Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 104-114
Received: 04-09-2019
Accepted: 08-10-2019

Novidzro Kosi Mawuéna
Laboratory of Engineering Process and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01 BP 1515 Lomé, Togo

Melila Mamatchi
Laboratory of Engineering Process and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01 BP 1515 Lomé, Togo

Houndji Bidossessi Victor Saturnin
School of Nutrition, Food Sciences and Technologies, Faculty of Agricultural Sciences, University of Abomey-Calavi, 01BP 526 Cotonou, Bénin

Wokpor Kodjo
Laboratory of Engineering Process and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01 BP 1515 Lomé, Togo

Dotse Kokouvi
Laboratory of Engineering Process and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01 BP 1515 Lomé, Togo

Koumaglo Kossi Honoré
Laboratory of Engineering Process and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01 BP 1515 Lomé, Togo

Osseyi Elolo
Laboratory of Engineering Process and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01 BP 1515 Lomé, Togo

Corresponding Author:
Novidzro Kosi Mawuéna
Laboratory of Engineering Process and Natural Resources (LAGEPREN), Department of Chemistry, Faculty of Sciences, University of Lomé, 01 BP 1515 Lomé, Togo

Fatty acids composition of the oil, and quantification of 5-HTP, chlorophylls (a & b) and total carotenoids, of *Griffonia simplicifolia* Seeds

Novidzro Kosi Mawuéna, Melila Mamatchi, Houndji Bidossessi Victor Saturnin, Wokpor Kodjo, Dotse Kokouvi, Koumaglo Kossi Honoré and Osseyi Elolo

Abstract
Griffonia simplicifolia seeds are highly sought after in African herbal medicine and nutraceutical industries, thanks to their richness in 5-HTP. The seeds also contain other natural interesting substances such as fatty acids, but totally ignored by local populations. This study proposes to add value to the seeds exported by some African countries such as Ghana to US and EU. GC-MS analysis shows that its oil contains fatty acids such as: linoleic acid (73.19%); stearic acid (14.69%); palmitic acid (11.12%) and the remainder (1.00%). HPLC analysis indicates that the seeds contain 8.11±0.25% of 5-HTP, while UV-Visible spectrophotometry analysis reveals that the seeds contain 253.154±1.95 µg/g of chlorophyll A, 436.390±2.44 µg/g of chlorophyll B and 64.870±2.37 µg/g of total carotenoids. In short, the composition of *Griffonia simplicifolia* seeds in fatty acids, 5-HTP and phytopigments confers biological properties testifying its multiple therapeutic and nutritional virtues long emphasized by local populations of Togo.

Keywords: *Griffonia simplicifolia*' seeds, fatty acids, 5-HTP, phytopigments, nutritional and therapeutic virtues

Introduction
Griffonia simplicifolia is a leguminous plant, widespread in the forests and savannas of Central and West Africa. It is well known to local populations thanks to its many virtues. The uses of this plant by local populations are common both therapeutically, domestically and foraging domains (Lemaire and Adosraku, 2002; Addotey, 2009; Pathak *et al*., 2010; Giurleo, 2017) [1-4]. Indeed, all the organs of this plant such as roots, stem, bark, leaves, flowers and seeds are locally valued in traditional herbal medicine. Leaves in decoction and taken orally have, among others, anti-emetic, aphrodisiac, antitussive, but also purgative virtues. They would also be used to treat diseases of bladder and kidneys. Externally in the form of paste, the leaves serve also to treat syphilitic wounds, burns and ocular inflammations (Lemaire and Adosraku, 2002; Addotey, 2009; Pathak *et al*., 2010) [1-3].
Griffonia simplicifolia is also used as a good feed for animals that are looking for particularly and selectively its leaves during their pastures. Thus, the leaves are sought after by some animals such as sheep, goats, camels, oxen and horses. They would stimulate reproduction for these animals (Pathak *et al*., 2010) [3]. In addition, young leaves and flowers are used to treat gonorrhea, stomachaches, edema and fever. Most of these pharmacological properties are due to the high content of the plant in 5-HTP, a molecule whose content varies from 5% to 12% in the seeds, and from 0.3% to 1.2% in the leaves (Thérapeutes Magazine, 2017) [5].
The stem and branches of this plant are used in the treatment of anxiety, depression, insomnia, migraine and headaches, as well as for the regulation of appetite causing a reduction of weight in obese people. They are also used to brush teeth; while the hard parts are used as a cane with which people can lean to walk. In dry periods, stem and dry branches are also used as firewood (Lemaire and Adosraku, 2002; Addotey, 2009; Pathak *et al*., 2010) [1-3].
The seeds are used for aphrodisiacs, antibiotics and as a remedy against diarrhea, vomiting and stomach upset. It is used to treat insomnia, migraine and headaches (Lemaire and Adosraku, 2002; Adotey Addotey, 2009; Pathak *et al*., 2010) [1-3]. However, the seeds are the most popular organ because it is the only natural source recognized so far to have the highest 5-hydroxytryptophan (5-HTP) content. Thus, the seeds are usually harvested and exported after drying (Lemaire and Adosraku, 2002; Adotey Addotey, 2009; Pathak *et al*., 2010) [1-3] to the US and EU countries. 5-HTTP is the active ingredient involved in the prevention or treatment of fatigue and mental disorders due to depression, anxiety and stress (Giurleo, 2017) [4].

In Ghana, regions such as Volta Region, Ashanti Region, Brong Ahafo Region, Western and Eastern Regions are the main production, exploitation and marketing areas for *Griffonia simplicifolia* plant. In these areas, seeds are exported to United States and European Union countries (Pathak *et al*., 2010) [3].

In Togo, there are no formal studies on *Griffonia simplicifolia* territorial distribution. However, research and investigations carried out from the local populations assume that it is well known and used by local populations of "Maritime" and "Plateaux" Regions as a therapeutic and fodder plant. The leaves of the plant are also used by the School of Agronomy (ESA) of the University of Lomé-Togo to feed cattle, and sold at the market of Gbossimé-Lomé to feed the cattle.

Our interest in this study is to promote the use of *Griffonia simplicifolia* seeds in the production of edible vegetable oils and nutraceuticals (supplements and dietary supplements). The present work aims to determine the chemical composition of the vegetable oil in fatty acids and to evaluate the contents of 5-HTTP of the seed cakes, chlorophyllian pigments (A and B) and total carotenoids of *Griffonia simplicifolia* seeds harvested at Lomé-Togo.

Material and Methods

Collection of plant material used

The seeds of *Griffonia simplicifolia* were collected in the botanical garden of the Faculty of Sciences (FDS) of the University of Lomé-Togo, during the months of January and February 2018. After drying in the laboratory (28 ± 2 °C) followed by dehulling, the collected almonds are crushed using a grinder. The powder obtained was stored in a glass bottle, then put on hold for fat extraction.

Oil extraction

Fat extraction from *Griffonia simplicifolia* almond powder was performed with hexane in a Soxhlet extractor system. At the end of the extraction, the solvent is evaporated using a rotary evaporation system under vacuum by heating slightly about 40°C. The extracted oil was recovered and stored in a tinted glass bottle and stored in the freezer (-23 °C) for subsequent analysis.

