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Chemical analysis and antifungal activity of rubber seed oil (*Hevea brasiliensis*) from the Southern region of Cote d'Ivoire

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Abstract

In Cote d'Ivoire, most of the rubber seeds produced each year generally remain unexploited. The aim of this study is to evaluate the physicochemical properties, fatty acid composition and the antifungal activity of rubber seed oil (RSO), extracted by soxhlet method using *n*-hexane as a solvent. After extraction, the yield of rubber seeds oil obtained is 47.10 ± 0.20%. The physicochemical properties of this oil are following: acid value (3.27 ± 0.36 mg KOH/g), peroxide value (4 ± 0.01 meq O_2/g), saponification value (172.98 ± 0.36 mg KOH/g), ester value (169.70 ± 0.31 mg KOH/g), iodine value (141 ± 1.13 g I_2 KOH/g) and density at 25 °C (0.83 g/m^3). Moreover, the gas chromatographic profile indicates that this oil contains mainly made up of four (4) fatty acids which are palmitic acid (13.78%), linoleic acid (34.70%), linolenic acid (39.24%) and stearic acid (12.01%). In addition, RSO shows an inhibitory action against *Fusarium* sp. The characteristic features of RSO from this study shows that it can be exploited in the production of environmentally friendly oil and in other industrial application.

1. Introduction

Following its independence, Cote d'Ivoire based its economy on export crops such as coffee and cocoa. Anxious to diversify this economy, the Ivorian state has launched a vast program to develop various agricultural products, including rubber tree (*Hevea brasiliensis*) exploited for latex. This action has enabled Cote d'Ivoire to be ranked at the first latex producer in Africa and the sixth producer in the World [1]. Rubber tree cultivation occupies a preponderant place in the Ivorian economy with an ever-increasing production going from 172,000 tons of latex in 2006 to 380,000 tons in 2015 then to 624,000 tons in 2018. The high latex prices observed in 2009 to 2011 sparked a real frenzy in Cote d'Ivoire, resulting in a notable increase in producers, cultivated areas and production [2]. However, the particularly gloomy world prices for latex in recent years have led to sluggish sales in field funds and significant delays in payment to growers. In order to diversify the sources of income for the grower, technologies for valuing rubber tree by-products, in particular seeds have been developed in many rubber-producing countries such as Malaysia, Philippines and Nigeria [3]. Unfortunately, in Cote d'Ivoire, the auxiliary products of rubber tree, namely, wood and the seeds are neglected. The rubber seed doesn't find any major applications and hence even the natural production of seeds itself remains underutilized. The trees are planted on more than 550,000 ha land over the country and each hectare can give an approximate amount of 150 kg of rubber seeds [2]. These rubber seed oil (RSO), rich in oil (from 35 to 45%) has been found to have potential applications in many areas amongst which are in the production of biodiesel as fuel for compression engines [4, 5], foaming agent in latex foam, in the synthesis of alkyd resin used in paints and coatings and several other uses [6, 7]. Therefore, RSO has the potential to replace/substitute for edible oils such as palm oil, coconut oil, etc. as a raw material for industrial uses during periods of high food sector demand. In addition, the meal cake of these seeds could serve as feed for poultry and cattle [8, 9]. There is lack of information available about rubber seed oil from Cote d'Ivoire. This study aims to extract oil from seeds of rubber tree (*Hevea brasiliensis*) to also investigate it physiochemical properties and the antifungal activity to provide baseline data on their quality in order to give an indication for their suitable uses.

2. Methodology

2.1 Collection rubber seeds

The rubber seeds were collected from a plantation in the locality of Irobo (Lagune District, Cote d'Ivoire) in February 2021. The seeds were taken to the Bioorganic Chemistry and Natural Substances Laboratory for crushing. The almonds obtained after crushing were sorted to eliminate those which are defective and then gathered to constitute a sample of 5 kg. The almonds were then dried under the sun for one week, then in an oven at 40 °C for 24 hours until constant weight. The seeds were immediately ground using a blender fitted with a mesh sieve having a diameter of 1 mm and stored in air-tight polythene bags before use.

