteristics and chemical composition of the seed oil.

2. Materials and methods

2.1. Plant material

Mature seeds of *A. pavonina* was collected in Kinshasa in September 2011. The plant was identified by Mr N. Nlandu of the Institut National d'Etudes et de Recherches en Agronomie (INERA), Faculty of Sciences, University of Kinshasa where a voucher specimen AP 00432011 was deposited in the herbarium of this institute.

2.2 . Extraction of oil

150 g of powdered cotyledons of seeds were submitted to a Soxhlet extraction with petroleum ether (1000 ml) for 1 h. The extract was filtered and evaporated *in vacuum* yielding oil which becomes white translucent solid at room temperature (26.55 g, 17.70%).

2.3. Reagents

Ethanol, methanol were of HPLC quality and purchased form Fisher Scientific UK Limited, Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG, UK. Petroleum ether, acetonitrile, *n*-hexane, diethyl ether and acetic acid were of HPLC grade and obtained from Acros Organocs, New Jersey, USA. Standard amino acids such as Aspartic acid, Glutamic acid, Alanine, Aspargine, Cysteine, Glutamine, Glycine, Histidine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tyrosine and Valine in addition to the eluting solvents and derivatization agent were all purchased from Sigma Aldrich laboratories, Seeize, Germany. Highly purified water (milli-Q-Millipore) was used throught for preparation of all buffers and reagents.

2.4. Determination of different indices, ash, moisture, and unsaponifiable matter

Moisture, acid, iodine, peroxide and saponification indices, and unsaponifiable matter were evaluated according the respective methods described by Gustone et al. (5).

2.5. TLC analysis of lipids

Total lipids from *A. pavonina* seeds were extracted by Sox let extraction using diethyl ether as solvent (6). They were anlayzed by TLC on silica gel (Merck) plates (0.25 mm thickness layer) using benzene diethyl ether/*n*-hexane/acetic acid: 80:20:2 as mobile phase. The separated fractions of the total lipids were visualized by exposure the plates to iodine vapor after drying. All lipid fractions were identified on TLC by comparing their Rf values with those of known available lipid standards. For the quantitative analysis, different lipid fractions were scanned using Shimadzu TLC scanner (C-S-910). The area of each peach was measured by the triangulation method (7). The percentage of each class was calculated using the following formula:

% lipids = Area of each peach/Total peaks area x 100 (8)

2.6. Fatty acids analysis

2.6.1. Sample preparation

An amount of 7.1 g of oil were solublized in 1 ml *n*-hexane. From this stock solution, 100 µL was transferred into a tube and *n*-hexane was evaporated under nitrogen to dryness. The dried residue was solubilized in 50 µL methanol, vortexed for 20 secondes for dissolution and, 50 µL of

dimethylsilyldiazomethane 0.1 M in *n*-hexane was added. The mixture was vortexed for 20 secondes and then kept at 60°C for 30 minutes. After cooling at room temperature, the solvent was evaporated under nitrogen to dryness and the residue redissolved in 1 ml *n*-hexane which was transferred into a GC injection vial for analysis.

2.6.2. GC-MS analysis

It was performed on an Agilent 6890-5973 GC-MS apparatus operated in electron ionization (EI) mode, equipped with a DD-5 capillary column of 30 m x 0.25 mm x 0.25 µm (JC W Scientific), and a flame ionization detector. The ion source, quadrupole and interface temperatures were at 250, 150 and 300°C respectively. Helium was used as a carrier gas at constant flow (1.0 ml/min). The electron multiplier voltage was set at 2000 V. Once, 10 µL of the methylated sample was injected in pulsed splitless mode (injector temperature 300°C, pressure pulse: 20 psi, pulse time: 4.50 min, splites time: 1.50 min). The temperature of the DD-5 capillary column was programmed from 90°C (1.50 min) to 300°C at the rate of 5°C/min, stay for 10 min. Full scan EI spectra (m/z 50-750) were recorded during the whole analysis time. The methylated fatty acids were identified by their m/z compared to those reported in the literature and by comparing their retention time to those of standard fatty acids made in the same experimental conditions described above. The corresponding fatty acid to each methylated fatty acid was deduced (m/z-15) (5).