Parameters determined

In this study, the chemical composition of fatty acids, the contents of 5-HTP, chlorophyll A and B, and total carotenoids contained in the seed were evaluated.

Preparation of the methyl esters

A mass of 30 mg of *Griffonia simplicifolia* oil and a 1 mL of a mixture of methanol and sulfuric acid (97.5%-2.5%: V/V) were introduced into a hemolysis tube with a screw cap. After closing the tube, the previously mixture formed was homogenized and then heated in a water bath at a temperature of about 80 °C for 90 minutes. Then, a volume of 1.5 mL of a sodium chloride solution (NaCl: 0.9%) was added to the mixture. After vigorous stirring, the resulting final solution was extracted with 1 mL of hexane. The hexane fraction containing the fatty acid methyl esters was analyzed by GC-MS.

Analysis of the chemical composition in fatty acid of the oil

The methyl esters of the fatty acids were analyzed using GC TRACE 1,300 Series, equipped with DB5-MS capillary column (length: 50 m; internal diameter : 0.25 mm; film thickness: 0.25 µm) and with AIS/AS 1310 Autosampler injector, and coupled to an ISQ MS Tune mass spectrometer (SM) with an electronic impact detector and equipped with XCalibur software for data acquisition. The inlet temperature of the SM was set at 200 °C and the temperature transfer line was set at 250 °C. The spectra were recorded at 70 eV.

The analytical conditions are as follows. The injector temperature has been fixed at 250 °C. The oven temperature was initially maintained at 120 °C for 20 minutes; the temperature is increased from 120 °C to 250 °C, then it was kept at isothermal temperature equal to 250 °C for 15 min. The carrier gas was helium with a flow rate of 1 mL/min. The identification of the various constituents of the oil was made by comparing their mass spectra with those of the XCalibur software database compounds.

Quantification of 5-HTP in the seeds by HPLC analysis

The content of 5-HTP in the *Griffonia simplicifolia* seeds was determined by the high performance liquid chromatography (HPLC) technique using the seed cakes. The 5-HTP molecule used as standard was purchased in France. The calibration curve for the quantification of 5-HTP in the seed cakes is shown in Figure 1. The solutions of the samples analyzed were prepared with the total extracts obtained by seed cakes with methanol (100%). The conditions for by HPLC analysis of 5-HTP are described as follows. The pump used was PERKIN ELMER 250, equipped with a Waters degasser and PERKIN ELMER Bio UV/VIS LC290 Spectrophotometric detector, coupled to MERCK/D2500 CHROMATO-Integretor. The chromatographic column was EC 260/4.6 Nucleosil 100-5C18. The elution was made in isochratic mode and at the wavelength of the detector was fixed at 275 nm. Water-acetonitrile mixture (97%-7%: V/V) was used as a mobile phase (Adotey Addotey, 2009) [2] with a flow rate of 1 mL/min. The high value of the correlation coefficient (R ≈ 0.99965) obtained thus certified the effectiveness of the method.

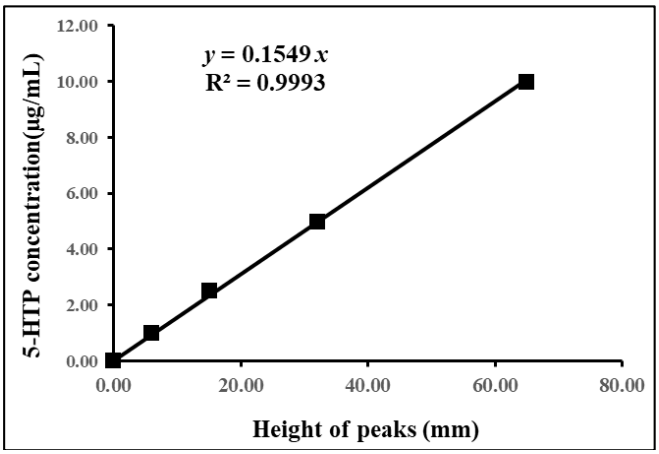


Fig 1: Calibration curve for quantification of 5-HTP

Pigment analysis

A mass of 50 mg of the seeds of *Griffonia simplicifolia* were ground in a porcelain mortar. The powder obtained was extracted by maceration in 10 mL of Methanol (100%). The mixture was introduced into a glass bottle. Then, the bottle was well closed and stirred vigorously before being left to rest. After 24 hours of rest, the previous mixture was filtered, and the collected filtrate was used for absorbance reading. The reading was done in triplicate. To ensure the efficiency of extraction, this operation was repeated under the same

conditions with two other solvents: Acetone (100%) and Acetone-Water mixture (80%-20%).

The contents of the three pigments (Chlorophyll A, Chlorophyll B and total carotenoids) contained in the filtrate were determined by spectrophotometric assay using METASH/UV-VIS spectrophotometer, equipped with MetaSpec Pro data acquisition software. Absorbance readings performed at three different wavelengths for each solvent as follows (Macckinney, 1941; Costache *et al*., 2012) [6, 7]:

- For Methanol (100%): 470 nm; 653 nm and 666 nm;
- For Acetone-Water mixture (80%-20%): 470 nm; 646 nm and 663 nm;
- For Acetone (100%): 470 nm; 645 nm and 662 nm.

The contents of chlorophyll A (C_a), chlorophyll B (C_b) and total carotenoid ($C_{(x+c)}$), measured in µg/g of seed, were calculated based on the formulas given in Table 1 (Costache *et al*., 2012; Macckinney, 1941) [6, 7].

Tableau 1: Formulas for $C_{(a)}$, $C_{(b)}$ and $C_{(x+c)}$ calculations

Solvents	Methanol 100 %	Acetone -Water (80 %-10%)	Acétone 100 %
$C_{(a)}$	$15.65A_{666}$-$7.34A_{653}$	$12.21A_{663}$-$2.81A_{646}$	$11.75A_{662}$-$2.35A_{645}$
$C_{(b)}$	$27.05A_{653}$-$11.21A_{666}$	$20.13A_{646}$-$5.03A_{663}$	$18.61A_{645}$-$3.96A_{662}$
$C_{(x+c)}$	$(1000A_{470}$-$2,86C_a$-$129.2C_b)/245$	$(1000A_{470}$-$3.27C_a$-$104C_b)/229$	$(1000A_{470}$-$2,27C_a$-$81.4C_b)/227$

Statistical analysis

The results of $C_{(a)}$, $C_{(b)}$ and $C_{(x+c)}$ were entered into an Excel spreadsheet and processed using the STATISCA 7.0 software for ANOVA variance analysis, at the threshold of 5%. A comparison of the averages was performed using 5% LSD test to identify the observed differences.

Results and Discussion

Chemical composition of *Griffonia simplicifolia* oil in fatty acids

The results of GC-MS analysis of the methyl esters of *Griffonia simplicifolia* oil allowed to obtain the pictogram in Figure 2 (A) and the chromatogram in Figure 2 (B).

The interpretation of the results (Figure 3-7) presented on the pictogram in Figure 2-A and the chromatogram in Figure 2-B shows that *Griffonia simplicifolia* oil mainly contained the fatty acids reported in Table 2 : Palmitic acid ((C16: 0): 11.12%); Linoleic acid ((C18: 2□6): 73.19%), Stearic acid ((C18: 0): 14.69%) and others (1.00%).

