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ORIGINAL RESEARCH PAPER

COMPARATIVE STUDY OF CHEMICAL COMPOSITION, PHYSICO-CHEMICAL AND ANTIOXIDANT PROPERTIES OF OILS EXTRACTED BY TRADITIONAL AND HEXANE METHODS FROM *TERMINALIA CATAPPA* L. KERNELS

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Abstract: The comparative study of physico-chemical characteristics of *Terminalia catappa L.* kernel oils extracted by two methods has been done. The oil yields were 28.13 % and 61.78 % respectively for traditional and hexane methods and the fatty acid profiles showed palmitic acid (40.79 % and 40.03 % respectively) oleic acid (25.55 % and 26.09 % respectively), linoleic acid (26.72 % and 26.64 % respectively) and stearic acid (4.35 % and 4.49 % respectively) as major components. The oils extracted by the two ways showed similar physico-chemical properties, good calorific values and non-toxicity against *Artemia salina* L. Oil obtained by traditional method exhibited more antioxidant capacity (1.40) than the hexane one (0.15). This traditional method helps to extract 45 % of the total oil. It gives oil free of organic solvent, with good physico-chemical properties that could be useful as edible oil and for industrial applications.

Keywords: *fatty acid profiles, larval toxicity, quality comparison, tropical almond, two extraction ways, vegetable oil*

INTRODUCTION

Several technics are used for the extraction of oils from seed and almonds. Among these, they are traditional (for shea butter, coconut oil, palm oil, palm kernel oil…) and industrial (by pressure or solvent extraction) methods [1]. Traditional methods using for most of the way, water extraction, are used in rural areas of developing countries, because of their non expensive costs and their non specific equipments. These methods give low yield oils. The influence of craft techniques of preservation and processing of seed has also been shown on the quality of extracted oils [2].

Terminalia catappa (Tropical almond) is a large, spreading tree now distributed throughout the tropics in coastal environments. The tree is tolerant to the strong winds, the salt spray, and moderately to a high salinity in the root zone. It mainly grows in freely drained, well aerated, sandy soils. The species has traditionally been very important for coastal communities, providing a wide range of non-wood products and services. It has a spreading, fibrous root system and plays a vital role in coastline stabilization [3]. It is widely planted throughout the tropics, especially along sandy seashores, for shade, ornamental purposes, and edible nuts.

Kernels from this tree contain oil generally extracted by pressing or solvent methods [4], with yields varied among 49 to 65 % [5, 6]. This oil showed a high level of unsaturated fatty acids including oleic (27.1 %) and linoleic acids (26.6 %) and saturated fatty acids such as palmitic acid (40.0 %) as well as several phytosterols and triterpenes. The kernels and their unsaturated oil are of interesting nutritional value and could also be used as a biofuel or lubricant [7].

The extraction and the transformation in good conditions of oils from unexploited species as *T. catappa*, would significantly increase the supply of edible oils. This could also contribute to the reduction of poverty among farmers through the diversification of their income generating activities [8]. Information was available on methods of organic solvent extraction and mechanical expression of *T. catappa* kernels oil. But to our knowledge, there are no reports to date concerning the traditional method of extraction of this oil using water. The aim of this study is to determine the chemical composition, the physic-chemical characteristics, the toxicity and the antioxidant properties of *T. catappa* kernels oil extracted by heating aqueous process (traditional way) and to compare this oil to that obtained by method requiring hexane as solvent (hexane conventional method).

MATERIALS AND METHODS

Materials

Chemicals and drugs

Dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-pycrilhydrazile (DPPH), 40 component fatty acid methyl esters (FAMEs) were purchased from Sigma-Aldrich (Steinhein, Germany), Acros Organics (New Jersey, USA), and Fluka Chemie (Buchs, Switzerland). Hexane was purchased from Fluka Chemie, anhydrous Na_2SO_4 from UCB (Bruxelles, Belgium), absolute ethanol and absolute methanol from Labotec (Bruxelles, Belgium). All compounds and solvent were of analytical grade.

Plant material, kernel obtaining and grinding

T. catappa nuts were obtained from fruits harvested in August 2013 in Calavi (South of Benin) and Voucher specimen (AA6627/HNB) was conserved at the University of Abomey-Calavi Herbarium. Kernels were obtained by manually crushing nuts dried in the sun for a week. After drying in an oven at 80 °C for 48 hours, they were skinned, milled in a domestic electric coffee-grinder (Moulinex KM1, type 27-2761743-85) and then sieved to obtain a fine flour useful for extraction and analysis. The whitish flour obtained was stored in self-seal polyethylene bags at 7 °C until further use.