2.7. Determination of proteins and carbohydrates

The determination of crude proteins was carried out in evaluating the nitrogen content by the standard micro-Kjedahl method using a digestion apparatus (Kjeldathern System KT 40) and titration system (9) .

Crude proteins content was calculated as N% x 6.25. For carbohydrates, proteins were removed from the ethanolic solution of the oil after treatment with lead acetate. Carbohydrates were determined using the anthron method as follows: 1 ml of the treated oil as described above was incubated with 5 ml anthron solutions (0.12 g anthron in 100 ml 6.5 M H_2SO_4) at 90°C for 10 min. The absorbance of the green product resulting was measured at 630 nm. Glucose equivalents were calculated from a standard curve obtained with pure analytical grade glucose. The identification of individual sugars was made by TLC on silica gel plates (Merck, thickness layer 0.25 mm) using methanol/ ethyl acetate/ acetic acid/water (1:6:5:1. Sugars were identified by spraying the plates with a solution of 50% diphenylanine in aniline (35 ml), 100 ml acetone and 15 ml phosphoric acid. Spots were revealed after heating plates at 110°C for 10 min. Glucose, rhamnose, galactose, sucrose and fructose with high analytical grade were used as reference sugars (10).

2.8. *Determination of amino acids*:

2.81. Derivatization

Amino acids were determined according to the procedure previously described by Montaňo et al. and Kabelová et al.(11,12). Briefly, dried sample (20 mg proteins) was hydrolyzed with 1 ml HCl 6 M in a glass tube and was put under nitrogen for 1 min to remove air. The hydrolysis was carried out at 110°C for 24 h and then filtered through a 0.45 µm filter. An aliquot of 100 µL was evaporated to dryness under nitrogen. The dried residue was vortexed into 20 µL HCl 20 mM containing L-α-amino-*n*-butyric acid as an internal standard. The **derivatization** was carried out with 6-aminoquinolyl-N-hydroxysuccinimethyl carbonate (AQC). Then 60 µL of borate buffer 200 mM and 20 µL of ACCQ-Fluor reagent were added. The mixture was vortexed for 2 min. The vial was heated in a heating block at 550°C for 10 mim. For standard amino acids, 10 µL of the solution was combined with 70 µL of borate buffer and 20 µL of ACCQ-Fluor reagent. They were future treated in the same conditions described above. Then, 10 µL of sample and 5 µL of standard amino acid solutions were injected into HPLC.

2.8.2. *Analysis*

The analysis of amino acids was conducted on a HPLC system with Muth λ fluorescence detector (Water, Milford, Massachussetts, USA) equipped with a C_{18} column (4 µm x 150m x 3.90 mm) helded at 37°C, was used for the analysis (excitation at 250 nm and emission at 395 nm). The system of eluents was constituted with 3 solvents: A: acetate-phosphate buffer, B: acetonitrile and C: Milli-Q-watter. The separation programm was follows: 0 min A-100%, B-0%, C-0%; 0.5 min: A-99%, B-1%, C-0%; 18 min: A-95%, B-5%, C-0%; 19 min: A-91%, B-9%, C-0%, 29.5 min: A-83%, B-17%-C-0%; 33 min: A-0%, B-60%, C-40%; 36 min: A-100%,

B-0%, C-0%. The HPLC peaks were identified by comparing the retention time data obtained with those of amino acids reported in the literature (13).

Sulfurs containing amino acids were identified after performic acid oxidation (9) while tryptophan was colorimetrically determined after alkaline hydrolysis (4.2 M NaOH) according the method previously described by Basha and Roberts (14).

2.9. Mineral analysis

5 mg of *A. pavonina* seed oil was incinerated at 450°C for 12 hrs in a moufle furnace. The acid digest was prepared by oxiding the sample with nitric/perchloric acids (2:1). Sodium (Na) and potassium (K) amounts were evaluated by flame photometer (Flame Photometer model-EEC). The minerals, such as calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) were determined with an atomic absorption spectrophometer (Perkin-Elmer Model 5000) while phosphorus (P) was determined by phosphovanado-molybdate (yellow) method according to AOAC (9). All mineral elements were quantified against standard solutions of known concentrations that were currently analyzed in the same experimental conditions (8).