2.2 Oil extraction method by Soxhlet

The recommended methods of AFNOR [10] were adopted to determine oil yield. Thus, 100 g of the milled seeds were loaded into a thimble and placed in the refluxing unit of the Soxhlet apparatus with 300 mL of n-hexane as extraction solvent. The extract was de-solventized with the rotary evaporator to obtain the seed oil and stored in amber bottles in the refrigerator before analysis. The oil yield was calculated using the expression below:

Yield = Weight of oil extracted x 100/ Weight of sample **Eqn. 1**

2.3 Physicochemical Characteristics

The physicochemical characteristic of the oil was carried out. The percentage yield was obtained as ratio of the weight of oil extracted to the weight of sample, multiplied by 100. The acid value (AV), iodine value (IV), peroxide value (PV) and saponification value (SV) were determined using the official method of analysis AOAC [11]. The relative density of oilseeds was determined at 25°C following the IUPAC method by using a pycnometer [12]. Ester value (EV) value was determined by mathematical expression; EV= SV- AV.

2.4 Fatty acid composition

The fatty acids were converted to their methyl esters (FAMEs) as described by Chunhieng [13]. About 0.1 g of rubber seed oil sample was mixed with 1.5 mL of n-hexane and 1.5 mL of a methanolic solution of boron trifluoride. The whole mixture was shaken up for 30 s and then placed in a water bath at 50 °C for 20 seconds. After cooling to room temperature, 1 mL of hexane and 2 mL of distilled water were added to the mixture and then stirred under nitrogen. The top layer containing the FAMEs was used for gas chromatography (GC) analysis. FAMEs solution (1 µL) containing the internal standard (erucic acid) was injected into a gas chromatograph (Hewlett-Packard HP 5890 Series II) equipped with a flame ionization detector (FID) and a capillary column CP-Select CB, type WCOT Fused Silica (50 m X 0.25 mm i.d. x 0.25 µm film thickness). The carrier gas was helium and the flow rate was adjusted to 1.2 mL/min. Temperatures of detector and injector were 250°C. The initial column temperature was set at to 100°C and programmed to increase by 5°C per min intervals until 220°C and, kept for 10 min at this temperature. The fatty acid methyl esters peaks were identified by comparing their retention times with those of standards. After adjusting areas with the internal standard (erucic acid), the yield of each fatty acid was calculated as follow:

Yield of each fatty acid= area of the fatty acid/areas of total fatty acids in the oil sample × 100 (%) **Eqn. 2**

2.5 Antifungal activity of rubber seed oil.

The antifungal activity of rubber seed oil was carried out by adopting protocols of those described in previous reports [14]. A tri-series oil concentration range in the proportions of 1/4, 2/4 and 3/4 was prepared in Potato Dextrose Agar (PDA) (50 °C) and poured into plates. The media surface is inoculated with a mycelia disc of *Fusariu*m sp and the plates were incubated at 30 °C for 5 days. A control was also carried out under the same conditions without adding oil sample.

3. Results and Discussion

3.1 Physicochemical characteristics

The physicochemical properties of rubber seed oil are summarized in **Table 1**. The oil content of RSO is 47.10 ± 0.20%. This value is higher than oil seeds exploited industrially, in particular cotton seed (13%), soybean (14%) and palm oil (20%) [15]. In addition, this yield is similar to the values reported in previous studies [16, 17, 18]. These authors found rates between 42.97 ± 0.59 and 52 ± 0.1%. Thus, the rubber seeds could constitute a judicious alternative for an industrial production of vegetable oil. In fact, RSO constitute a more important lipid source than certain unconventional oilseeds exploited such as *Canarium schwenfurthii* (36.1%), *Solanum nigrum* (38%) and *Dacroydes edulis* (43.20%) [19].

The relative density at 25 ºC was determined using a pycnometer and was found to be 0.83 g/cm^3, which implies that the oil is less than water with the absence of heavy elements. This value does not differ from those found into the vegetable oils [12]. However, higher values have been determined by several authors [20, 21] with values ranging between 0.857 and 0.943 g/cm^3.

Acid value is a measure of the amount of free fatty acids present in fat and oils. It gives an indication of the deterioration, rancidity, or edibility of the oil. The acid value of RSO is less than 6 mg KOH/g which is the recommended limits value advised by Codex [22]. This value is also lower than those determined in previous studies by Onoji [17] and Maliki and Ifijen [18] who reported values of 18.20 ± 0.14 and 32 mg KOH/g, respectively. This index value is also lower than those of certain edible oils such as coconut oil (4-7 mg KOH/g), palm kernel (4-7 mg KOH/g), peanut (0.8-6 mg KOH/g) and soybean (7 mg KOH/g) [34].

