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Comparative Physicochemical, Spectroscopic and Elemental Analyses of the Seed Oils of *Parinari excelsa* Sabinus and *Chrysobalanus icaco* Linn., Chrysobalanacea

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ABSTRACT

African cultures commonly rely on a variety of spices and herbs in the preparation of their usually tasty cuisines and delicacies. *Parinari excelsa* Sabinus and *Chrysobalanus icaco* L, members of the Chrysobalanacea family, produce edible nuts that are widely used in Nigeria as spices. Their nuts contain potentially rich and nutritious oils. The oils of *P. excelsa* and *C. icaco* were extracted successively using n-hexane and dichloromethane and concentrated *in vacuo*. The physico-chemical parameters of the oils were determined using standard methods. Chemical compositions were evaluated using Gas Chromatography/Mass Spectroscopy (GC/MS), while elemental contents were determined using Atomic Absorption Spectroscopy (AAS). *P. excelsa* oil had higher acid values (29.2, 42.1) than *C. icaco* (5.6, 8.4), while the saponification values of *C. icaco* (460, 471.2) were higher than *those* of *P. excelsa* (350.6, 465.6). Elemental analysis revealed the presence of minerals such as Sodium, Potassium, Magnesium, Calcium, and Iron in both crude samples, while heavy metals such as Lead, Arsenic, and Mercury were undetectable. GC/MS analysis showed that oils from both plants contain common fatty acids such as arachidonic acid and oleic acid, vitamin E, and stigmasterol.

INTRODUCTION

Herbs and spices are used for both culinary and medicinal purposes for centuries. Food has been known to be the very basis of human health and wellbeing, since time immemorial [1, 2]. These substances have been used in different cultures as flavouring agents to enhance taste, aroma and acceptability by the people. Nigeria is a country with diverse cultural heritage among the black race, with over 371 ethnic nationalities with different cultures and traditional systems[3].

Parinari excelsa sabinus and *Chrysobalanus icaco* L Chrysobalanacea are species that produce edible nuts which are used by the Itsekiris in Delta State, Nigeria as spices in preparing pepper soup, which is widely consumed by Nigerians. The kernels have been reported to contain oily substances which could be responsible for their use as spices. The oil could be a source of vitamins, fatty acids, minerals, vehicle in pharmaceutical preparations and a raw material for industrial purposes [4, 5].

Chrysobalanus icaco has been used in traditional medicine as treatment for chronic diarrhea, diuretic, hypoglyceamic, and antiangiogenic effect, while *Parinari excelsa* is used in Africa as a remedy for dysentery, epilepsy, malaria, toothache and venereal diseases [6]. These medicinal plants have been reported to contain various chemical constituents such as flavonoids [7, 8].

Oils are produced by plants and are usually sequestered in specialized pockets or glands and could act as an insect's attractants or repellants. The purpose of this research includes to evaluate the physicochemical parameters, GC-MS analysis of the oils, elemental assessment of the seeds to ascertain the chemical composition and nutritional values of the oils in the seeds.

Fig. 1 : Leaves and fruit parts of Parinari *excelsa* Sabinus

Fig. 2 : Leaves and fruits of *Chrysobalanus icaco* L Chrysobalanacea

MATERIALS AND METHODS

Collection

The fruits of *Parinari excelsa* (Fig. 1), and *Chrysobalanus icaco* (Fig. 2), were bought on the 11th of November, 2019 at Akenfa new market in Yenagoa metropolis. The nuts were broken and the kernel collected, grounded and weighed.

Extraction

The dried powdereds seed weighing 736 g and 449 g of *Parinari excelsa* and *Chrysobalanus icaco* respectively were extracted via successive maceration at room temperature using 2.2 L each of n-Hexane, and dichloromethane for 7-days respectively. Each of the extracts were concentrated *in vacuo* at 50°C using rotary evaporator. The concentrated oils were subjected to physicochemical analysis [9].