3. Results and discussion

The obtained amount of oil from the seeds of *Adenanthera pavonina* collected in Kinshasa in 2011 was 17.70%. This amount of oil was low compared that from the same plant collected in Mbandaka in 1975 (25%) (4), two different provinces of DRCongo. The variation in the oil content within the same country can be attributed not only to environmental conditions, time of collection and geographical areas (15), but also to some extents to the age of the plant material (seeds), the nature of solvent and method used for the extraction. In the present study, the extracted oil was characterized by its white translucent colour, odourless and becomes immediately in solid state at room temperature (30°C). The colour of this oil was different to that extracted form the seeds of *A. pavonina* collected in Nigeria (light yellow) (2) and Sri Lanka (pale yellowish) (16) ; these oils becomes solid after a storage at 4°C and are liquid at room temperature.

The physico-chemical characteristics of the extracted oil are presented in Table 1. With a specific gravity of 0.96 g/cm^3, *A. pavonina* seed oil is denser than most drying oils. This value was slightly higher than that from the same plant part collected in Nigeria (0.94) and soybean (0.92) (2). The relative index (RI) (1.49) was closed to that of *A. pavonina* seed oil from Nigeria (1.467) (2). The oil had a high iodine indice (94.17 ± 2.07g Iodine/100 g) and high RI reflecting its high degree of unsaturation. The iodine indice value was higher than that from the same plant collected in Nigeria (83.3 g Iodine/100 g) and lower than that of soybean oil (126.0 mg/100 g) (2).

The amount of peroxide indice (30.12 ± 1.62 mEq/kg) and unsaponifiable matter (4.15% ± 0.14) of the oil were comparable to that of previously reported from Nigeria (29.6 mEq/kg and 4.10% (2), but were higher than that seen for soybean oil (0.20 mEq/kg and 1.50% respectively) (2). In comparing with high peroxide indice values reported in other oils (17), the Codex Alimentarius Commission (18) stipulated a permitted maximum peroxide indice level more than 10 mEq/kg such as oils from coconut, cottonseed rapeseed and soybean oils. Thus amount of unsaponifiable matter was favourably comparable to that of *A. pavonina* seed oil from Nigeria (4.10%) (2). According to Ezeagu et al. (2), the high values of proxide and unsaponifiable matter point to a resemblance to oils processed for food. The high amount of peroxide indice may result from the extraction procedure or conservation conditions. (19)

The acid indice of *A. pavonina* seed oil reported in the present study (2.97 ± 0.06 mg KOH/g) was higher than that of soybean oil (1.0 mg KOH/g), but is in agreement with the limits recommended for virgin and non virgin edible oils (< 10 mg KOH/g). Maximum acid values of 10 mg KOH/g and 4 mg KOH/g was stipulated for virgin palm oil and coconut oil respectively (18).

The saponification indice (166.3 mg KOH/g) was lower than the values reported in the literature for cottonseed oil (189-198 mg KOH/g) and coconut oil (248-265) (18) and other common oils (20). It was however lightly higher than that of *A. pavonina* seed oil from Nigeria (164.1 mg KOH/g) (2).

Crude proteins (31.04 ± 2.34 g/100 g) in this oil was lower than that found in Soybean oil (36.70 g/100 g) and other edible oils from Watermelon seed kernel and Pumpkin seed kernel flour (35.7 and 36.5 g/100g), but higher than seen in Paprika seed oil (24.4 g/100 g) (21), cowpea, maize (23.1 and 8.9 g:100 g) and some cultivated legume oils (22,23,24) but slightly high compared to that of *A.pavonina* seed oil from Nigeria (29.44 g/100g) (2).

In addition, the oil contained appreciable amount of carbohydrates (53.05 g/100 g) which were high compared to that seen in soybean (33.95%). It was however low compared to that of cowpea and maize oils (67.8 and 74% respectively, and *A. pavonina* seed from Nigeria (41.95%) (2). Simple sugars were identified by TLC on silica gel plates (Merck, thickness layer 0.25 mm) as glucose, fructose and sucrose in the presence of standards sugars. It's worth noting that some simple sugars were identified in *A. pavonina* seed oil for the first time in this investigation.

