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Extraction of vegetable oil from *Cissus populnea* Guill and Perr. Seeds and determination of its fatty acids content

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Abstract
The objective of this work was to highlight the oleaginous nature of seeds of *Cissus populnea*, a plant of the tropical flora and to determine the profile of fatty acids. Extraction of the oil was done by the soxhlet method using petroleum ether as a solvent. The fatty acid composition was determined by gas chromatography by derivatization. We noted an oil extraction yield of 20.3 ± 1.64%. This oil contains palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and myristic acid (C14:0). The unsaturated fatty acids are: oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6), vaccenic acid (C18:1 n-7) and 11-Eicosenoic acid (C20:1 n-9). The four major compounds encountered in this oil are palmitic acid (40.1 ± 0.2%), oleic acid (27.1 ± 0.2%), stearic acid (16.5 ± 0.14%) and linoleic acid (11.9 ± 0.1%). Unsaturated fatty acids ω6 (linoleic acid) and ω9 (oleic acid) were found in high proportion. This oil could be used like edible oil following further studies.

Keywords: *Cissus populnea*, oil, saturated and unsaturated fatty acids, seeds

1. Introduction
Many oilseed plants in sub-Saharan Africa are used in the food, pharmaceutical and cosmetic industries [1, 2]. However, many other species that are found in the natural forests are still under exploited and even unrecognized by the populations as oleaginous [3]. This is the case of *Cissus populnea* Guill. And Perr. Oleaginous are groups of plants whose seeds or fruits are rich in oil. No studies conducted on this specie mention it as an oleaginous specie.
Cissus populnea Guill. and Perr. belongs to the *Vitaceae* family. It is a plant of perennial stalk, with herbaceous and voluble annual stems that can become almost woody at the base. The leaves are alternate and whole. This plant is found in wooded savanna in tropical areas. Its geographical area extends from Senegal to Mozambique [4].
The specie is widely used for its medicinal properties as remedies for many diseases: The mucilaginous root preparations would be anti-helminthic. The decoction of the leaves in drinks would calm the intestinal pains and expel intestinal parasites [4]. Mature fruits are edible [2] and immature fruits are used as condiments for sauces [5, 2]. Ethnobotanical surveys, carried out among Bobo ethnic populations in Burkina Faso, showed that seeds of this specie were consumed [6].
Cissus populnea is very abundant in the Bobo-Dioulasso region of Burkina Faso. The production of vegetable oil of this specie is very important for the local people.
The objective of this work is to highlight the oleaginous nature of this plant and to determine the fatty acid profile of this oil. This work is part of the characterization and valorization of the plant resources of Burkina Faso.

2. Materials and methods
Plant material
The fruits of *Cissus populnea* were harvested in the nature reserves of Dindérosso and Kua, two sites around the city of Bobo-Dioulasso in Burkina Faso. In this region, the flowering of *Cissus populnea* begins in May and immature fruits (Figure 1A) are available as early as June. Fruit maturity begins in August and is available until November. The fruit is an ovoid berry, smooth, 20- 25 x 10-15mm, purple black at maturity [2] (Figure 1B).

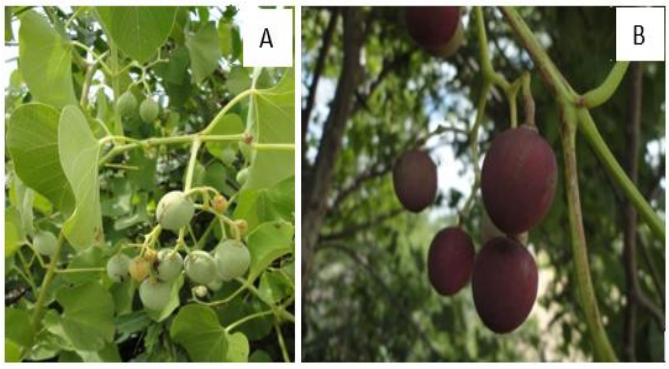

Fig 1: *Cissus populnea*; The whole plant with immature fruits (A) and mature fruits (B) Vernacular names: Kièh in Bobo ethnic group; oseille des pygmies in French; food gum in English

Seeds sampling

The fruits were harvested during the month of August. About 200 to 500 g of fruit were harvested per individual depending on the availability of the fruit and transported to the Plant Biology Laboratory located in Bobo-Dioulasso. In the Laboratory, the seeds were extracted manually and dried in the dark for at least two weeks.