Physicochemical Determination

The following physicochemical parameters such as density, refractive index, viscosity, acid value, saponification value, iodine value, peroxide value and ester value of the samples were determined using standard guidelines [4,5,10,11,12,13,14,15, 16,17,18].

GC-MS and Elemental analysis

The oils were analyzed using GC-MS Agilent Technologies 5977AA MSD GC-MS machine, while elemental analysis of the crude seeds was determined using Agilent Technologies 200 Series AA.

RESULTS AND DISCUSSION

The acid values obtained from *P. excelsa* extracts were 29.2 mg KOH/g oil (Pe-Hex) and 42.1 mg KOH/g oil for Pe-Dcm, while *C. icaco* extracts had acid values of 8.4 mg KOH/g oil and 5.6 mg KOH/g oil for Ci-Hex and Ci-Dcm respectively. Saponification values determined for the two plants were 350.6 mg KOH/g oil (Pe-Hex), 465.6 mg KOH/g oil (Pe-Dcm), 471.2 mg KOH/g oil (Ci-Hex) and 460 mg KOH/g oil (Ci-Dcm). The ester values of the different extracts were obtained as differences between the saponification values and the corresponding acid values, as shown in Table 1.

Table 1 shows the physicochemical parameters of the oils obtained from *Parinari excelsa* and *Chrysobalanus icaco*. Highest yield was obtained in Ci-Hex fraction (28.54%), while lowest was Pe-DCM fraction (5.76%). Saponification, ester and perioxide values were also higher in the Ci-Hex fraction. The P-value obtained from the One-way Analysis of Variance (ANOVA) in the data on table 1, is 0.9758, considered not significant, F = 0.06934.

Keys; Pe-Hex *(Parinari excelsa* n-Hexane Extract); Pe-Dcm (*Parinari excelsa* Dichloromethane Extract); Ci-Hex (*Chrysobalanus icacao* n-Hexane extract; Ci-Dcm (*Chrysobalanus icacao* dichloromethane extract)

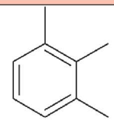
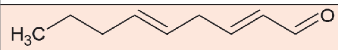
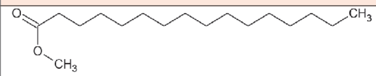
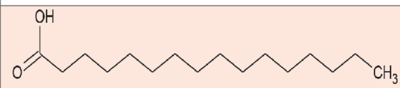
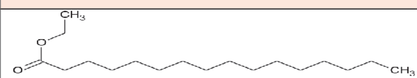
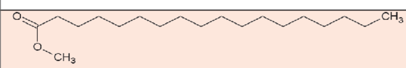
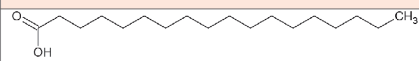
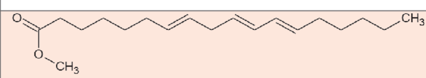
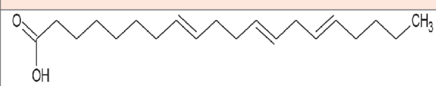
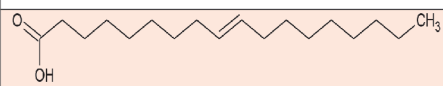
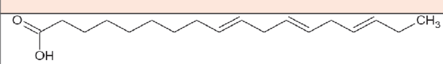
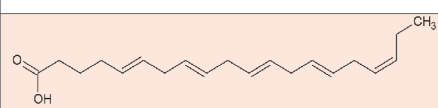
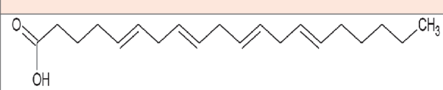
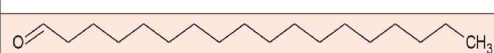
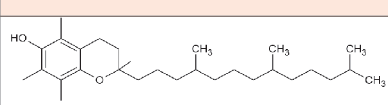
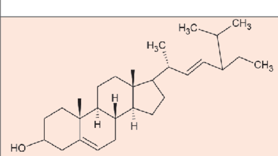
Table 1 : Physicochemical analysis of oils obtained from *Parinari excelsa* and *Chrysobalanus icaco*