Crude lipids or fats (18.34 ± 1.41 g/100 g) was comparable to that of *A. pavonina* seed oil from Nigeria (17.99 g/100g); and soybean oil (20.10 g/100 g), but higher than that of cowpea and maize oil (5.0 and 3.9 g/100 g (23). The composition of lipid showed high appreciable amounts of phospholipids (37.3 ± 1.7%) and triglycerides (34.6 ± 1.2). The amounts of diglycerides such as 1,2-diglycerides, 2,3- diglycerides, 1,3-diglycerides and sterols were ranged from 0.2 to 3.4% (Table 2). The level of the total lipids or fats was high compared to that of cowpea oil (15.0%) and maize oils (3.9%), but lower than that of soybean oil (20.10%) oils (2). To our best knowledge, it is the first time to report the presence of these classes of lipids in *A. pavonina* seed oil.

The high amount of the total ash (4.47 ± 2.40 g/100 g) indicated that the oil is a good source of mineral elements. The reported level of the total ash in the present study was higher than that seen in *A. pavonina* seed oil from Nigeria (2.37 g/100g) (2), watermelon seed kernel, pumpkin seed kernel seed oils (3.60 and 3.21 g/100g), but was slightly lower than that of Paprika seed oil (4.23 g/100g). It has been indicated that the amounts of potassium (1284.22 mg/100 g) and sodium (842.35 mg/100 g) are the most abundant of the mineral elements in *A. pavonina* seed oil. The levels of these two elements seem to be higher than those found in basic cultivate products (21,25) and particularly in *A.pavonina* seed oil from Nigeria (1252.85 and 512.52 mg/100g respectively), soybean and cowpea oils (10 and 20 mg/100 g, and 192 and 96 mg/100 g respectively) (2). The amount of other analyzed mineral element being ranged from 2 to 12 mg/100 g (Table 3).

Table 1. Physico-chemical characteristics of the oil of *Adenanthera pavonina* seeds

	A. pavonina seed oil	*Soybean*
State (28.5°C)	Solid at room temperature	Liquide
Colour	White translucent	Brown
Odour	Odourless	n.d
Specific gravity	0.96 ± 0.02	0.92
Moisture (%)	7.39 ± 1.04	-
Refractive index	1.49 ± 0.34	1.47
Iodine indice (g Iodine/100 g)	94.17 ± 2.07	126.0
Peroxide indice (mEq/Kg)	30.12 ± 1.62	0.20
Saponification indice (mg KOH/g)	166.31 ± 0.04	193.0
Acid indice (mg KOH/g)	2.97 ± 0.06	1.00
Unsaponifiable matter (%)	4.15 ± 0.14	1.50
Carbohydrates (g/100 g)	55.03 ± 1.06	-
Starch (g/100 g)	44.75 ± 1.04	

n.d : not determined

Table 2. Composition and amounts (%) of crude proteins, crude lipids, free fatty acids and lipid classes in the oil of *A. pavonina* seeds.

Classes	Amounts
Crude proteins	31.04 ± 2.3
Crude lipids	11.03 ± 1.41
Free fatty acids	1.51 ± 0.02
Phospholipids	37.34 ± 1.73
Monoglycérides	1.37 ± 0.21
Dithylglycerols	
1,2-diglycerides	0.60 ± 0.21
2 ,3-diglycerides	0.42 ± 0.13
1,3-diglycerides	0.34 ± 0.11
Triclycerides	34.65 ± 1.24
Monoethylglycerols	3.47 ± 0.43
Stérols	2.09 ± 0.67

Table 3. Composition and amounts of mineral elements (mg/100 g) identified in the oil of *A. pavonina* seeds**.**

Composés	*A. pavonina*	Graine de soya*
Ash (g/100 g)	4.47 ± 1.04	-
Sodium	842.35	10.0
Potasssium	1284.22	192.0
Iron	11.71	-
Copper	2.04	-
Zinc	4.21	-
Magnesium	4.63	4.3
Calcium	204	
Manganese	2.41	-
Phosphourus	5.02	-
Aluminium	9.65	-

FAO (1982). Food composition tables for the Near East. Food and Nutrition paper 26, FAO/UN, Rome. -: non disponible.

Free fatty acid content of the analyzed seed oil in the present investigation (1.51 ± 0.02%) was very close to that of corn oil (1.5%), *A. pavonina* seed oil from Nigeria (1.4%) (2) and other several seed oils .(21,26) This amount was however very higher than the range of 0.14 to 0.60% reported for groundnut, sesame and coconut seed oils (20) .