Extraction of crude oil from *Cissus populnea* seeds

After drying the seeds, they were crushed with a GM-200 Retsch grinder. The crushed products obtained were used for the extraction of lipids. The extraction was carried out using a soxhlet type extractor according to the standard NF EN ISO 659, 2009. For this purpose the cartridges (HM1004-33x94mm) were filled with 200g (Table 1) of crushed seeds and placed in the column of the extractor. As extraction solvent, petroleum ether (CARLO ERBA REAGENT-SDS), an apolar solvent was used. After 4 hours, the extract (mixture of solvent and fat) was subjected to rotary evaporation (BUCHI ROTAVAPOR R-200) to remove the solvent. The experiment was repeated three times. The crude oil was recovered and the average yield of the extraction calculated according to the following formula:

$$Y = \left(\frac{m}{M}\right) * 100$$

Y: extracting yield, *m:* the mass of oil and *M*: the mass of ground seeds.

The oil obtained was stored at a temperature of 4° C before analyzing the profile of fatty acids by gas chromatography.

Table 1: Crushed seeds masse used by trial

	Trial 1	Trial 2	Trial 3	Total
Sample weight (g)	66	66	68	200

Analysis of the fatty acids profile

The crude oil obtained by soxhlet extraction was analyzed by gas chromatography after derivatization [7]. Briefly, 100 microliters of a solution of extracted oil in hexanes (10.7 mg/mL) containing 77.3 micrograms of triheptadecanoin as an internal standard were dried under nitrogen then hydrolyzed with 400 microliters of KOH (0.5 M) at 100° C for 15 min. Then the fatty acids were transmethylated with 1 ml of boron trifluoride (14% in methanol) at 100° C for 10 min. The resulting fatty acid methyl esters (FAMEs) were then extracted by partitioning between 2 ml of hexanes and 2 mL of saturated sodium chloride. After mixing with a vortex, the organic phase was concentrated under nitrogen and resuspended in 150 uL hexanes, transferred to a GC vial and analyzed by gas chromatography using a Thermo Trace gas chromatograph (Thermo Electron Corporation, Mississauga, ON, Canada). The volume injected was 1 microliter. The injection was made in split mode 1/40 onto a Trace-FAME column (Thermo) with flame ionization type detector. The fatty acids were identified by comparing their retention times with those of authentic standards, and a standard curve was used for quantification [7]. All analyses were performed in triplicate.

Data analysis

XLSTAT 7.5.2 was used as data analysis software. The oil extraction yields and percentages of fatty acids are expressed as mean plus or minus standard deviation values.

3. Results

Extracting yield

After extracting, we obtained an average oil yield of 13.55 ± 1.26 g corresponds to a yield of 20.31 ± 1.64% (table 2). This percentage is quite high compared to that of several oilseeds. This oil is yellow (Figure 2).

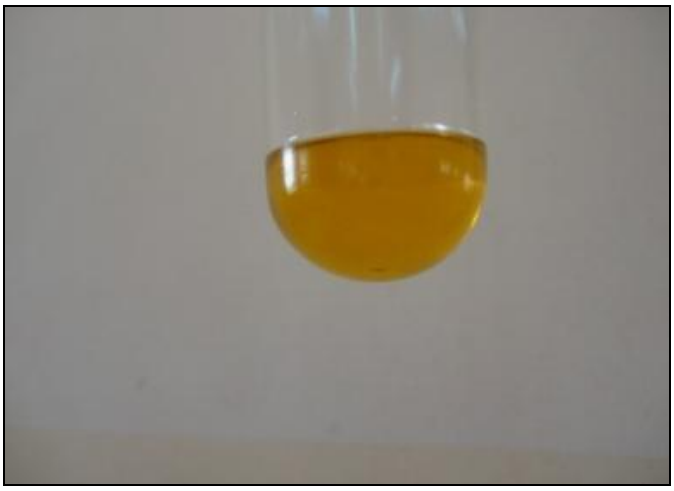

Fig 2: *C. populnea* seeds oil

Table 2: Extraction yield of *C. populnea* seeds oil

	Trial 1	Trial 2	Trial 3	Average
Sample weight (g)	66	66	68	66.67 ± 1.15
Oil weight (g)	13.88	12.16	14.61	13.55 ± 1.26
Extracting Yield (%)	21.03	18.43	21.48	20.31 ± 1.64

Fatty acids composition of the crude oil

Figure 2 shows the abundance and retention times of the different fatty acids contained in *C. populnea* seeds oil. This allowed us to detect 9 peaks in the chromatogram.

