

Physicochemical Analysis and Characterization of the Lipid Fraction of the Tubers of *Cyperus esculentus* L. var. *sativus* from Côte d'Ivoire

ABSTRACT

Aims: this work consists in determining the physical, physico-chemical parameters, the composition in fatty acids and unsaponifiables of the oil of tuber of *Cyperus esculentus* of Côte d'Ivoire.

Methodology: After extraction of the fat by the Soxhlet method with hexane, the physico-chemical parameters were determined by colorimetric assay. The analysis of the fatty acid and unsaponifiable acid composition was carried out by GC-MS.

Results: The fat content ranged from 23.64 to 27.66%, those of fatty and unsaponifiable acids were from 90.41 to 95.20% and 1.59 to 1.60%, respectively. As for the values of the acid, peroxide, saponification, iodine, ester, refraction and density indices, they varied respectively from 2.81 to 10.95 mg KOH/g, 1 to 2 meq of O₂/Kg oil, 198.72 to 216.78 mg KOH/g, 54.64 to 73.86 g I₂/100g oil, 193.11 to 205.83 mg KOH/g, 1.453 to 1.461, and 0.76 to 0.83. In the fatty acid composition of oils, the predominance of unsaturated fatty acids (78.8-92.14%) was revealed, mainly oleic acid prevails (59.14-82.83%). Additionally, petroselinic (0.23-10.15%,) and 2,6-di-O-palmitoyl-L-ascorbic (8.58-8.84%) acids known for their medicinal properties (anti-inflammatory, antimicrobial, antioxidants), were first discovered in the oil of *Cyperus esculentus*. As for the unsaponifiables, they are mainly dominated by phytosterols (22.28-44.03%) and hydrocarbons (21.32-50.8%).

Conclusion: These results suggest that oil from *Cyperus esculentus* tubers from Côte d'Ivoire can be used as an excellent source of edible and medicinal oil.

Keywords: *Cyperus esculentus*, oil, physico-chemical parameters, GC-MS, Côte d'Ivoire

1. INTRODUCTION

Vegetable oils also occupy an important place in the diet of men, in cosmetics or in certain soft medicines. They have pharmaceutical properties and are very important energy reserves [1]. Their properties are determined by the plurality of fatty acids and unsaponifiables which they contain. Essential fatty acids (arachidonic, linoleic, linolenic) have an antioxidant effect, their consumption prevents oxidative stress and slows down the aging process. We know that the human need for essential fatty acids is 2 g/day [2; 3]. In addition, oleic (monounsaturated) and linoleic (polyunsaturated) acids increase HDL cholesterol, which reduces the risk of cardiovascular disease, diabetes, asthma and cancer [4]. As for the constituents of the unsaponifiables of vegetable oils, they have many interesting properties. Indeed, polyphenols have antioxidant, antibacterial and anti-inflammatory properties. Phytosterols, good healing and regenerating agent, improve the "barrier" function of the skin and microcirculation, slow down skin aging. As powerful natural antioxidants, carotenoids regenerate and repair the skin by stimulating the synthesis of collagen and photoprotectors [2; 5]. There are limited sources of linoleic and linolenic acids in the plant kingdom, but almost nothing has been found for arachidonic acid. But the African continent is full of oilseeds, most of which are unused or little used. However, they may be important new sources of these mono- and polyunsaturated fatty acids.

In recent years, *Cyperus esculentus* L. var. *sativus* (nutsedge, tiger nut, chufa) has been the subject of many studies. The increased interest in this crop is explained by the nutritional value of its tubers [6; 7; 8; 9]. *Cyperus esculentus* is a weed plant of tropical and Mediterranean regions. It is a monocotyledonous root vegetable that grows in humid places and belongs to the Cyperaceae family [10]. Tigernut tubers are one of the oldest cultivated plants [11]. In ancient Egypt, nuts have been found in archaeological excavations where its tubers have been used as a source of food, medicine and incense for fumigating homes and clothes, as well as incense for myrrh. From Egypt, Arab traders spread tiger nuts to northern and western Africa, Sicily and Spain [12]. At present, given the composition of lipids, starch, proteins, sugars, vitamins E, C, as well as trace elements (magnesium, calcium, phosphorus, iron, etc.), tiger nut is very popular. Several works have shown that the tubers of *C. esculentus* produce edible oil (up to 33%) rich in unsaturated fatty acids [7; 8; 9]. Moreover, the unsaponifiable part of *C. esculentus* oil is rich in vitamin E and phenolic compounds [13]. As a result of research conducted by doctors, it was found that 100 g of tiger nut cover the daily norm of useful vitamins and microelements [14]. Such consumption contributes to effective weight loss and improvement of metabolic disorders in obese diabetics [14]. In Côte d'Ivoire, tiger nut is sold everywhere, commonly called tchongon in the Malinké, Bété, Dida and Bambara languages; atadjo in apolo; shop in koulan go; maguélé in Sénoufo (Korhogo) and consumed after soaking in water or blanched as in the traditional case, and also used in powder form in drinks [15].