The identification profile of fatty acids (FAs) from *A. pavonina* seed oil collected in Kinshasa and their amount are illustrated in Figure 1 and Table 4 respectively.. There are wide variations in the content of FAs in the studied seed oil leading to differences in total saturated, total unsaturated, monounsaturated and polyunsaturated fatty acids. The FAs profile of the oil is dominated by the high amount of unsaturated fatty acids (UFAs) of 83.5% constituted with mono- and polyunsaturated FAs explaining its solid state at room temperature. The total UFAs content was higher than those of Mbandaka (49.9%) (4) and Nigeria (79.6%) (2).

Among the USFAs, results indicated that linoleic acid (52.60%) and oleic acid (18.70% were the most abundant FAs making up 70.3% of the total fatty acids. The level amount of linoleic acid was slightly lower than that found in soybean oil (53.7%), and *A. pavonina* seed oil from Nigeria (53.5%) (2). However, it was higher than that reported by Kabele et al.,(2) for the sample collected from Mbandaka (45.0%). This amount was close to values reported by Balogun and Fetuga (27) for other edible oils. The amount of oleic acid (18.7%) was comparable to that of soybean oil (17.2%). In other respects, the identified saturated acids (SFAs) represented 16.2% of the total FAs. Among them, palmitic acid (7.5%) was the dominant saturated fatty acid,

followed by lignoceric acid (2.7%). The amount of palmitic acid was almost in the same range while that of lignoceric acid was lower (3.5%) compared to those reported in *A. pavonia* seed oil from Nigeria (7.7%) (2). The amounts of other identified SFAs and USFAs were ranged from 0.2 to 2.5% (Table 4).

Table 4. Retention times (RT, minutes) and amount (%) of identified fatty acids in the oils of *A. pavonina* seed**.**

Fatty acids	RT	Amount (%)
1. Palmitic acid	24.10	7.5
2. Margaric acid	25.25	1.6
3. Linoleic acid	27.25	53.7
4. Oleic acid	27.40	18.7
5. Elaidic acid	27.45	0.2
6. Stearic acid	28.00	2.6
7. Methyl-ester linoleic acid	28.45	0.8
8. Cis-10-nonadécanoïc acid	28.61	1.4
8a. Unidentified acid	30.45	0.3
8b. Unidentified acid	30.80	0.1
9. Gadolic acid	31.00	7.3
10. Arachidic acid	31.39	0.6
10a. Unidentified acid	32.00	0.1
11. Erucic acid	34.20	2.3
12. Behenic acid	34.65	0.2
13. Nervornic acid	37.25	2.2
14. lignocéric acid	37.65	2.7
14a. Unidentified acid	38.68	0.2
14b. Unidentified acid	39.10	0.1
15. Cerotic acid	40.41	0.2
16. Myristic acid	-	
17. Palmitoleic acid	-	
18. Eicosadien acid	-	
19. Linolenic acid	-	

- : not detected fatty acids

The absence of lignoceric acid in the oil of some members of Leguminosae family previously reported by Balogun and Fetuga (27) is not in agreement with the results of the present study and other studies (2,16)., Sotheeswaran et al. (28) have reported a higher amount of lignoceric acid (20-30%) in the seed oil of *A. pavonina*, a Fijan plant species than to that from Nigeria (3.5%) and that reported in the present study (2.9%). This difference in the amount of this fatty acid can be due to the geographical areas, time of the collection of the plant material, age of the seeds and environmental conditions (15). The amounts of other identified SFAs were ranged from 0.6 to 1.6% (Table 4). However, the total amount of SFAs from seed oil of *A. pavonina* collected in Kinshasa was lower than the one from the same plant part collected in Mbandaka(4), but was similar to that reported for the same plant part collected in Nigeria (2) and other consumed oils (25,29)