Oils extraction

Hexane method

T. catappa kernel oil was extracted from 40 g of kernel flour with 240 mL of hexane in a Soxhlet apparatus for 4 hours [9]. After evaporation under reduced pressure, the crude oil was dried in an oven at 103 °C for 20 minutes to remove traces of hexane. The dried crude oil was cooled, weighed to calculate lipid potential and kept in a freezer. The extraction was carried out in triplicate.

Traditional method

Flour (40 g) was introduced with 160 mL of tap water into a two-necked flask. The mixture was heated at 100 °C for 15 minutes. The supernatant (mixture of oil and water) was decanted and the lipid phase was collected. Boiling water was added to the solid portion in the flask to extract the remaining oil from the flour. The supernatant was recovered after decantation and the procedure was repeated three time until there was practically no oil extracted from the cake. All the oil collected was dried in an oven at 103 °C for 30 min to remove traces of water then the dried oil was cooled, weighed to calculate the extraction yield and kept in a freezer.

Oils analysis methods

Physico-chemical analysis

Density, acidity (IA), saponification (IS), iodine (II) and peroxide values (PV) were determined according to standard methods (NFT 60 - 214 standard, NF T60 - 204, NFT 60 - 206, NFT 60 - 203 and NFT 60 - 220 respectively) of the "Association Francaise de Normalisation" [10]. The ester value (IE) was calculated on the basis of analytical data using the formula: IE = IS - IA. The calorific value was calculated using the approximate formula of Batel et al. [11]: CV [$kJ \cdot kg^{-1}$] = 47645-4.187xII - 38.31xIS. Content and isolation of the unsaponifiable matter was performed by the method described by Kpoviessi et al. [12] and in our previous work [7].

GC-FID determination of fatty acid profile

Fatty acid profiles were obtained by gas–liquid chromatography of the fatty acid methyl ester derivatives by the method described in our previous work [7]. A total of forty pure FAME standards were used.

Pharmacology

Antioxidant activity

Evaluation of the antioxidant activity was carried with 2,2-diphenyl-1-pycrylhydrazyl (DPPH) according to a modified method of Mensor et al. [13] and Lee Mei Ling et al [14] as described in our previous work [7].

Toxicity test against Artemia salina L.

The test was performed against *Artemia salina* Leach by the method of Michael et al. [15] summarized by Vanhaecke et al. [16] and by Sleet and Brendel [17]. The eggs of *Artemia salina* Leach obtained from JBL society (JBL Gmbh&Co.KG, Germany) were incubated in sea water until hatching of young larvae (48 hours). Then, series of solutions of tested oils and extracts at increasing concentrations were prepared. A defined number of larvae (16) were introduced into each solution. All solutions and control solution (containing no active substance) were left under stirring for 24 hours. Counting under a microscope the number of dead larvae in each solution was used to evaluate the toxicity of the solution. In the case where there was death in the control medium, the data was corrected by Abbott's formula: % death = [(test - control) / control)] x 100 [18]. Camptothecin (Sigma) was used as positive reference compound.
Data (dose-response) were transformed by logarithm and the LC_{50} were determined by linear regression [19]. Tests were carried out in triplicate.

Statistical analysis

Student's *t*-test was used to test the significance of differences between results obtained for different samples, and between results for samples and controls (GraphPad Prism 4.0; GraphPad Software Inc., San Diego, USA). Statistical significance was set at $P < 0.05$ [20, 21].

RESULTS AND DISCUSSION

Comparison of oil yields

The extraction with hexane of oil from *T. catappa* kernels gave a yield (61.76 %) higher than that obtained by the traditional method (28.13 %) (Table 1). This difference can be explained by the oil affinity to the hexane. The high solubility of the oil in hexane facilitates its extraction with this solvent. The hexane method used, gave the oil content of the kernels according to standard AFNOR method [9]. When considering it as 100 %, the traditional method helped to extract 45.54 % of the total oil from the kernels. This value was higher than that (27.36 %) obtained by Womeni et al. [2] for *I. gabonensis* oil extracted by the same method but separated by centrifugation. With this performance, this method using available and inexpensive equipments with non organic solvent would be more profitable for operation in rural areas.

Table 1. *Oil yield of T. catappa kernel extracted by traditional method and hexane method*

Extraction methods	Traditional (heating aqueous)	Hexane
Oil yield % w/w [g per 100 g of meal]	28.13 ± 0.03	61.76 ± 1.01

Physico-chemical characteristics

T. catappa kernels oils extracted by both methods were liquid at room temperature with a yellow color and a pleasant smell of roasted almonds. The characteristics of oils are resumed in Table 2.