Table 2: Chemical composition of *Griffonia simplicifolia* oil in fatty acids

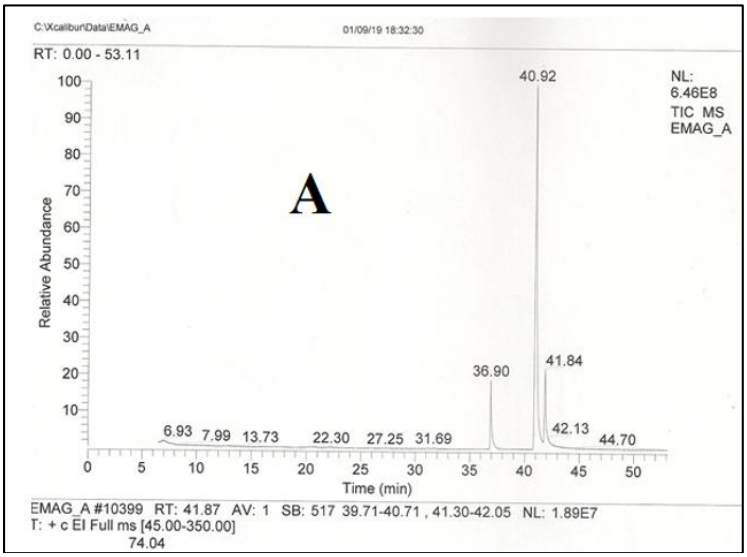
Types of Fatty Acids in *Griffonia Simplicifolia* seed oil	Proportions
Palmitic acid (C16 : 0)	11.12%
Linoleic acid (C18 : $2\omega^6$)	73.19%
Stearic acid (C18 :0)	14.69%
Saturated fatty acids (SFA)	25.81%
Unsaturated fatty acids (USFA)	73.19%
Others	1.00%
USFA/SFA	2.84

Legends: SFA = Saturated fatty acids and USFA = Unsaturated Fatty Acids

The seed oil of *Griffonia simplicifolia* harvested in Togo is very rich in linoleic acid (73.19%).

Therefore, this oil can be comparable to soybean, sunflower and hemp oils, which are very popular worldwide. Linoleic acid is a fatty acid having 18 carbon atoms and a molecular structure with two carbon-carbon double bonds; the first one is in position 6 with respect to the last carbon of the chain, called Omega carbon; hence the name of $2\omega^6$.

The chemical composition of *Griffonia simplicifolia* oil, characterized by a predominance of linoleic acid, an unsaturated fatty acid, shall confer hypocholesterolemic and antiatherogenic properties (Charrouf, 2002; Berrougui *et al*., 2003) [8, 9]. The ratio of polyunsaturated fatty acids to saturated fatty acids for this oil is equal to 2.83. This ratio favorably exceeds the values between 1.25-1.50 recommended by nutritionists.



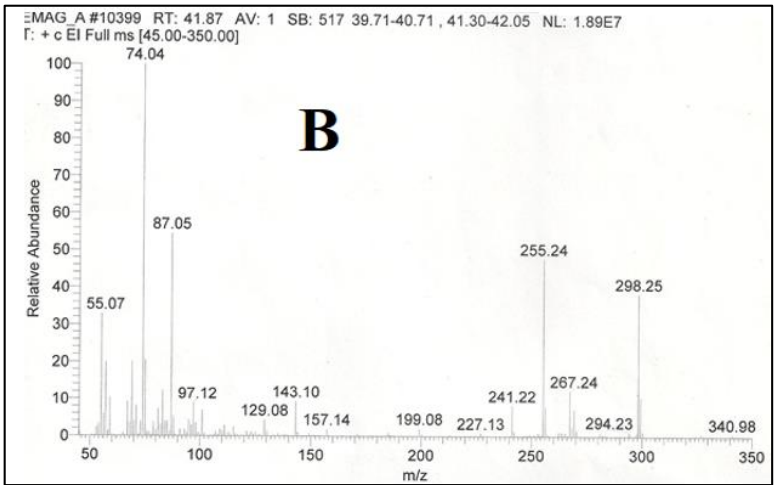
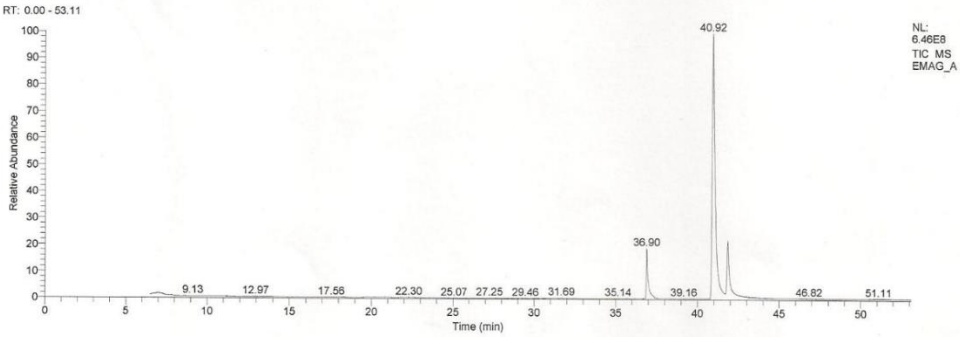


Fig 1: Pictogram (A) and mass spectrum (B) obtained by GC-MS analysis of methyl esters of the oil of *Griffonia simplicifolia* seeds

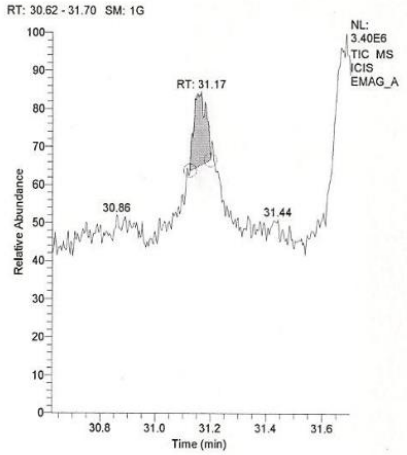
Data File:	EMAG_A	Original Data Path:	C:\Xcalibur\Data
Current Data Path:	C:\Xcalibur\Data	Sample Type:	Unknown
Sample ID:	1	Sample Name:	
Operator:	ISQ1612505	Acquisition Date:	01/09/19 06:32:30 PM
Run Time(min):	46.61	Comments:	
Vial:	1	Injection Volume(µl):	1.00
Scans:	13706	Low Mass(m/z):	45
High Mass(m/z):	350	Sample Volume(µl):	0.00
Sample Weight:	0.00	ISTD Amount:	0.000
Calibration Level:		Dilution Factor:	1.00
Instrument Method:	C:\Xcalibur\methods\ESTER_4.meth	Original Processing Method:	
Current Processing Method:	N/A		