Properties	Pe-Hex	Pe-Dcm	Ci-Hex	Ci-Dcm
% yield	12.95	5.76	28.54	5.76
Density g/ml	0.9140	0.9653	0.9469	0.9258
Refractive index	1.420	1.349	1.330	1.374
Acid value	29.2	42.1	8.4	5.6
Saponification value	350.6	465.6	471.2	460
Peroxide value	24	26	101	63
iodine value	16.75	23.34	12.9	18.65
Ester value	321.4	423.5	462.8	462.8

GC-MS analysis of the Pe-Hex fraction revealed the presence of Vitamin E, Stigmasterol, Arachidonic acid and several other fatty acids and fatty acid esters, as shown in Table 2.

Table 2 shows a list of compounds found to be present in the n-hexane extract of *P. excelsa* (Pe-Hex). Notably present are Vitamin E, Stigmasterol and Arachidonic acid. The

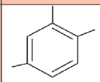
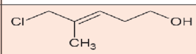
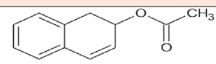
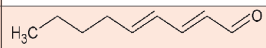
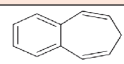
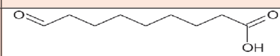
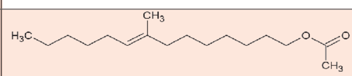
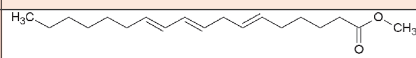
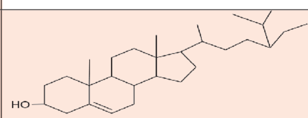
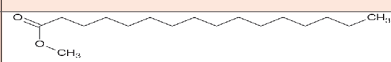
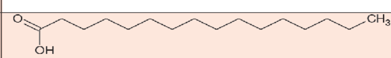
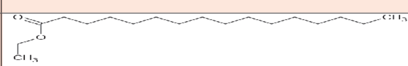
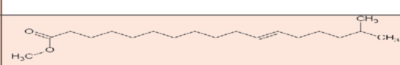
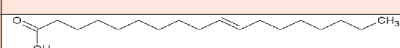
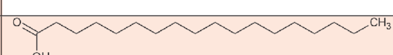
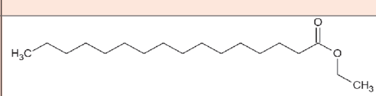
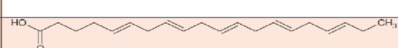
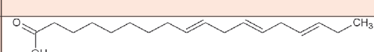
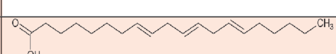
Table 2 : GC-MS analysis of n-Hexane oily fraction obtained from seeds of *Parinari excelsa*

S/N	Chemical Structure	Formular	Name
1		C_9H_{12}	Trimethylbenzene
2		$C_9H_{14}O$	2,4-Nonadienal
3		$C_{17}H_{34}O_2$	Hexadecanoic acid methylester
4		$C_{16}H_{32}O_2$	Hexadecanoic acid
5		$C_{18}H_{36}O_2$	Hexadecanoic acid ethylester
6		$C_{19}H_{38}O_2$	methylstereate
7		$C_{18}H_{36}O_2$	Octadecenoic acid
8		$C_{19}H_{32}O_2$	Methyl,6,9-cis,11-trans-octadecatrienoate
9		$C_{20}H_{34}O_2$	8,11,14-Eicosatrienoic acid
10		$C_{18}H_{34}O_2$	Oleic acid
11		$C_{18}H_{30}O_2$	9,12,15-Octadecanoic acid
12		$C_{20}H_{30}O_2$	Cis-5,8,11,14,17-Eicosapentaenoic acid
13		$C_{20}H_{30}O_2$	Arachidonic acid
14		$C_{18}H_{36}O$	Octadecanal
15		$C_{29}H_{50}O_2$	Vitamin-E
16		$C_{29}H_{48}O$	Stigmasterol

dichloromethane extract of *P. excelsa* was found to contain Sitosterol, Eicosanoids and several other fatty acids as shown in Table 3.