Table 2. *Physico-chemical characteristics of T. catappa kernel oils extracted by hexane and traditional ways*

Characteristics	*T. catappa* oil	
	Hexane [7]	Traditional
Unsaponifiable matters % w/w [g per 100 g of oil]	1.76 ± 0.32	0.97 ± 0.04
Density [g·mL^{-1}] at 25 °C	0.88 ± 0.01	0.88 ± 0.01
Physical condition	liquid	liquid
Color	Yellowish	Yellowish
Odor	Toasted almond	Nice
Acid value [mg KOH·g^{-1}]	2.24 ± 0.01^{a}	2.58 ± 0.33^{a}
Saponification value [mg KOH·g^{-1}]	175.33 ± 0.42^{a}	179.52 ± 0.01^{a}
Calculated ester value	173.09^{a}	176.94^{a}
Peroxide value [mequiv O_2·kg^{-1}]	3.71 ± 1.2^{a}	2.07 ± 0.25^{a}
Iodine value [g I_2·100g^{-1}]	74.48 ± 0.65^{a}	77.34 ± 0.01^{a}
Calorific value [kJ·kg^{-1}]	40616.78	40443.76

Data in the same line followed by different letters ($^{a,b,c,\cdots}$) are statistically different by Student's *t*-test ($P < 0.05$). Values are means ± standard deviation of three different experiments

The acidity values of oils extracted by traditional and hexane method (2.58 and 2.24 mg KOH·g^{-1} respectively) were in the standards values (≤ 4 mg KOH·g^{-1}) set by the Codex Alimentarius [22] for crude edible oils. These data were not significantly different according to student's *t*-test. Oils had low free fatty acid levels and then would possess according to this point, a proven nutritional quality for food use [7].

The saponification value of oil extracted by traditional method (179.52 mg KOH·g^{-1}) was higher than that of the oil extracted by hexane method (175.33 mg KOH·g^{-1}). These values which were not significantly different according to student's *t*-test, were higher than the value (168.27 mg KOH·g^{-1}) obtained by Atsu barku for oil from Ghana [23] and slightly less than those (196 to 207 mg KOH·g^{-1}) obtained by Matos *et al.* [6] for oil from Congo. This difference can be due to different factors such as climate, place, period of harvest and extraction methods [6, 7, 12].

The iodine values (77.34 and 74.48 g I_2·100 g^{-1}) obtained (respectively for traditional and hexane method) were not significantly different according to student's *t*-test and

were close to values reported by Matos et al [6]. The values found were higher than 65 g I_2·100 g^{-1} mentioned by Olatidoye et al. [24].
Values obtained 2.07 mequiv O_2·kg^{-1} (traditional) and 3.71 mequiv O_2·kg^{-1} (hexane) for peroxide were close (Table 2) but lower than the 15 mequiv O_2·kg^{-1} prescribed for the raw food oils but in accordance with previous value (2.8 mequiv O_2·kg^{-1}) and less than 10 mequiv O_2·kg^{-1}, the minimum value specified for the rancid oils [25], demonstrating the good quality of these oils.
Calorific values of *T. catappa* oils extracted by hexane and traditional process were greater than 35000 kJ·kg^{-1} (Table 2) with the higher value (40616.78 kJ·kg^{-1}) for hexane extracted method. They could therefore be used as a biofuel and lubricant [8].
Physico-chemical characteristics of the oils such as: density, color, acidity values (2.58 and 2.24 mg KOH·g^{-1}), saponification values, iodine values (77.34 and 74.48 g I_2·100 g^{-1}), peroxide values (2.07 and 3.71 mequiv O_2·kg^{-1}) and calorific values (40443.76 and 40616.78 kJ·kg^{-1}) were not so influenced by extraction methods (traditional and hexane). Their data were not significantly different according to student's *t*-test.

Fatty acids composition

Fatty acid profile of *T. catappa* oils extracted by traditional and hexane methods showed the presence of 17 fatty acids with a variation according to extraction method (Table 3).

Table 3. *Fatty acid composition of the T. catappa kernels oil obtained by traditional and hexane extractions expressed in % of total fatty acids (% TFA) and the recommended daily intake (RDI)*

Fatty Acids	Traditional	Hexane [7]	RDI
Caproic acid (C6:0)	0.06	0.08	
Capric acid (C10:0)	0.03	0.03	
Lauric acid (C12:0)	0.15	0.16	
Myristic acid (C14:0)	0.13	0.14	
Pentadecylic acid (C15:0)	0.02	0.02	
Palmitic acid (C16:0)	40.79	40.03	
Margaric acid (C17:0)	0.11	0.12	
Stearic acid (C18:0)	4.35	4.49	
Arachidic acid (C20:0)	0.51	0.52	
Palmitoleic acid (C16:1, C9)	0.40	0.39	
Oleic acid (C18:1, C9)	25.55	26.19	
Vaccenic acid (C18:1, C11)	0.78	0.82	
Linoleic acid (C18:2, c9c12)	26.72	26.64	11-17 g/day[a]
Linoleic acid (C18:2, t9t12)	0.01	0.00	
Linolenic acid (C18:3, C9C12C15)	0.09	0.09	1.1-1.6 g/day[a]
Gadoleic acid (C20:1, c11)	0.07	0.08	
Behenic acid (C22:0)	0.15	0.12	
SFA/UFA	0.86	0.84	<1[b]
MUFA/PUFA	1.00	1.03	
w-6/w-3	297	269.09	4[C]

[a]Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids; [b]FAO (2008); [c]Schaefer (2002).