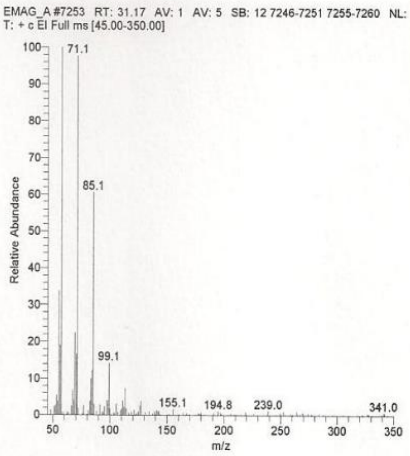


RT	Peak Area	Peak Height	S/N	Area %
31.17	2081281.33	658685.77	94.36	0.05
31.69	4236373.92	1165651.83	166.99	0.10
36.90	481293708.01	106435328.16	15248.02	11.12
37.04	809613.35	1333865.85	191.09	0.02
37.06	505354.94	1524695.34	218.43	0.01
37.07	643019.62	1330269.39	190.58	0.01
37.12	208703.10	410329.10	58.78	0.00
37.17	152120.14	592478.10	84.88	0.00
37.29	102089.68	226433.93	32.44	0.00
40.92	3169173963.31	484339420.82	69386.88	73.19
41.05	4673424.41	6848353.19	981.10	0.11
41.10	8346007.39	7160802.86	1025.86	0.19
41.12	2089292.79	2997785.08	429.47	0.05
41.16	2118670.76	4253083.89	609.30	0.05
41.18	640340.12	1376992.23	197.27	0.01
41.20	870996.37	1438404.09	206.07	0.02
41.23	211461.10	684034.00	98.00	0.00
41.25	759899.35	1918425.80	274.84	0.02
41.27	298524.32	774254.71	110.92	0.01
41.29	1178704.64	2982558.37	427.28	0.03
41.31	1864771.86	2873160.98	411.61	0.04
41.33	100800.32	434102.92	62.19	0.00
41.36	845685.28	1568978.29	224.77	0.02
41.37	302403.12	927407.10	132.86	0.01
41.44	464477.75	829445.13	118.83	0.01
41.46	111347.64	413463.49	59.23	0.00
41.50	131174.83	519592.57	74.44	0.00
41.54	192960.00	670391.51	96.04	0.00
41.60	273400.50	409594.56	58.68	0.01
41.64	249619.35	772719.99	110.70	0.01
41.69	284216.43	621373.72	89.02	0.01
41.84	635920329.30	103868553.56	14880.30	14.69
41.95	4349620.60	4171254.35	597.58	0.10
42.01	1137970.31	1896896.68	271.75	0.03
42.04	310558.90	783095.46	112.19	0.01
42.07	180681.59	683998.66	97.99	0.00
42.10	205110.63	408247.20	58.49	0.00

Fig 3: Pictogram and data provided by GC-MS analysis of the oil of *Griffonia simplicifolia* seeds

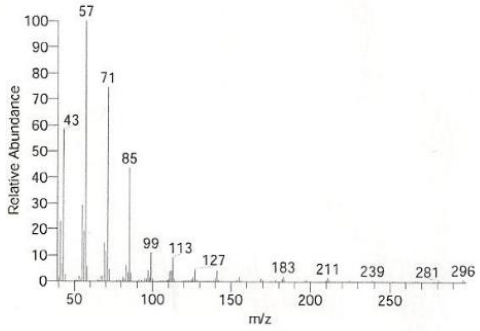
RT	Peak Area	Peak Height	S/N	Area %
42.13	1509433.09	1359744.14	194.80	0.03
42.16	581319.43	1440111.08	206.31	0.01
42.23	270572.92	522668.58	74.88	0.01
42.26	83486.07	346769.94	49.68	0.00
42.41	94306.23	399895.32	57.29	0.00

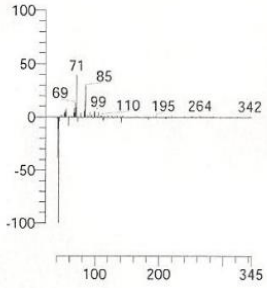




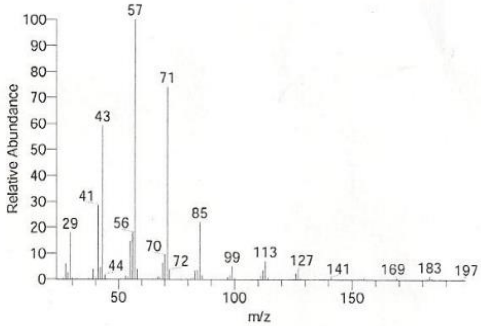
SI	RSI	Compound Name	RT	Library	Probability	Molecular Formula
765	820	Heptadecane, 2,6,10,15-tetramethyl-	31.17	mainlib	7.06	C21H44
751	847	Dodecane, 2,7,10-trimethyl-	31.17	mainlib	4.41	C15H32
748	824	Decane, 2,3,5,8-tetramethyl-	31.17	mainlib	3.90	C14H30

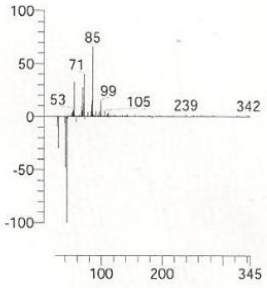
Hit Spectrum | Delta | Compound Structure





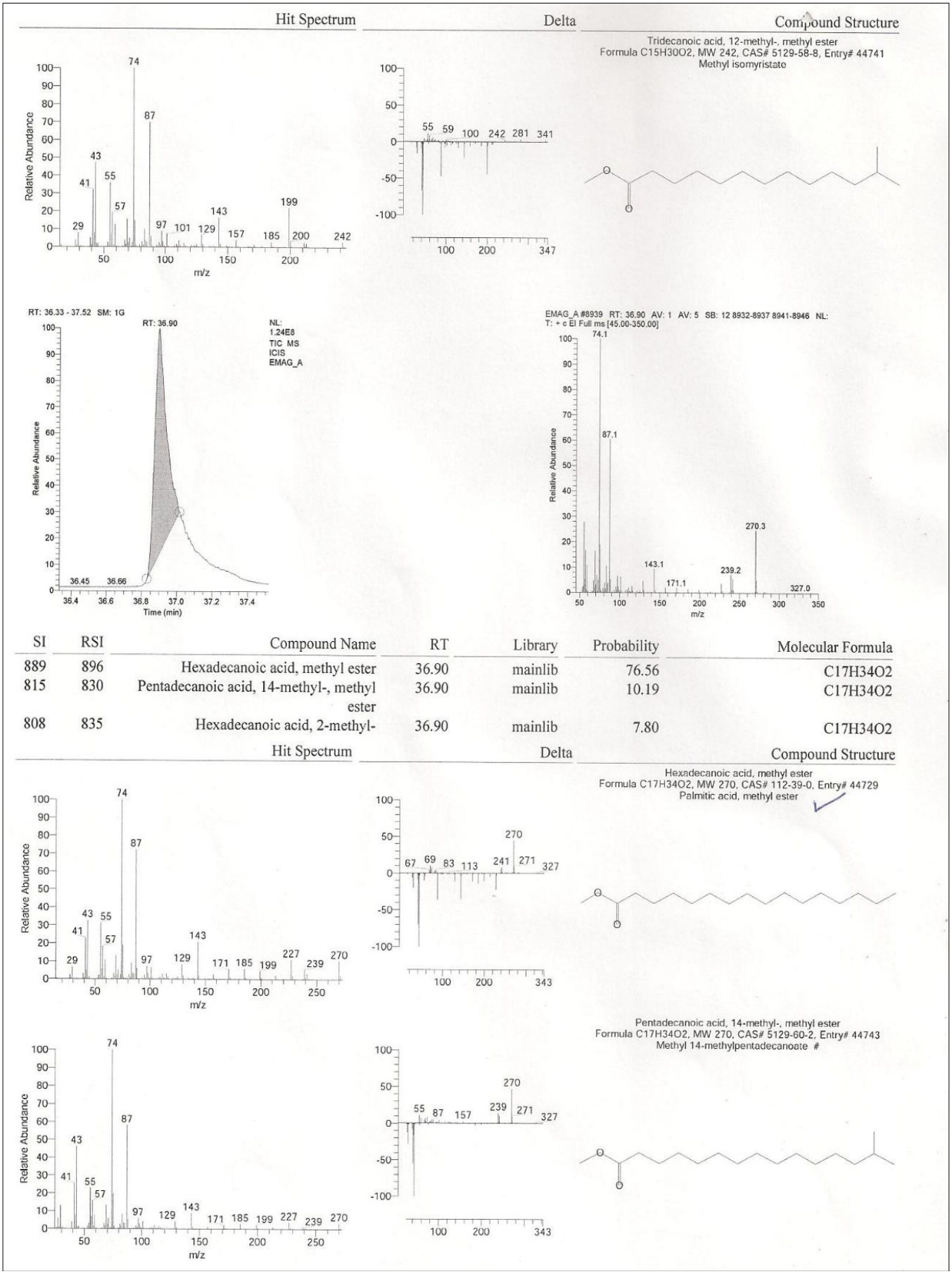
Heptadecane, 2,6,10,15-tetramethyl-
Formula C21H44, MW 296, CAS# 54833-48-6, Entry# 25344
2,6,10,15-Tetramethylheptadecane #