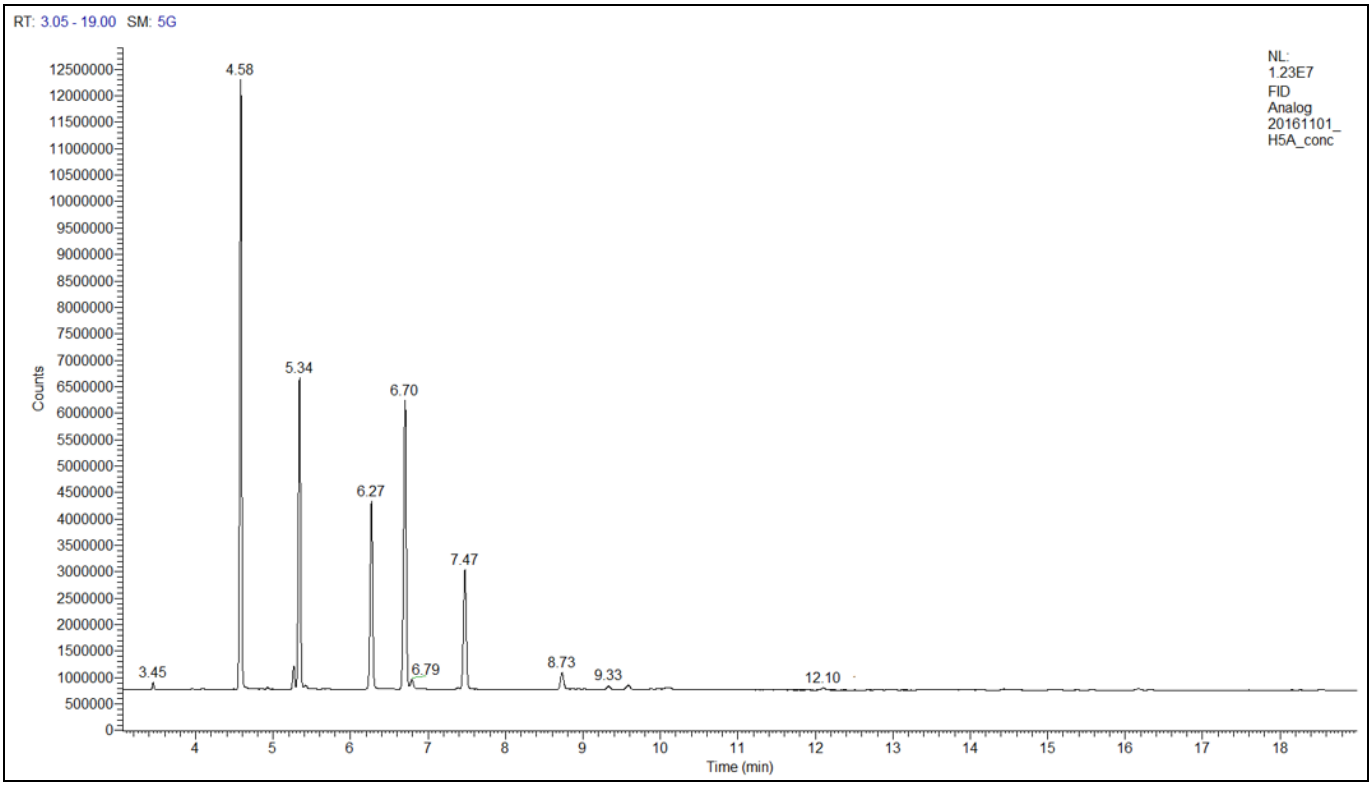

Fig 3: Chromatogram of fatty acids of *C. populnea* seeds oil

Nine fatty acids were found in the crude oil of *Cissus populnea*, including five saturated fatty acids and four unsaturated fatty acids (Table 3). The saturated fatty acids can be classified as follows in terms of proportion: palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and myristic acid (C14:0). The proportion of unsaturated fatty acids decrease from oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6), vaccenicacid (C18:1 n-7) to 11-eicosenoic acid (C20:1 n-9).

Table 3: Fatty acid profile of the oil of *Cissus populnea* seeds.