Despite these advantages, the oil from the tubers of *Cyperus esculentus* is still not part of the local diet. However, in the face of changing demographics and the complexity of health issues and trends, the high cost of living is increasingly felt. From this point of view, tiger nut can contribute to poverty reduction among vulnerable populations, especially rural African women. Thus, the aim of this work is to determine the physico-chemical parameters (refractive indices, acid, peroxide, saponification, ester and iodine) and the fatty acid and unsaponifiable acid composition of the tuber oil of *Cyperus esculentus* from Côte d'Ivoire.

2. MATERIAL AND METHODS

2.1 Plant material

The yellow tubers of *Cyperus esculentus* were collected in April 2020 in three villages in Côte d'Ivoire: Klolékaha (in the department of Sinematiali, 9° 35' North 5° 23' West), Lélékaha (in the department of Korhogo, 9° 27' 41" North 5° 38' 19" West) and Sokala Sobara (in the department of Dabakala, 8° 21' North 4° 31' West). They have been authenticated at the Center National de Floristique (CNF) of the University FELIX HOUPHOUËT-BOIGNY (herbarium N°UCJ004623). Tubers are sampled according to harvest locations and designated TKS, TLK and TSD for tubers from Klolékaha, Lélékaha and Sokala Sobara respectively. Arrived at the laboratory, the tubers are cleaned with running water, dried in an oven (50°C) for 24 hours then pulverized and stored in glass bottles.

2. 2 Methods

2.2.1 Physical analysis of *Cyperus esculentus* tubers

TSK, TLK and TSD tubers were characterized by the shape (round, oval or elongated), the size (small, medium or large) and mass. Tuber size was determined by measuring the length (L, cm) and diameter (thickness, T, cm) of 300 tubers from each batch using an electronic digital caliper (Mitutoyo Body, accuracy 0.01mm). When the L/E ratio ≤ 1.24 the tubers are said to be round; oval if $L/T < 1.25$ and elongated if $L/T \geq 1.45$ [16]. Concerning the size, the tubers are said to be small if $E \leq 0.5$ cm; medium if $0.5 < T \leq 1.0$ cm and large if

T>1.0 cm [17]. The mass (M, g) of each tuber was determined using an electronic balance (Adventure Pro, precision 0.001 g).

2.2.2. Fat extraction

The tuber oil was obtained by continuous Soxhlet extraction for 2 h from 15 g of powder mixed with 3 g of anhydrous Na₂SO₄ using hexane as extraction solvent. The hexane was removed after extraction using a rotary evaporator (BÜCHI). The yield (Yield) of fat matter extraction (FM) was calculated according to the formula:

$$\text{Yield (\%)} = (m_0/m_1) \times 100$$

m₀: mass of the extracted fat (g), m₁: mass of the initial vegetable powder

2.2.3. Determination of physical and physicochemical parameters

For each parameter measured, three tests were carried out, and the average of the three tests was taken into account.

- The refractive index (I_r) was determined according to the ISO 6320: 2000 standard at 20° C. with a refractometer (Leica AR 200 Barolworld brand).
- The density (D) was determined by calculating the ratio of the weight of oil and the weight of water taken in the same volumes according to the protocol described by NF ISO 6883.
- The saponification index (I_s) was determined according to the method described by **Bamba et al., 2015 [18]** and standard NF T 60-206 [19].
- The acid index (I_a) was determined according to standard NFV 03-906 [19].
- The peroxide index (I_p) was determined following the protocol described by the **AOAC standard, 1981 [20]**.
- The iodine value (I_i) was determined according to the method described by **Bamba, 2016 [21]** and the **AOAC standard, 1981.[20]**
- The Ester Index (I_e) value is equal to the Saponification Index (I_s) for pure glycerides and it was calculated based on the analytical data using the formula I_e = I_s - I_a.
- The calorific value (CP) was calculated using the following expression:

$$CP = 47645 - 4.187I_i - 38.31I_s \text{ (kJ/kg) [22; 23]}$$

2.2.4 Determination of the content of unsaponifiables

The fat (5 g) was dissolved in a solution (2N) of KOH in ethanol (50 mL). The resulting mixture was boiled and heated for 1 hour under reflux. After cooling, add 100 ml of distilled water. The organic part was extracted with diethyl ether (3×60 mL) then washed with distilled water (5×50 mL) until neutral pH. The organic phase was then dried with MgSO₄ anhydrous, filtered and evaporated to dryness under reduced pressure using a rotary evaporator after distillation of the solvent [24]. The residue obtained, constituting the unsaponifiable (I_{ns}) fraction, is weighed and its content is calculated according to the following formula:

$$\text{Ins (\%)} = (m_1/m_2) \times 100$$

Ins: content of unsaponifiables; m₁: mass of the unsaponifiable fraction (g); m₂: mass of the FM (g).

2.2.5. Preparation of methyl esters (ME) of fatty acids

After extraction of the unsaponifiables, the aqueous phase is treated by 5 mL of HCl (5N). Fatty acids (FA) were extracted with ethyl acetate (3 × 25 mL). The organic fractions were collected and dried over anhydrous MgSO₄. The fatty acids were obtained after removal of the solvent using a rotary evaporator [25]. The extraction yield is calculated using the formula below.

$$\text{FA (\%)} = (m'_1/m'_2) \times 100$$

FA: fatty acid content; m' 1 (g): mass of fatty acids; m'2 (g): mass of FM.

To facilitate GC/MS analysis, fatty acids have been methylated to form methyl esters (ME). To do this, 1 mL of hydrochloric methanol (2N) was added to the fatty acids. The whole is brought to the boil and heated for 1 hour on a heating plate with slow stirring at a temperature of 45 to 50°C. After cooling, 20 mL of distilled water was added to the reaction mass, then the EMs were extracted with 25 mL ethyl acetate. After solvent removal using a rotary evaporator, EMs were obtained for analysis.

2.2.6. GC/MS analysis of the unsaponifiable fraction and methylated fatty acids

1 mg of methylated fatty acid and 1 mg of unsaponifiables to be analyzed are dissolved in 2 ml of hexane and 2 ml of a pyridine/ acetic anhydride mixture (50/50, v/v), respectively. The analyzes were carried out on a GC/MS (brand SHIMADZU, model QP2010SE), equipped with a column with a Zebron ZB-5 ms, 20 m long, with an internal diameter (0.18 mm) and a film thickness of the stationary phase (0.18 μm). Helium (He) was the carrier gas at linear velocity (0.9 ml/s). The temperature of the oven (50° C.) is then increased in gradient at the rate of 4° C /min up to 250° C and maintained for 15 min. The injector and detector temperatures were set at 250°C and 280°C, respectively. Split mode injection was performed for 30 min at an ionization energy of 70 eV, scan rate (50 scans/s) and acquisition rate (10.000 amu/s). The phytocomponents were identified by comparing their retention time (TR) with the retention time of authentic compounds and the spectral data from the internal library spectrometer.

3. RESULTS AND DISCUSSION

3.1. Physical characteristics of tubers of *Cyperus esculentus* (*C. esculentus*)

The mass, length, and thickness of the tubercles of *C. esculentus* were measured and the linear dimensions obtained were used to classify the tubers according to shape and size (Table I). The results show that the length of the TKS, TLK and TSD tubers does not differ significantly (p>0.05) on the other hand their thickness differs markedly (p<0.05). Tubers TKS (0.83 ± 0.009 cm) and TLK (0.824 ± 0.007 cm) have almost the same thickness, however TSD tubers are significantly thicker (1.025 ± 0.011cm).

Table I Physical parameters of *C. esculentus* tuber

Parameters	Samples		
	TKS	TLK	TSD
Mass (g)	0.52 ± 0.03 ^a	0.32 ± 0.007 ^b	0.76 ± 0.007 ^c
Thickness (T) (cm)	0.83 ± 0.009 ^a	0.824 ± 0.007 ^a	1.025 ± 0.011 ^b
Length (L) (cm)	1.371 ± 0.012 ^a	1.009 ± 0.012 ^a	1.453 ± 0.032 ^a
(L/T)	2.56 ± 0.85 ^a	1.24 ± 0.017 ^a	1.48 ± 0.038 ^a
Shape	Elongate	Round	Elongate
size	Medium	Medium	Wholesale