Dodecane, 2,7,10-trimethyl-
Formula C15H32, MW 212, CAS# 74645-98-0, Entry# 25403
2,7,10-Trimethyldodecane #

Fig 4: Spectrogram and data provided by GC-MS analysis of the methyl esters of the oil of *Griffonia simplicifolia* seeds



SI	RSI	Compound Name	RT	Library	Probability	Molecular Formula
889	896	Hexadecanoic acid, methyl ester	36.90	mainlib	76.56	C17H34O2
815	830	Pentadecanoic acid, 14-methyl-, methyl ester	36.90	mainlib	10.19	C17H34O2
808	835	Hexadecanoic acid, 2-methyl-	36.90	mainlib	7.80	C17H34O2

Fig 5: Spectrogram and structure of the methyl ester of palmitic acid, proposed after GC-MS analysis of the methyl esters of the oil of *Griffonia simplicifolia* seeds

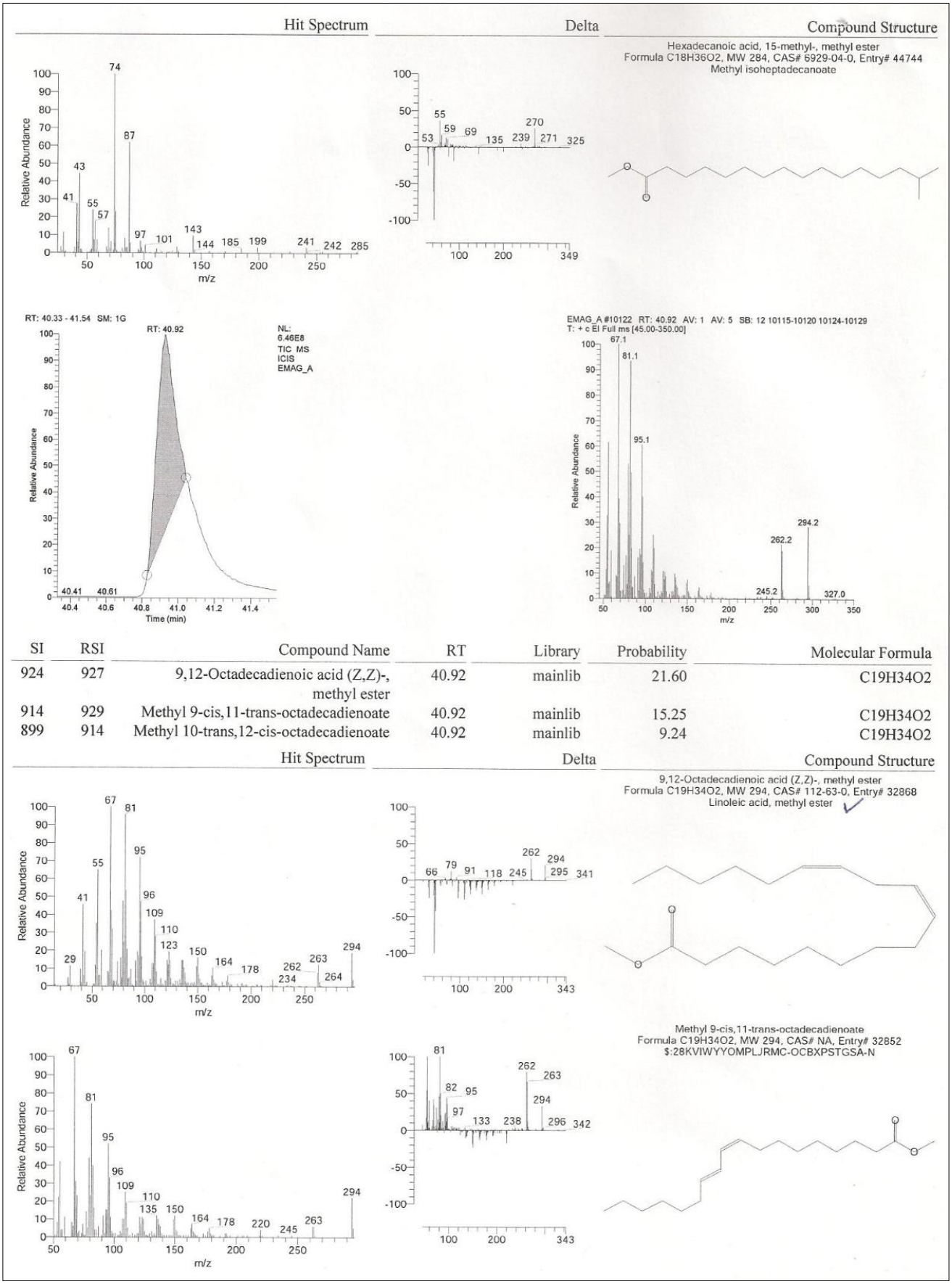
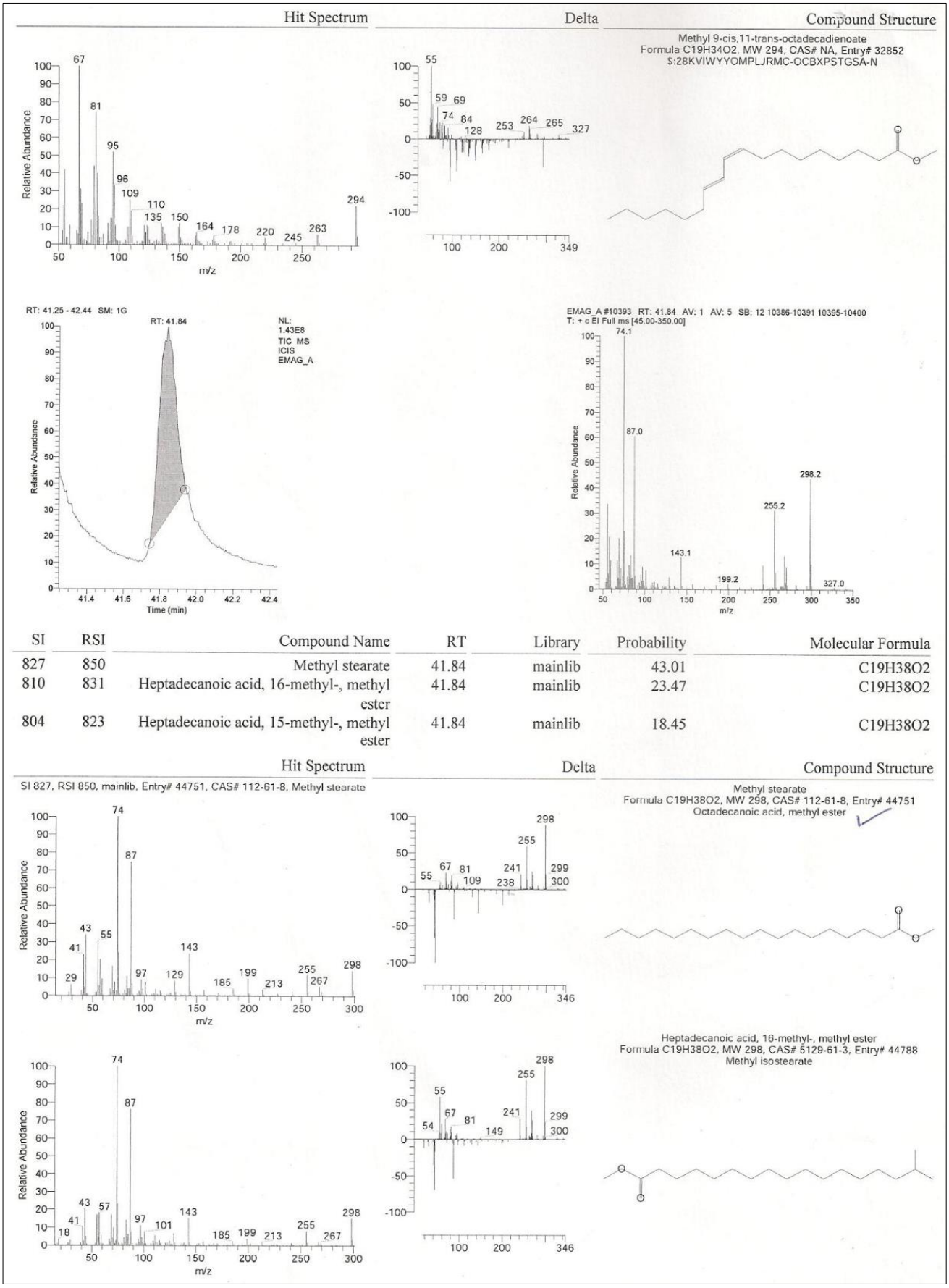