Peroxide value (PV) gives an indication of the quality and stability of oil. The peroxide value determines the extent to which the oil has undergone rancidity when stored, heated, or in contact with air and could be used to assess the quality and stability of oils. Oils become rancid when the PV ranges from 20–40 meq O_2/kg oil [23]. The PV obtained for rubber seed oil in this study is 4 meq of O_2/kg oil. This low value would testify to a high resistance to oxidation. In addition, this value is within the range reported in literature for RSO (1.6–16 mequiv O_2/kg oil) [17] and close to FAO standards for edible oils [22].

The saponification value (SV) indicates the amount of alkali required to convert the oil into soap and is an index of average molecular mass of fatty acid in the oil sample. As oil is mainly triglycerides, it allows for comparison of the average fatty acid chain length. The SV of 172.98 mg of KOH/g obtained is lower than those determined by authors [16, 17]who found respective values of 187 and 195.30 ± 0.28 mg of KOH/g. This value is also lower than those of usual oils such as soybean (189-195mg / g), peanuts (187-196 mg / g), cotton (189-198mg / g), palm (195-205) and olive (184-196mg / g) [22]. However, the saponification value find in this study is close to that reported by Abubakar et al. [24] who recorded a value of 179.6 mg KOH/g oil.

The iodine value is a measure of the degree of unsaturation of oils. High iodine value is attributed to high unsaturation. The iodine value of rubber seed oil (141 ± 1.13 g I_2 KOH/g) was estimated by Wijs' method and falls within the range specified for semi-drying oils (100–150 g I_2/100 g) [25]. This value is similar to those reported (139.70 g I_2 / 100g and 141 ± 0.5 g I2 / 100 g) reported by Maliki et al [18] and Ochou et al [16]. However, it is slightly higher than that of common oils, such as soybean (120-143), sunflower (110-143), sesame (104-120), cotton seed oil (105), jatropha (100), rapeseed oil (94-120) and olive (75-94). Also, this index is higher than those of other unconventional oilseeds such as *Coula edulis* (90-95) and *Canarium schwenfurthii* (71.1) [26]. The higher iodine value may be due to high-unsaturated fatty acid content, as observed in GC-MS analysis of oil.

The ester value recorded in this study of 169.70 mg KOH/g oil is greater than the value of 135.33 ± 3 mg KOH/g determined by Maliki et al [18]. This high index indicates a strong presence of ester in the oil [27].

Table 1. Physicochemical properties of rubber seed oil

Parameters	Values
Yield (%)	47.10 ± 0.20
Relative density at 25°C	0.83
Acid value (mg KOH/g)	3.27 ± 0.36
Peroxide value (meq O_2/Kg)	4.00 ± 0.01
Saponification value (mg KOH/g)	172.98 ± 0.36
Iodine value (g I_2/100g)	141.00 ± 1.13
Ester value (mg KOH/g)	169.70 ± 0.31

3.2 Fatty acid composition

The qualitative and quantitative fatty acid composition of the rubber seed oil is given in **Table 2** and **Figure 1**. The gas chromatographic profile indicates that the oil contains mainly made up of four (4) fatty acids which are palmitic acid (13.78%), linoleic acid (34.70%), linolenic acid (39.24%) and stearic acid (12.01%). This oil is unsaturated because the proportion of unsaturated fatty acids (PUFA) in RSO (73.94%) is higher than that of the saturated fatty acids (SFA) which is 26.07%. Although the composition of this oil did not follow the trend of some of the reported fatty acid profile for rubber seed oil in the literature [17, 21]. These differences in the results can be explained by the varieties of rubber tree clones used in different countries [22]. The content of polyunsaturated fatty acids in this study is higher than those reported for tropical vegetable oils such as shea butter (6.9%), avocado oil (15.5%), safou oil (25.2%) and aiele oil (28.8%) [28]. The high proportion of PUFAs in this oil could make it possible in cosmetics to ensure good fluidity of skin cell membranes [29]. In view of the linolenic acid content (39.24%) in the RSO, it can be considered as linolenic oil. Indeed, this content is higher than that of soybean oil (7.8%), considered to be a significant source of linolenic acid [30]. Therefore, the RSO could be used in the food, cosmetic, biofuel, paint and coating industries [25, 31].