Fatty acids		Mean % of total ± SD			Retention time (min)
14:0	Myristic acid	0.40	±	0.03	3.45
16:0	Palmitic acid	40.07	±	0.24	4.58
16:1n-7	Palmitoleic acid	ND			-
18:0	Stearic acid	16.50	±	0.14	6.27
18:1n-9	Oleic acid	27.08	±	0.16	6.70
18:1n-7	Vaccenic acid	0.82	±	0.09	6.79
18:2n-6	Linoleic acid	11.86	±	0.09	7.47
18:3n-3	Linolenic acid	ND			-
20:0	Arachidic acid	2.27	±	0.07	8.73
20:1n-9	11-eicosenoic acid	0.55	±	0.08	9.33
22:0	Behenic acid	0.46	±	0.05	12.10
22:1n-9	Erucic acid	ND			-

In the first column we have the symbols of fatty acids. These symbols include the number of carbon atoms, the number of unsaturation and the omega family of the fatty acid. For example, 18: 0 means a fatty acid with 18 carbon atoms and 0 unsaturation and 18: 2n-6 means an unsaturated fatty acid having 18 carbon atoms with a first unsaturation in position 6 (omega 6) and a second unsaturation in position 9.
ND: Not detected

4. Discussion

The extraction of the oil from the seeds of *Cissus populnea* allowed us to have an average yield of 20.31 ± 1.64%. This oil yield is comparable to that of several oilseed species that are the subject of industrial food processing. Cotton and soybean seeds contain respectively 15-25% and 15-22% of oil [8]. This result confirms that *Cissus populnea* has a quantitatively valuable oleaginous potential.

Nine fatty acids were detected in the crude oil of *Cissus populnea*, namely; five saturated fatty acids and four unsaturated fatty acids. The saturated fatty acids include: palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and myristic acid (C14:0). The unsaturated fatty acids in terms of proportion were identified as: oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6), the vaccinic acid (C18:1 n-7) and 11-eicosenoic acid (C20:1 n-9). The four major compounds encountered in this oil are respectively palmitic, oleic, stearic and linoleic acids comprising more than 95% of the fatty acids present. We note the presence of ω6 (linoleic acid) and ω9 (oleic acid) unsaturated fatty acids, with no detection of ω3 unsaturated fatty acids. This fatty acids profile shows a certain specificity of *C. populnea* seeds oil because a similar study carried out on *Cissus tiliacea* Kunth seeds oil [9] showed a different fatty acid profile. The main fatty acid encountered in the oil of *C. populnea* is palmitic acid (40.07%) whereas linoleic acid (54%) is the major fatty acid in *C. tiliacea* seeds oil [9]. Furthermore, the second major fatty acid encountered in the oils of these two species is oleic acid but, the oil of *C.*

populnea is much rich in oleic acid (27.08%) than the oil of *C. tiliacea* (18%) (Franco-Mora et *al*., 2016). Hence the need to continue research on the oil of the under-used species to establish their fatty acid profile. This will allow to have a wide range of oils where each one can be valorized according to its specificity.

Given the unsaturated fatty acid diversity of this oil (ω9, ω6) it could be interesting in nutritional terms. The proportions of polyunsaturated fatty acids to saturated fatty acids is 0.68,which is less than 0.7 considered by some authors as ideal for a dietary oil [10]. In addition, the major fatty acid in this oil is palmitic acid, which favors its use in the food industry as suggested in previous studies using other oils (Milk lipids, *C. azarolus* seeds oil [11, 12].

Oleic acid (monounsaturated fatty acid), which accounts for about 25% of the fatty acids of this oil, is cited by some authors as having beneficial properties with regards to extended shelf life and high thermal stability [1].

Linoleic acid (polyunsaturated fatty acid omega 6), with a proportion of 11.8% of the total fatty acids, plays a role in the oxidative stability of an oil. The lower the linoleic acid content of an oil, the higher its thermal stability [13]. The content of linoleic acid in this oil is less important than in peanut oil [13] which contains 30% of linoleic acid. Peanut oil is one of the most consumed oils in the West African sub-region [13].

The oil would not be a source of omega 3 fatty acids that are considered to be beneficial to health. On the other hand, the absence of these highly polyunsaturated fatty acids in a vegetable oil is rather favorable for oils whose use would include heating at high temperature as in a frying pan or for frying since these fatty acids are very susceptible to oxidation and the production of undesirable odors of rancid nature [14]. Therefore, the oil of *Cissus populnea* would have versatile uses as an edible oil.

5. Conclusion and perspectives

Cissuspopulnea is a plant which, due to its seeds, has a very marked oleaginous character in view of the oil yields obtained. The most important fatty acids in this species are palmitic acid, oleic acid, linoleic acid and stearic acid. *Cissus populnea* could be used as edible oil or food supplement because it is commonly used in Burkina Faso. Further studies on the profile of glycerides, vitamins and dyes likely to be present in this oil can be carried out. It would also be interesting to evaluate the toxicity of the oil. This would allow us to have a more complete indication of the nutritional potential of this oil.

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