Table 3 shows compounds present in the Pe-Dcm extract, including Sitosterol, Oleic acid and eicosanoids. These were confirmed to be present by GC-MS analysis.

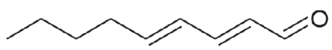
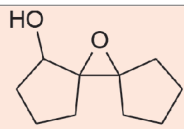
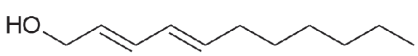
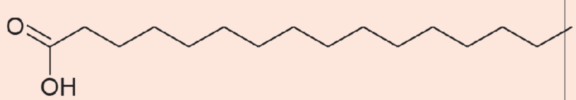
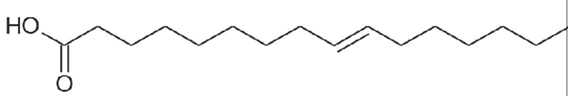
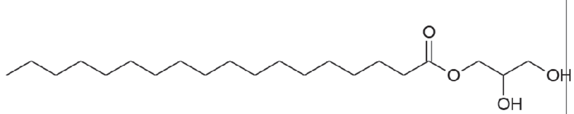
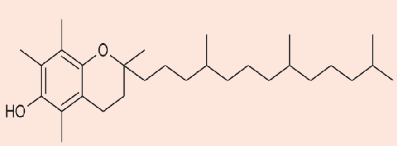
Table 3 : GC-MS analysis of Dichloromethane oily fraction obtained from seeds of *Parinari excelsa*

S/N	Chemical Structure	Chemical Formular	Name of Chemical Structure
1		C_9H_{12}	1,2,5-Trimethylbenzene
2		C_5H_9ClO	4-Chloro-3-Methylbut-3-en-1-ol
5		$C_{12}H_{12}O_2$	2-Napthalenol,1,2-dihydro acetate
6		$C_9H_{14}O$	2,4-Nonadienal
8		$C_{11}H_{10}$	Benzocycloheptatriene
9		$C_9H_{14}O_2$	3-Nonynoic acid
11		$C_9H_{16}O_2$	Oxononanoic acid
15		$C_{17}H_{32}O_2$	7-Methyl-Z-tetradecen-1-ol acetate
16		$C_{19}H_{32}O_2$	Methyl-6-cis,9-cis,11-trans-octadecatrienoate
17		$C_{17}H_{50}O$	Sitosterol
18		$C_{17}H_{34}O_2$	Hexadecanoic acid methylester
19		$C_{16}H_{32}O_2$	Hexadecanoic acid
20		$C_{18}H_{36}O_2$	Hexadecanoic acid ethylester
22		$C_{19}H_{38}O_2$	16-methylheptadecanoic acid methyl ester
23		$C_{18}H_{34}O_2$	Oleic acid
24		$C_{18}H_{34}O_2$	Octadecanoic acid
25		$C_{18}H_{36}O_2$	Hexadecanoic acid ethylester
26		$C_{20}H_{30}O_2$	Cis-5,8,11,14,17-Eicosapentaenoic acid
27		$C_{18}H_{30}O_2$	9,12,15-Octadecatrienoic acid
29		$C_{20}H_{34}O_2$	8,11,14-Eicosatrienoic acid

Chemical constituents found to be present in the n-hexane extract of *C. icaco* (Ci-Hex) include Tocopherol, Glycerol monostearate and other saturated and unsaturated fatty acids (Table 4).

Table 4 is a list of compounds confirmed by GC-MS analysis to be present in the Ci-Hex extract. They include dl-α-Tocopherol and Glycerol monostearate.