For each tuber, values in the same row followed by superscripts (a–c) differed significantly ($P < 0.05$). TKS: tubers from Kholékaha department of Sinematiali ; TLK: tubers from Lèlèkaha department of Korhogo; TSD tubers from Sokala Sobara department of Dabakala

In addition, TKS and TLK tubers are respectively medium and elongated-rounded while those TSD are large and elongated. Regarding the mass, variability is observed, TKS (0.32 ± 0.007 g) has the smallest mass while TLK has the highest mass (0.76 ± 0.007 g). Also note that the mass of the tubers depends on their shape. Indeed, the elongated tubers TSD (0.76 ± 0.007 g) and TKS (0.52 ± 0.03 g) are heavier than the TLK tubers round (0.32 ± 0.007 g). The evolution of the mass of the tubers depends on the thickness, but at equal thickness, the increase in mass is consistent with the increase in length. In addition, the physical parameters of TKS, TLK and TSD tubers (length 1.009-1.453 cm; thickness 0.82-1.025 cm and mass 0.32-0.76 g) are similar to those of Spanish tubers (length 1.0-1.437; thickness 0.821-0.905; mass 0.3-0.51g). However, they are higher than those of Burkina Faso and Nigeria, whose parameters fluctuate respectively between 0.762-0.1203 and 0.649-0.806 cm in length; 0.77-1.119 and 0.645-0.788 cm for the thicknesses; 0.23-0.29 and 0.24-0.3g for the masses [16].

3.2 Fat, fatty acid and unsaponifiable content of *Cyperus esculentus* tubers

The oils extracted from tubers (TKS, TLK and TSD) are golden yellow in color with a pleasant smell and have a content of 23.64 ± 0.25 ; 24.72 ± 0.05 and $27.66 \pm 0.17\%$ respectively (Table II). these oil contents are lower than those of some conventional oils such as olive (29-50%) [26], peanut (45-50%) [27], copra and palm kernel (48%) [28; 29]. The results show that the tubers TSD contained more FM ($27.66 \pm 0.17\%$) than TKS ($23.64 \pm 0.25\%$) and TLK ($24.72 \pm 0.05\%$) tubers. The fat content obtained is close to that of the tubers of *C. esculentus* studied by the Agricultural Institute of Turkey (22.8 to 32.8%) [30]. Variability in fat content can be linked to the climatic conditions in which the species evolves [31].

Table II: Contents of fat, fatty acids and unsaponifiables

Content (%)	Samples		
	TKS	TLK	TSD
Fat	23.64 ± 0.25	24.72 ± 0.05	27.66 ± 0.17

Unsaponifiable	1.59 ± 0.01	1.60 ± 0.01	1.60 ± 0.005
fatty acids	95.20 ± 0.4	92.07 ± 0.23	90.41 ± 1.04

TKS: tubers from Klolékaha department of Sinematiali ; TLK: tubers from Lèlèkaha department of Korhogo; TSD tubers from Sokala Sobara department of Dabakala

TKS, TLK and TSD tubers showed no significant difference in content between the unsaponifiable fractions (1.59 ± 0.01 ; 1.6 ± 0.01 and 1.60 ± 0.005 %) and fatty acids (95.20 ± 0.4 ; 92.07 ± 0.23 and 90.41 ± 1.04 %). The average content of unsaponifiables (Ins) obtained in this study (1.56% or 15.6 g/kg) was superior to those of conventional oils such as peanut and palm kernel oil $\text{Ins} \leq 10\text{g/Kg}$, palm oil $\text{Ins} \leq 12\text{g/Kg}$, coconut, cotton and soybean $\text{Ins} \leq 15\text{g/Kg}$ [32]. Moreover, this average is higher than that of *C. esculentus* tubers from Nigeria (0.50 - 0.82%) [9] and from Turkey (0.5 - 0.59%) [33]. Furthermore, the average percentage of unsaponifiables is 0.2 to 2% , as reported in the literature [34]. These high values of unsaponifiables can indicate the presence of large amounts of polyphenols, phytosterols, vitamins or pigments in the tubers, which can give *C. esculentus* oil stability and interesting biological properties.

3.3. Physical and physico-chemical characteristics by FM

The physical and physico-chemical parameters of fat (FM) are listed in Table III. The refractive index is an indicator of oil purity and the degree of fatty acid unsaturation. An oil with a high content of unsaturated fatty acids has the highest refractive index. It also provides information on the degree of oil degradation, increased free fatty acids, reduced the refractive index considerably [34]. For most oils, it ranges from 1.44 to 1.48 .