Fig 2: Spectrogram and structure of linoleic acid methyl ester after GC-MS analysis of the methyl esters of the oil of Griffonia simplicifolia seeds



SI	RSI	Compound Name	RT	Library	Probability	Molecular Formula
827	850	Methyl stearate	41.84	mainlib	43.01	C19H38O2
810	831	Heptadecanoic acid, 16-methyl-, methyl ester	41.84	mainlib	23.47	C19H38O2
804	823	Heptadecanoic acid, 15-methyl-, methyl ester	41.84	mainlib	18.45	C19H38O2

Fig 3: Spectrogram and structure of stearic acid methyl ester, proposed after GC-MS analysis of the methyl esters of the oil of *Griffonia simplicifolia* seeds

With a content of 73.19% approximately, linoleic acid, one of the essential fatty acids for human health that the body can not synthesize and which must be obtained from vegetable sources, is therefore mainly represented in the *Griffonia simplicifolia* oil studied in this work. Precursor of unsaturated fatty acids of the ω^6 series, linoleic acid is indirectly responsible for the synthesis of prostaglandins and leukotrienes of series 1 and 2 who play an important role in

the nervous system, cardiovascular balance, immunity, wound healing, and allergic and inflammatory reactions (Rahmani, 2005) [10]. Thus, it has been recognized that linoleic acid and α-linolenic acid are two fatty acids that serve as precursors to prostaglandins (Garrett and Grisham, 2006) [10] acting as signaling hormones that help to regulate vasodilatation, platelet aggregation, inflammation and contraction of smooth muscle (Garrett and Grisham, 2006) [10]. In addition, linoleic acid also plays a role in cell permeability and its deficiency causes, among other things, skin aging which results in drying and loss of elasticity of the skin, with wrinkle appearance (Rahmani, 2005) [10].

By quantifying 2% of the total energy of the diet for the need of linoleic acid, a person consuming a daily ration of 2700 kcal would therefore need 54 kcal of linoleic acid, which corresponds to 6 grams (Rahmani, 2005) [10]. This person can therefore satisfy his needs for linoleic acid by consuming only 8.200 g of *Griffonia simplicifolia* oil per day, approximately one tablespoon. However, it should be noted that our *Griffonia simplicifolia* oil is not a good source of ω^3 fatty acids whose protective role against atherosclerosis has been well established (Giurleo, 2017) [4], because this acid, belonging to the class of ω^3 fatty acids, is practically absent in the oil. Alpha-linolenic acid (C_{18}: $3\omega^3$) which is often found in vegetable oils and other fatty acids of this series (Eicosapentaenoic acid (EPA, C_{20}: $5\omega^3$) and Docosahexenoic acid (DHA, C_{22}: $6\omega^3$), found almost exclusively in fish oils, are almost absent in *Griffonia simplicifolia* oil studied here. It has been shown that, for a balanced diet, ω^6/ω^3 ratio must be close to 4, and in any case be less than 10. Thus, a diet based on *Griffonia simplicifolia* oil should then be supplemented by a supply of ω^3 fatty acids in the form of vegetable oils providing α-linolenic acid (nut oil or colza oil) or under form of fish oils (rich in EPA and DHA).

The presence of fatty acids in the *Griffonia simplicifolia* seed would constitute a major asset for local populations if the extracted oil does not contain any toxic compounds for humans. Thus, it has been argued that fatty acids are important for the vitality of plant and animal cells (Giurleo, 2017) [4]. Indeed, a large amount of energy is stored in the carbon-carbon bonds of the fatty acid molecules and this energy can be released to direct the metabolic processes of the cells.

Energy for metabolic processes can also be obtained by breaking down sugars and proteins, but these molecules produce only about half of the amount of energy per gram of fatty acids, because most carbons of sugars and some proteins are already partially oxidized (Nelson and Cox, 2008)[12]. Fatty acids are also important precursors in the production of some biomolecules such as phospholipids, pigments, co-factors, detergents, carriers, hormones, chemical messengers and anchors for membrane proteins (Lehninger *et al*., 1992) [13]. It is also very important to know that myristic, palmitic, stearic, linoleic, oleic and arachidic acids are the most commonly found fatty acids in the plant kingdom and all these fatty acids have been reported in *Griffonia simplicifolia* seeds from other sources (Ramazanov and Petkov, 2003) [14].