Moreover, by comparing the chemical composition of the seed oil of *A. pavonina* of this study to other previous studies on the same plant material, it was observed that Kabele et al. (4) had identified docosadien-13,16-dione acid (C22:1), palmitoleic acid (16:1), dihomolinoleic acid (C20:2), in the oil of the plant part collected from Mbandaka; Zarnowski et al., (16) had identified myritic acid (14:0), pentadecanoic acid (15:0), eicosanoic acid (20:0), eicosenoic acid (20:1), eicodienoic acid (20:2) docosanoic acid (22:0) in small amount in *A pavonina* collected from Kandy district, Sri Lanka, Eicosadienoic and benhenic acids were detected in the seed oil of *A. pavonina* collected in Nigeria (2). Curiously, these acids were not detected in the oil sample analyzed in the present study or in other *A. pavonina* seed oil collected in other regions (4,2). In contrast, in the seed oil sample from Kinshasa, elaidic acid (18:1), cis-nonadecanoic acid (19:1), erucic acid (22:1), margaric acid and nervornic acid (24:1) and methyl-ester linoleic acid ‘19/2) were identified, but were not previously

found in the oil samples from Mbandaka (4), Kandy district, Sri Lanka (16) and Nigeria (2). This finding can be explained by the environmental conditions, time of the collection of plant part, geographical areas, and in some extents to the age of seeds, the nature of solvent, the method of extraction, and the capacity of the plant part to synthesize different metabolites (15). In the present study, it was not detected the presence of polyamines and 5-alkylresorcinols as also previously reported by Zarnowski et al.(16)