Unsaturated fatty acids represent 53.65 % and 54.23 % of total fatty acids respectively for traditional and hexane extraction with linoleic and oleic acid as predominant unsaturated fatty acids (Table 3). The major components of these oils were palmitic (C16:0; 40.03 and 40.79 %), linoleic (C18:2; 26.64 % and 26.72 %), oleic (C18:1; 25.55 % and 26.19 %) and stearic acids (C18:0; 4.35 % and 4.49 %). These values showed that they are no significant difference between fatty acid content of oils extracted by the both methods. The saturated to unsaturated fatty acid ratios (SFA/UFA) obtained in the present study (0.86 for traditional extraction and 0.84 for hexane extraction) are close to the dietary recommendations [26]. The monounsaturated to polyunsaturated fatty acid ratio (MUFA/PUFA) was low (1.00 and 1.03 respectively), whereas the Ω-6 to Ω-3 ratio was much higher than the maximum recommended value of 4 for [27]. The contents of fatty acids of these oils were similar to those mentioned by Dos Santos et al [5]. These oils with their important unsaturated fatty acid content may be useful in human consumption.

Pharmacology

The antioxidant activity and the larval toxicity of oils obtained by the both extraction methods were summarized in Table 4.

Antioxidant activity

The antioxidant activity of crude oils obtained by both methods were determined and expressed as EC_{50} (Table 4).

Table 4. *Antioxidant activity (EC_{50}), antiradical power (ARP) and toxicity (LC_{50}) against Artemia salina Leach of T. catappa kernel oil extracted by traditional method and hexane*

Sample		Antioxidant activity (EC_{50}, [mg·mL^{-1}]) mean ± standard deviation	Antiradical power ARP (ARP = 1/EC_{50})	Toxicity (LC_{50}, [µg·mL^{-1}]) mean ± standard deviation
Traditional extract		0.71± 0.05^{b}	1.40^{b}	7580.50±0.13
Hexane extract		6.61± 0.70^{c}[7]	0.15^{c}[7]	8000.50±0.02
Positive controls	Ascorbic acid	0.02±0.00^{a}	50^{a}	nd
	Camptothecin	nd	nd	13.27±0.02

EC_{50} = concentration of sample producing 50 % scavenging of the DPPH radical, ARP = Antiradical power, LC_{50} = sample concentration providing 50 % death of larvae, nd = not determined

Data in the same column followed by different letters ($^{a,b,c,\cdots}$) are statistically different by Student's *t*-test ($P < 0.05$). Values are means ± standard deviation of three different experiments.

These values, significantly different according to student's *t*-test, were 0.71 and 6.61 mg·mL^{-1} respectively for traditional and hexane extraction methods. Oil extracted by traditional method show high antioxidant power (1.40). This may be due to the extraction of more antioxidant components with the oil during this method. *T. catappa* oil had low capacity of neutralizing DPPH free radical, since his antiradical power is at least 30 times lower than that of ascorbic acid used as positive control, but is not devoid of antioxidant activity.

Larval toxicity

The toxicity against *Artemia salina* Leach larvae of oils showed LC_{50} values higher than 7 $mg \cdot mL^{-1}$ for both extraction methods (Table 4). These data were not statistically different according to the extraction methods but were very higher than the value (13.27 $\mu g \cdot mL^{-1}$) obtained for camptothecin, the positive control used in this test. These oils are then non toxic. This test helps to predict cytotoxicity against human cells. There is a correlation between shrimp larvae toxicity and cytotoxicity of human cells lines particularly lung carcinoma (A-549) and colon carcinoma (HT-29) [28].

CONCLUSIONS

In this study, detailed physicochemical characteristics of *T. catappa* kernels oils extracted by traditional method and with hexane have been studied. The results showed that these kernels which possess 61.76 % of unsaturated edible oil can also be defatted with traditional method with oil yield of 28.13 %. Physicochemical properties and chemical compositions of these oils obtained by both methods were not significantly different according to student's test. These parameters were in accordance with Codex Alimentarius recommendations for crude edible oils. Oil obtained by traditional way showed the highest antioxidant power. *T. catappa* oil rich in palmitic, oleic and linoleic acids can be extracted using traditional way with preservation of physicochemical, chemical properties and good antioxidant activity. This way of extraction which is less expansive and accessible for farmers, could be benefic for people in rural areas.

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