There is possibility to produce linoleic acid, with a purity of 95%, from our oil by separating the other fatty acids present in the oil by simple addition of urea as realized by Petkov and Ramazanov, 2003) [14]. So, the seeds can therefore serve as an excellent production of this vital nutrient in Togo.

In comparison with other legumes, linoleic acid accounts for 21-53% of total free fatty acid content (Grela and Gunter, 1995; Ryan *et al*. 2007) [15, 16] and plants of Caesalpinioideae legume subfamily of which *Griffonia simplicifolia* is a member, are known to have higher levels of this fatty acid (Bagci and Sahin, 2004) [17]. In fact, the richness of the oil of *Griffonia simplicifolia* seeds in linoleic acid gives him nutritional virtues much sought after. Its specific composition also predestines it for cosmetic uses. Whereas its specific biochemical composition gives him then interesting properties nutritionally, cosmetically or therapeutically. The effort to valorize *Griffonia simplicifolia* oil must be continued because it can, by its positive impact on the incomes of the local populations, play a decisive role in the development of grassroots communities.

In fine, the oil of *Griffonia simplicifolia* seeds harvested in Lomé-Togo contains mainly three fatty acids which are: linoleic acid in a higher proportion compared to stearic acid and palmitic acid which are poorly represented.

Quantification of 5-HHTP in seed cakes

The result of HPLC analysis reveals that 5-HTP is present (Figure 8) in the seed cakes of *Griffonia simplicifolia*, with a content of 8.11 ± 0.25% compared to dried mass of the seeds.

This result corroborates with the work done by many researchers according to which *Griffonia simplicifolia* is the species that contains the highest 5-HTP content, ranging between 3% and 7% (Lemaire and Adosraku, 2002; Ramazanov and Petkov, 2003; Adotey Addotey, 2009) [1,2, 14]. According to the works carried out by other researchers, this rate can reach up to 20% depending on the countries and regions of harvesting (Lemaire and Adosraku, 2002; Mehta *et al*., 2015; Giurleo, 2017) [1, 14, 18]. However, it has been reported that 5-HTP concentrations in *Griffonia physocarpa* and *Griffonia speciosa* seeds are not as negligible (Pathak *et al*., 2010) [3].

We also investigated whether the oil of *Griffonia simplicifolia* seeds, extracted with hexane, could contain 5-HTP. This investigation shows that 5-HTP molecule is absent in the oil.

In short, it should be remembered that 5-HTP is contained in the seed cakes, so in the seeds of *Griffonia simplicifolia* harvested in Lomé-Togo, but it is absent in the seed oil extracted with hexane.

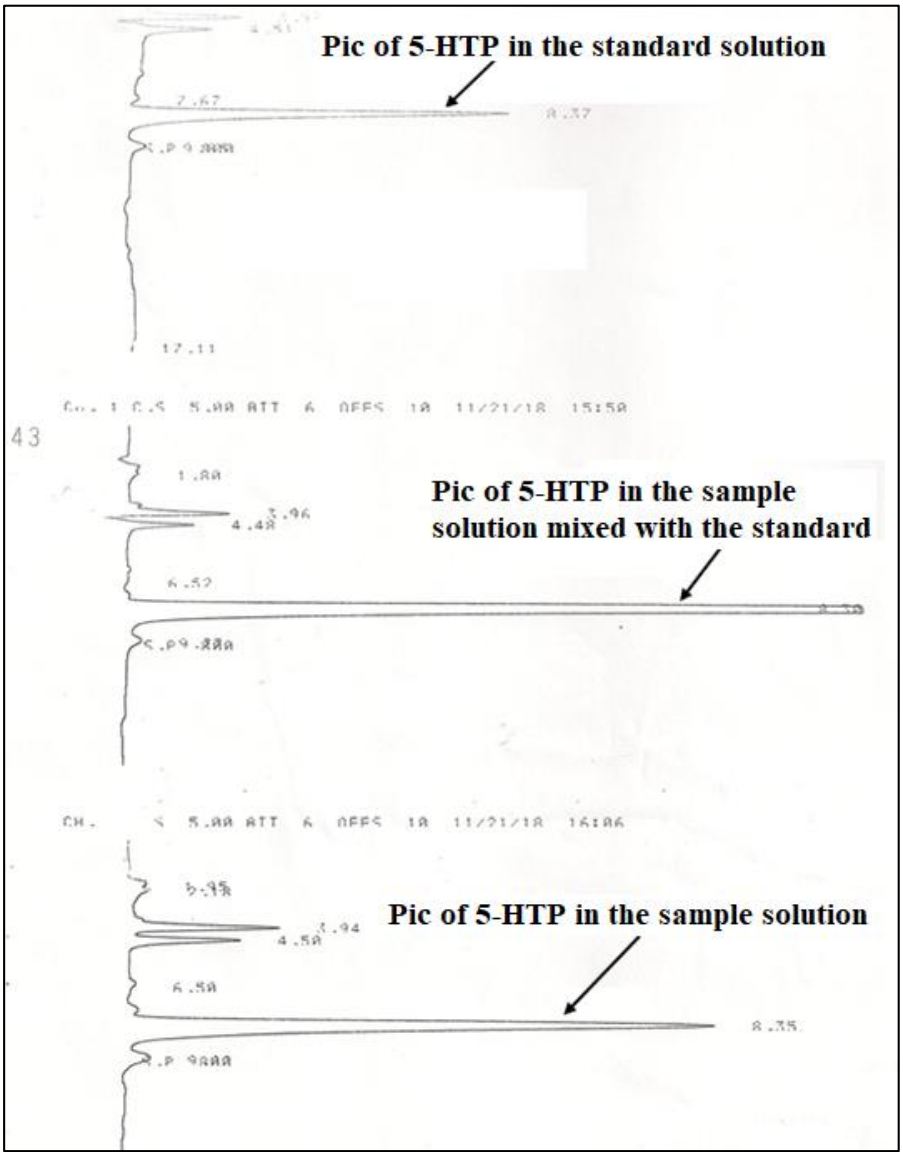


Fig 8: Pictogram obtained by HPLC analysis of *Griffonia simplicifolia* seed cakes Contents of Chlorophyll A and B, and Total Carotenoids in *Griffonia simplicifolia* seeds

The analysis results for the contents chlorophyll A and B, and for total carotenoids of *Griffonia simplicifolia* seeds are recorded in Table 3.

Table 3: Chlorophyll A (C_a), Chlorophyll B (C_b) and Total Carotenoid (C_{x+c}) Contents of *Griffonia simplicifolia* seeds

Solvent types	$C_{(a)}$: µg/g	$C_{(b)}$: µg/g	$C_{(x+c)}$: µg/g
Methanol (100 %)	$30.26 \pm 2{,}20^{b}$	65.50 ± 2.15^{b}	$63.07 \pm 1{,}89^{a}$
Acetone-Water (80 %-20%)	$253.15 \pm 1{,}95^{a}$	436.39 ± 2.44^{a}	$64.87 \pm 2{,}37^{a}$
Acetone (100 %)	17.38 ± 1.15^{c}	27.08 ± 0.45^{c}	$47.12 \pm 1{,}17^{b}$

Legends: The values recorded in Table 3 are the means of 3 tests ± standard deviations for each of the contents C (a), C (b) and C (x + c). In the same column, the same superscript letter indicates that there is no significant difference between the data in this column by performing with LSD test at the 5% significance level.