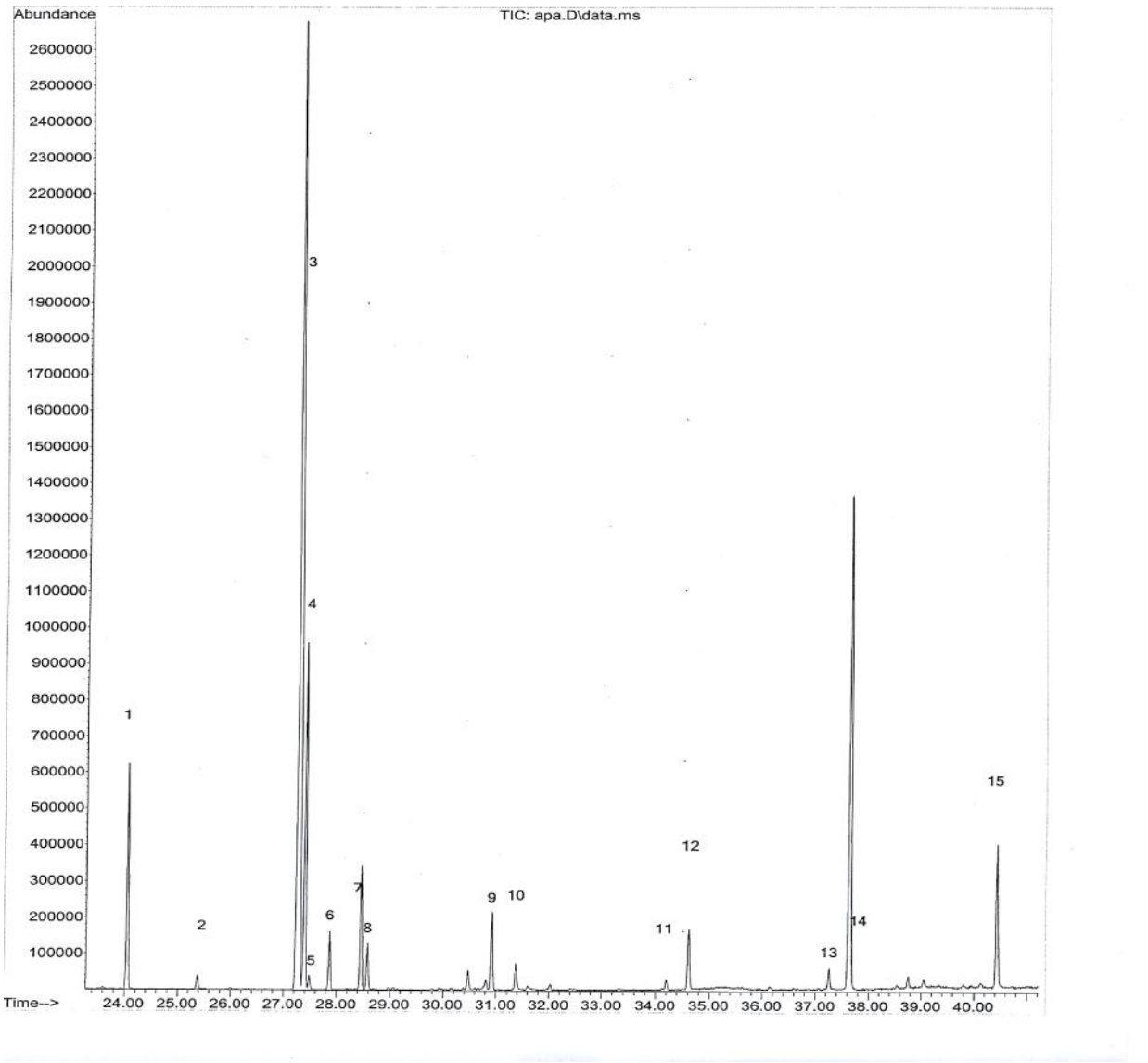

Figure 1. Chromatographic profile of fatty acids identified in the oil of *A. pavonina* seeds

1. Palmitic acid, **2**. Margaric acid, **3**. Linoleic acid, **4**. Oleic acid, **5**. Elaidic acid, **6**. Stearic acid, **7.** Methyl-ester linoleic acid **8**: Cis-10-monadecenoïc acid, **8a** and **8b** : Unidentified acids, **9**. Galodeïc acid, **10**. Arachidic acid, **10a** : Unidentified acid, **11** Erucic acid, **12**. Behenic acid, **13**. Nervornic acid, **14**. Lignoceric acid, **14a** and **14b** : Unidentified acids **15**. Cerotic acid.

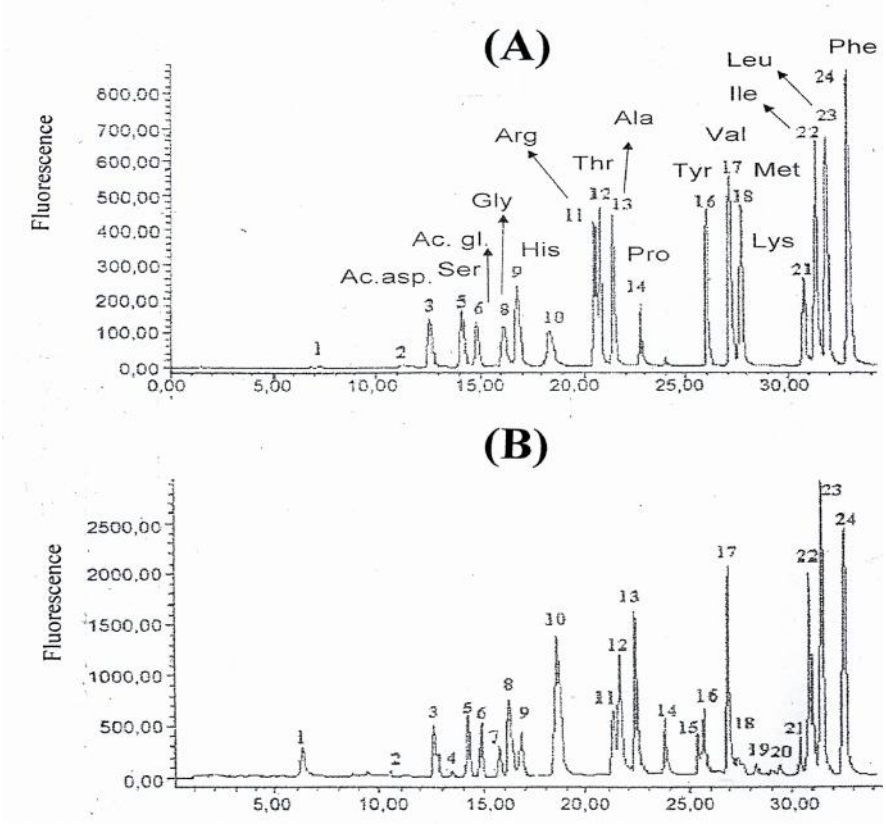

Figure 2. Chromatrographic profile (HPLC) of amino acid standards **(A)** and amino acids detected in the oil of *A. pavonina* seeds **(B).**