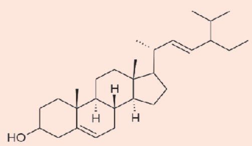
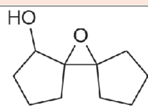
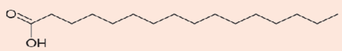
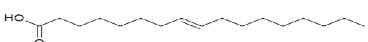
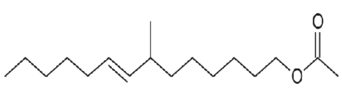
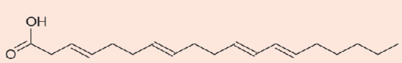
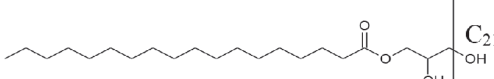
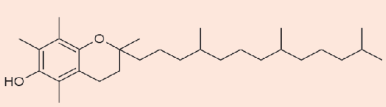
Table 4 : GC-MS analysis of n-Hexane oil obtained from *C. icacoa* seeds

S/N	Structure	Chemical Formula	Name
1		$C_9H_{14}O$	2,4-Nonadienal
2		$C_{10}H_{16}O_2$	11-Oxadispiro(4.O.4)1]undecan-1-ol
3		$C_{11}H_{20}O$	2,4-Undecadien-1-ol
4		$C_{16}H_{32}O_2$	n-Hexadecanoic acid
5		$C_{18}H_{34}O_2$	Oleic acid
6		$C_{20}H_{32}O_2$	Arachidonic acid
7		$C_{21}H_{42}O_4$	Glycerol monostearate
8		$C_{29}H_{50}O_2$	dl-α-Tocopherol

Compounds found in Ci-Dcm were dl-α-Tocopherol, Stigmasterol, Arachidonic acid, as well as other fatty acids (Table 5).

Table 5 shows chemical compounds present in the oily dichloromethane extract of *C. icaco.* This fraction also contained dl-α-Tocopherol, Stigmasterol, Arachidonic acid and Oleic acid.

Table 5 : GC-MS analysis of dichloromethane extract of *C. icaco*

S/N	Structure	Chemical Formula	Name
1		$C_9H_{14}O$	2,4-Nonadienal
2		$C_{29}H_{48}O$	Stigmasterol
3		$C_{10}H_{16}O_2$	11-Oxadispiro[(4.O.4)1]undecan-1-ol
4		$C_{16}H_{32}O_2$	n-Hexadecanoic acid
5		$C_{18}H_{34}O_2$	Oleic acid
6		$C_{18}H_{36}O_2$	Octadecanoic acid
7		$C_{17}H_{32}O_2$	7-methyl-Z-tetradecen-1-ol acetate
8		$C_{20}H_{32}O_2$	Arachidonic acid
10		$C_{21}H_{42}O_4$	Octadecanoic acid 2,4-dihydroxypropyl ester
11		$C_{29}H_{50}O_2$	dl-α-Tocopherol

Elemental analysis of the seeds of *P. excelsa* and *C. icaco* revealed the presence of sodium, potassium, magnesium, calcium, and traces of manganese, while heavy metals such as lead, arsenic and mercury had less than 0.001 mg/Kg in both plants (Table 6).

Table 6 summarizes the elemental composition of the seeds of *P. excelsa* and *C. icaco*. Both seeds were found to contain sodium, potassium, magnesium, calcium, phosphorus and manganese.

DISCUSSION

Percentage yields were 12.95% (n-hexane extract, or Pe-Hex) and 5.76% (dichloromethane extract, or Pe-Dcm) for *Parinari excelsa*; 28.54% (n-hexane extract, or C-Hex) and 5.76% (dichloromethane extract, or Ci-Dcm) for *Chrysobalanus icaco*.

The relative densities of all the oils were lower than the density of water. This applies to most oils used for cooking, and also conforms to the report of the FAO/WHO's Codex Alimentarius Standard for named vegetable oils [19].

The refractive indices of *P. excelsa* oils ranged from 1.349 - 1.420, while those for *C. icaco* ranged from 1.330 - 1.374 as shown in table 1. When compared with olive oil which has a refractive index of 1.460 and arachis oil, 1.468-1.472 it shows that light would travel faster in the samples than standard [5]. These values were slightly lower than the range of refractive indices recorded in the Codex Alimentarius Standard for vegetable oils [19]. These values are also lower than results that have been previously reported for both plants[20].