The data recorded in Table 4 show that there are significant differences between the values measured for the same content when changing solvent, either chlorophyllian pigment A or B, according to the LSD test performed at the threshold of 5%. On the other hand, for the value of the total carotenoid content, no significant difference is observed when using methanol (100%) or acetone (80%), whereas the value obtained with acetone (100%) is significantly different from that obtained with methanol (100%) or acetone (80%). Overall, chlorophyll A and B pigments are extracted to the maximum with acetone (80 %), with contents of 253.15±1.95 µg/g and 436.39±2.44 µg/g of seeds, respectively. However, it was by acetone (80%) or methanol (100%) that maximum extraction of carotenoids was done, with a concentration of 64.87 ± 2.37 µg/g and 63.07 ± 1.89 µg/g of seeds.

In this study, acetone (100%) gave the lowest values obtained for chlorophyll A and B contents, and total carotenoids, respectively: 17.38 ± 1.15 µg/g; 27.08 ± 0.45 µg/g and 47.12 ± 1.17 µg/g of seeds. Among the three solvents used for pigment extraction, acetone (80%) was the best, followed by methanol (100%), while acetone (100%) is the bad solvent.

The results presented here are comparable to the work done by Costache *et al*. (2012) [7] after which they concluded that methanol and acetone are the best extraction solvents for

spectrophotometric determination of chlorophyllian pigments A and B, and total carotenoids in certain plants such as: tomatoes, peppers and the cucumbers.

The presence of chlorophylls (A and B) and carotenoids in *Griffonia simplicifolia* seeds harvested in Lomé-Togo confirms the results obtained by Novidzro *et al*. (2019) [19] on the oil of seeds of this plant and which revealed that the oil contained chlorophyll A, chlorophyll B and total carotenoids with respective contents of 95.55 µg/g; 156.28 µg/g and 26.01 µg/g of oil.

In view of these results, *Griffonia simplicifolia* seeds are a good source for the three pigmented phytoconstituents, thus conferring on its appreciable nutritional qualities, because these biomolecules are endowed with antioxidant powers.

Conclusion

In this study, a characterization of fatty acids in the oil and an evaluation of 5-HTP, chlorophylls A and B, and total carotenoids contained in the *Griffonia simplicifolia* seeds harvested from Togolese flora, were made. The investigations revealed that the oil of *Griffonia simplicifolia* seeds contains fatty acids predominated by the presence of linoleic acid, whereas stearic acid and palmitic acid are poorly represented. The seeds are potentially rich in 5-HTP and chlorophyllian pigments A and B, and total carotenoids. The extraction, isolation and purification of these various natural substances of food and therapeutic interest on the spot could create added value for the benefit of the local populations and the actors who would be involved in the seed marketing.

This study could be pursued in the direction of conserving plant biodiversity by developing optimal methods for the agroforestry culture of the *Griffonia simplicifolia* plant in order to increase seed production yields and with contents of natural substances for nutritional and therapeutic interests.

References

1. Lemaire PA, Adosraku RK. An HPLC method for the direct assay of the serotonin precursor, 5-hydroxytrophan, in seeds of *Griffonia simplicifolia*: Phytochem Anal. 2002; 13(6):333-337.
2. Adotey Addotey JN. Local production of 5-HTP from the seeds of *Griffonia simplicifolia*, Master of Philosophy Pharmaceutical Chemistry College of Health Sciences Kwame Nkrumah University of Science and Technology Kumasi, Ghana, 2009, 99.
3. Pathak SK, Tahilani P, Jain NP, Banweer J. A review on *Griffonia simplicifolia* - an ideal herbal anti-depressant. IJPLS. 2010; 1(3):174-181.
4. Giurleo DJ. Phytochemical Exploration of Griffonia simplicifolia Seeds and Leaves, a thesis of The State University of New Jersey in partial fulfillment of the requirements For the degree of Master of Science Graduate Program in Medicinal Chemistry; 2017, 232.
5. Thérapeutes Magazine. UPAC Compendium of Chemical Terminology, 2nd ed. (The "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford, 1997.
6. Macckinney GJ. Biol. Chem. 1941; 140:315-322.
7. Costache MA, Campeanu G, Neata G. Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. Romanian Biotechnological Letters. 2012; 17(5):7702-7708.
8. Charrouf Z. Valorisation de l'huile d'argan. Résultats et perspectives. In : Collin & Garneau, ed. Produits naturels d'origine végétale. Actes du cinquième colloque de Sainte-Foy (Québec), Université du Québec à Chicoutimi (Canada), 2002.
9. Berrougui H, Ettaib A, Herrera Gonzalez MD, Alvarez de Sotomayor M, Bennani-Kabchi N, Hmamouchi M. Hypolipidemic and hypocholesterolemic effect of argan oil (*Argania spinosa* L.) in Meriones shawi rats. J Ethnopharmacol. 2003; 89(1):15-18.
10. Rahmani M. Composition chimique de l'huile d'argane « vierge ». Cahiers Agricultures. 2005; 14(5): 461-465.
11. Garrett RH, Grisham CM Principles of Biochemistry with a Human Focus. Student edition. Thompson Corporation: Mason, OH, 2006.
12. Nelson DL, Cox MM. Principles of Biochemistry. Fifth Edition. W. H. Freeman and Company: New York, NY, 2008.
13. Lehninger AL, Nelson DL, Cox MM, Principles of Biochemistry with an extended discussion of Oxygen Binding Proteins. Second edition. Worth Publishers: New York, NY, 1992.
14. Ramazanov Z, Petkov G, Biochemical characteristics of Griffonia simplicifolia and its chemical composition. Journal of Subtropical Plants Sciences. 2013; 42(01):86-90.
15. Grela ER, Gunter KD, Fatty acid composition and tocopherol content of some legume seeds. Animal Feed Science and Technology. 1995; 52:325-331.
16. Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM, Phytosterol. squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. Plant Foods for Human Nutrition. 2007; 62:85-91.
17. Bagci E, Sahin A. Fatty acid patterns of the seed oils of some *Lathyrus species* L. (Papilionideae) from Turkey, a chemotaxonomic approach. Pakistan Journal of Botany 2004; 36(2):403-413.
18. Mehta HD, Mangrulkar SV, Chourasia AJ. A revew on Griffonia simplicifolia – A natural anti-depressant. International Journal of Phytopharmacology. 2015; 6(2):76-79.
19. Novidzro KM, Wokpor K, Amoussou Fagla B, Koudouvo K, Dotse K, Osseyi E *et al*. Etude de quelques paramètres physicochimiques et analyse des éléments minéraux, des pigments chlorophylliens et caroténoïdes de l'huile de graines de Griffonia simplicifolia. Int. J. Biol. Chem. Sci. 2019; 13(4):2360-2373.