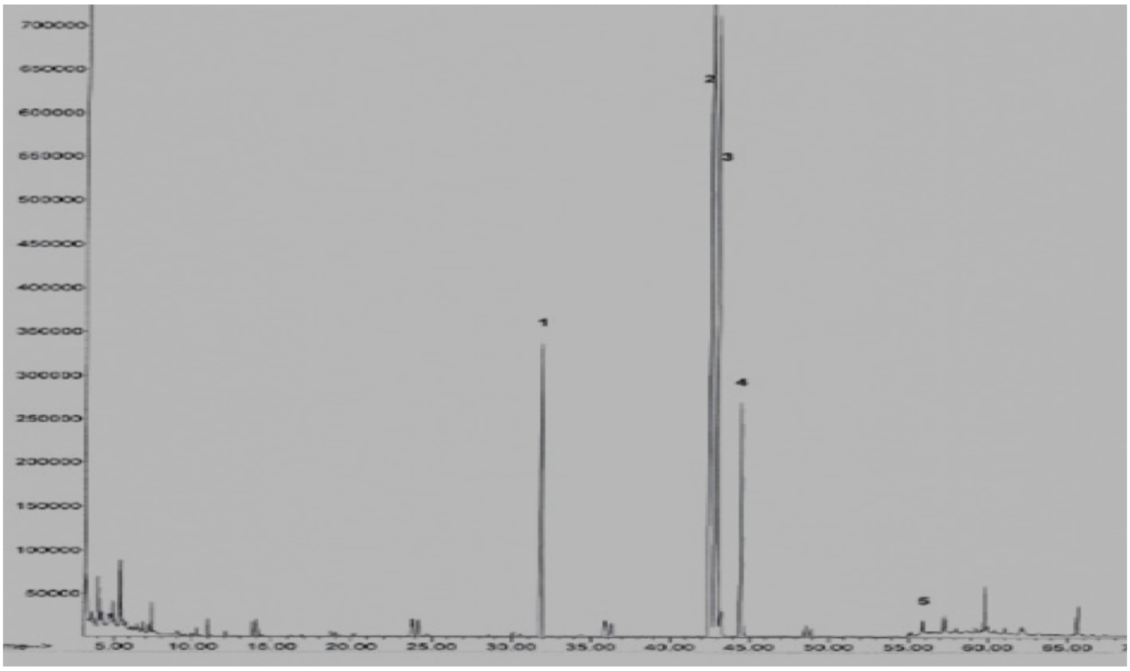

Figure 1. Gas chromatogram of rubber seed oil with identified peaks:
1: Palmitic acid; **2**: Linoleic acid; **3**: Linolenic acid; **4**: Stearic acid; **5**: Arachidic Acid

Table 2. Fatty acid composition of RSO

Compounds	Quantities (%)	Retention time (min)
Palmitic acid (C16:0)	13.78	31.83
Linoleic acid (C18 :2n6)	34.70	42.50
Linolenic acid (C18 :3n3)	39.24	42.90
Stearic acid (C18:0)	12.01	44.48
Arachidic Acid (C20:0)	0.28	55.89
SFA	**26.07**	
PUFA	**73.94**	
PUFA/SFA	**2.84**	

SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid

3.3 Antifungal activity

The antifungal activity of oil extracted from the rubber seeds on the fungal strain of *Fusarium* sp. is presented in Figure 2. The isolate of *Fusarium* sp does not show any mycelial development on the oil-amended media unlike tested the control after 7 days. However, depigmentation of the fungal inoculum was observed in the culture medium. The antifungal activity of this oil is thought to result from the fatty acid composition of oil and also the presence of numerous volatile aromatic compounds (aldehydes, alcohols, acids, esters) [32, 33]. Furthermore, after sampling and subculturing the inocula from the oil-amended media on oil-free PDA medium, the development of white fungal colonies was observed after five (5) days of inoculation (Figure 3).