Figure 2 shows the chromatogram profile corresponding to a mixture of reference amino acids (A) and those of *A. pavonina* seed oil (B). The identification of all amino acid in both samples was achieved with a good resolution using the HPLC apparatus in experimental conditions described above. In the studied oil, a total of 20 amino acids were unambiguously identified among which 10 essential amino acids (eaa) and 10 no essential amino acid (neaa) representing 41.67% each and 16.6% of unidentified amino acids of the total amino acids. Their relative retention times and relative corrective factors are presented in Table 5. In the studied sample, some peaks no identified compared to those of the amino acid standards detected in the chromatogram (B) and can be ascribable to aspargine (Rt= 13.4 min) and glutamine (Rt = 15.9), Two other peaks with the retention times at 28.50 and 29.53 min can tentatively be assigned to unknown; tryptophan was not detected in *A. pavonina* seed oil by HPLC method, but was identified by colorimetric method in low amount 0.1%. The content of amino acids in the sample collected in Kinshasa ranged with glutamic acid having the highest amount (22.6%) followed by leucine (9.48%), aspartic acid (8.76%), serine (5.10%) glycine (4.84%), proline (4.61%), lysine (4.54%), tyrosine (4.52%) and phenylalanine (4.46%). The total amount of essential amino acids was 45.92% with arginine and leucine as the abundant (11.33 and 9.48% respectively) while that of non-essential amino acids was 53.83% with glutamic and aspartic acids as the abundant (22.26 and 8.76% respectively). The amount of other amino acids were ranged from 1.7 to 2.64% (Table 6). The amino acids identified as the components of the trypsin isoinhibitor from the seeds of *A. pavonina* from Fortaleza in North-East Brazil (30) and *A.pavonina* seed oil from Nigeria (2) were also found in the studied oil in the present investigation. In general, the fatty acids composition of *A. pavonina* seed oil collected in Kinshasa is no similar to that previously reported from the same plant material collected in Mbandaka (DRCongo) (4) or in Nigeria (2).

Table 5. Identification of amino acid in the oil of *A. pavonina* seeds

Amino acids	RRT	RCF
1. Unidentified amino acid	6.52	-
2. Iunidentified amino acid	10.50	-
3. Aspartic acid	11.71	1.19
4. Aspargine	13.52	1.65
5. Serine	14.51	1.85
6. Acide glutamique	14.97	1.95
7. Glutamine	15.85	2.24
8. Glycine	16.53	1.40
9. Histidine	16.93	1.01
10. Amoniac	18.51	-
11. Arginine	21.35	1.28
12. Thréonine	21.63	1.51
13. Alanine	22.52	1.29
14. Proline	23.92	1.05
15. Cystéine	25.53	0.98
16. Tyrosine	25.84	0.61
17. Valine	27.32	1.07
18. Méthionine	27.23	0.96
19. Tryptophan	-	-
20. Unidentified amino acid	28.53	-
21. Lysine	29.51	0.74
22. Isoleucine	30.00	0.84
23. Leucine	30.50	0.98
24. Phenylalanine	32.72	0.64

RRT: Relative Retention Time, RCF, Relative Corrective Factor, - : no detected amino acids by HPLC

Table 6. Amounts (%) of amino acids identified in *A. pavonia* seed oil

Amino acids	*A. pavonina*
Essential amino acids	
Arginine	11.33
Histidine	2.30
Isoleucine	3.94
Leucine	9.48
Lysine	4.54
Methionine	2.64
Phenylalanine	4.46
Threonine	1.70
Valine	3.53
Total	**45.92**
Non-essential amino acids	
Alanine	3.57
Glutamic acid	22.26
Aspartic acid	8.76
Cysteïne	0.17
Glutamine	1.70
Glycine	4.84
Proline	4.61
Serine	5.10
Tyrosine	4.52
Total	**53.83**

In conclusion, the present study demonstrates that A.povonina seed oil is rich in crude lipids (fats), carbohydrates, proteins, essential and non-essential amino acids, fatty acids dominated by unsaturated fatty acids, carbohydrates and mineral elements. The extracted oil is characterized by its white translucent colour, odourless and becomes solid at room temperature (30°C). The wide spectrum and high levels of different identified nutrients of the oil, makes it for a supplementation of the high starchy diets. A.pavonina can provide extra income to the rural farmers where the plant grows and can be favorably used as a nutritive food for humans and animal and also as a pharmaceutical excipient.

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