The acid values of *P. excelsa* derived oils were very high: 29.2 mg KOH/g (Pe-Hex) and 42.1 mg KOH/g (Pe-Dcm), far exceeding that recorded in literature (2.58 mg KOH/g) [20], and the report of the Codex Alimentarius Standard for vegetable oils [19]. The relatively lower acid values of the oils obtained from *C. icaco* (8.4 and 5.6 mg KOH/g), were also higher than recommended value of 4.0 mg KOH/g oil [19] (CXS 210 - 1999). The high acid values could be indicative of significant hydrolysis of the glycerides [21].

Oils obtained from both plants had very high saponification values; 350.6 mg KOH/g oil (Pe-Hex), 465.6mg KOH/g oil (Pe-Dcm), 471.2mg KOH/g oil (Ci-Hex) and 460mg KOH/g oil (Ci-Dcm). This highlights their potential to be valuable in the soap and detergent industry [22].

The oils extracted from *P. excelsa* had about double the recommended peroxide levels (10-15 meq/kg oil). Peroxide values for the *C. icaco* oils were however, excessively high. The high peroxide values is indicative of high susceptibility to oxidative rancidity [10]. All the oily samples gave low iodine values, indicating very low levels of unsaturation. This agrees with the high saponification values [23]. The ester values of all the oil samples were very high (as shown in Table 1). The high ester values indicate that the oily samples contain high amounts of low molecular weight fatty acids [24].

The results of elemental assay bring to light the presence of Sodium, Potassium, Phosphorus, Magnesium and Calcium, while Zinc is present at low concentration. Although these elements are much more concentrated in *C. icacoa* compared to *Parinari excelsa* seed. However, the result of elemental analysis showed

Table 6 : Mineral composition of *Parinari excelsa* and *Chrysobalanus icacoa* seed

S/N	Parameter(mg/Kg)	*P. excelsa*	*C. icaco*
1.	Sodium	2.399	4.152
2.	Potassium	2.971	4.851
3.	Magnesium	3.402	6.872
4.	Calcium	1.847	2.048
5.	Phosphorus	0.204	0.732
6.	Manganese	0.065	0.027
7.	Chromium	0.001	<0.001
8.	Zinc	0.072	<0.001
9.	Iron	0.757	0.113
10.	Lead	<0.001	<0.001
11.	Arsenic	<0.001	<0.001
12.	Boron	<0.001	<0.001
13.	Cadmium	<0.001	<0.001
14.	Mercury	<0.001	<0.001
15.	Copper	0.006	<0.001
16.	Selenium	<0.001	<0.001

that Arsenic, Boron, Cadmium, Lead, Mercury, Copper, Iron, Chromium and Selenium were below the minimum detectable level. This showed that the samples were free from high heavy metals contamination, thus relatively safe for human consumption.. Some are essential nutrients that are required for various biological effects [25, 26]. The GC-MS analysis of the oils reveals that the oils contain common fatty acids, steroids and vitamins found in edible oils [27]. The n-hexane extract of *Parinari excelsa* was found to contain stigmasterol and vitamin E with a potent antioxidant effect while dichloromethane fraction of the oils contained sitosterol which could play an important role in men's prostate health and also prevent heart diseases. It also has an anti-inflammatory property [28, 29, 30].

CONCLUSION

Most of the physicochemical parameters assessed did not fall within recommended standards. Even though literature suggests that oils obtained from these plants have characteristics suitable for use in cooking, the results of this study indicate that the oil samples obtained from both *P. excelsa and C. icaco* do not meet the recommended standards for them to be used for cooking. Elemental analysis showed that both plants contained similar elemental constituents and had undetectable levels of the toxic heavy metals. GC-MS analysis showed that the oily samples contained similar chemical constituents such as vitamin E, stigmasterol, sitosterol, oleic acid and octadecanoic acid.

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CONFLICT OF INTEREST

Nil

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