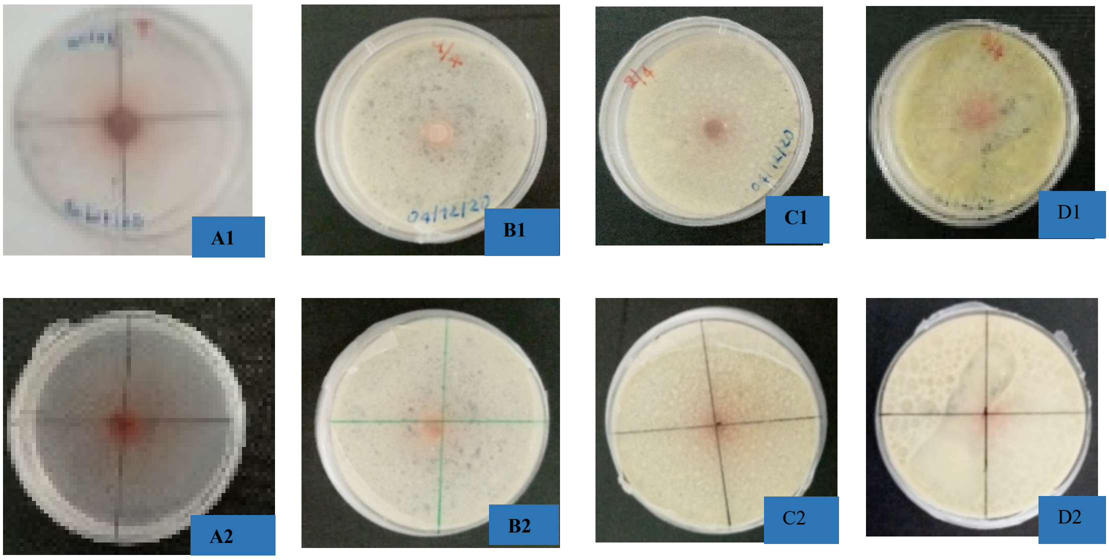

Figure 2. Antifungal activity of rubber seeds oil on *Fusarium* sp. strain after 7 days of inoculation: A1, A2: control test of *Fusarium* sp strain without RSO; B1, B2, C1, C2, D1, D3: inhibition of *Fusarium* sp strain development with rubber seed oil at different concentrations

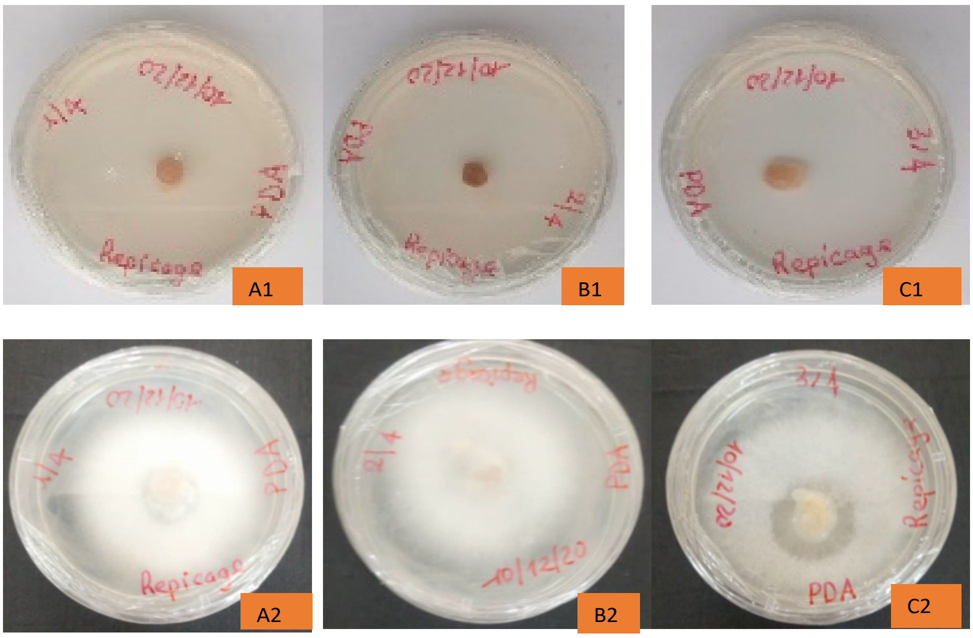

Figure 3. Fungal inocula of *Fusarium* sp. strain from agar media with RSO (A1, B1 and C1) and placed on PDA media 5 days after subculturing (A2, B2, C2)

In view of this result, the rubber seed oil could be considered to be a fungistatic agent on this isolate of *Fusarium* sp. It can be used as a fungicide to prevent the proliferation of pathogens. The oil extracted from the rubber seeds could be used as an antimicrobial agent in cosmetic preparations and in the formulation of biopesticides against *Fusarium* wilt.

Conclusion

This study was permitted to determine the physicochemical properties, fatty acid composition and antifungal activity of the rubber seeds oil. Results show that the yield of oil extract from rubber seed was 47.10 ± 0.20%. This oil presents good quality criteria as acid, iodine, peroxide, ester and saponification indices met the recommended limits for edible oils. Moreover, the chromatographic profile shows that RSO is mainly composed of polyunsaturated fatty acids (73.94%) with a high content of linolenic acid (**ω**-3) (39.24%). In addition, RSO shows an inhibitory action against *Fusarium* sp. Therefore, RSO should be developed for industrial applications such as in the production of biodiesel, cosmetics, and other pharmaceutical formulations.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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