Table III: Physical and physico - chemical characteristics of *Cyperus esculentus* FM

Parameters	Samples		
	TKS	TLK	TSD
Refractive index (25°C)	1.461 ± 0.001	1.457 ± 0.001	1.453 ± 0.001
Density	0.8 ± 0.002	0.76 ± 0.0012	0.83 ± 0.0011
Acid Value (mg KOH/g)	2.81 ± 0.01	5.61 ± 0.001	10.95 ± 0.001
Peroxide value (m_{eq} of O_2/Kg)	2 ± 0	2 ± 0	1 ± 0
Iodine index (g of $\text{I}_2/100\text{g}$)	54.64 ± 3.40	62.96 ± 1.59	73.86 ± 2.13
Saponification index (mg KOH/g)	208.63 ± 1.54	198.72 ± 4.51	216.78 ± 2.51
Ester number (mg KOH/g)	205.82 ± 1.54	193.11 ± 4.51	205.83 ± 2.51
Calorific value (KJ/Kg)	$39422.96 \pm$ 63.94	$39768.88 \pm$ 178.01	$39058.50 \pm$ 77.61

TKS: tubers from Klolékaha department of Sinematiali ; TLK: tubers from Lèlèkaha department of Korhogo; TSD tubers from Sokala Sobara department of Dabakala

The value of the oil refractive index of *C. esculentus* (1.453-1.461) (**Table III**) is more or less close to the refractive index of palm oils (1.454-1.456), cottonseed (1.458-1.466), peanut oil (1.460-1.465) [35], but lower than corn (1.458-1.466), sunflower (1.461-1.468) [35] and olive oil (1.469–1.470) [36] and higher than that of coconut oil (1.448-1.450) and palm kernel oil (1.448-1.452) [35]. They are also comparable to the refractive indices of tuber oils of *C. esculentus* from Egypt (1.466) [37]. However, they are lower than those of Ghana (1.47) [11].

The density of an oil is also considered as a physical criterion that allows its quality to be controlled. The values obtained in this study varying from 0.76 to 0.83 are lower than that of most vegetable oils (palm, coconut, cotton, peanut, but) which varies from 0.891 to 0.926. However, they are close to those of *C. esculentus* oil from Turkey (0.896) [33] and lower than those found for tubers from Ghana (0.912) [11].

Acid number is a measure of the amount of free acids in an oil. This indicator helps to determine the degree of oil change. Thereby, it is an important quality indicator in the production of edible oils [38]. The results obtained show that the acid values of the samples TKS, TLK and TSD range from 2.81 to 10.95 mg KOH/g. TKS tubers had the lowest acid number (2.81 mg KOH/g) and below the recommended range ($I_a < 4$ mg KOH/g) for edible oils [39]. This low acid number indicates a low content of free fatty acids, characterizing the purity and stability at room temperature of the TKS sample [40]. The acid value of the TSD tuber oil (10.95 mg KOH/g) was the highest and far exceeded the standard. However, this value is comparable to that obtained for the black variety (9.12 mg KOH/g) of *C. esculentus* tubers from Nigeria [9]. The acid index of TLK tubers (5.61 mg KOH/g), although higher than the standard, is close to the values of conventional oils: cottonseed ($I_a < 7$), palm kernel ($I_a = 4.7$) and a rachide ($I_a = 6$) [41].

As it concerns the peroxide index, the values varied from 1 to 2 meq of O_2 /Kg. These values were of the same order of magnitude and less than 10 meq of O_2 /Kg of oil found in most conventional oils [42]. This classifies the oils of the TKS, TLK and TSD tubers among the oils indicating an acceptable level of oxidation [43]. These oils can therefore be used in gastronomy [41].

As for the saponification index, the results show that the values varied from 198.72 ± 4.51 to 216.78 ± 2.51 mg KOH/g. The lowest values in the range of 198.72 ± 4.51 to 208.63 ± 1.54 mg KOH/g corresponded to TKS and TLK tuber oils, and the highest value (216.78 ± 2.51 mg KOH/g) to TSD tuber oil. These values are slightly higher than those of *C. esculentus* tuber oil from Africa: 180.30 mg KOH/g (Nigeria; [9]), 192.88 mg KOH/g (Egypt; [8]) and 180.24 mg KOH/g (Ghana, [11]) and from Europe 164.76 (Turkey, [33]). Because, according to **Mohammad, 2011** [38]; oils with a high saponification index would contain a high proportion of lower fatty acids. However, the saponification indices of oils presented in our study were much lower than those of coconut (248-265) and palm kernel (230-254) oils commonly used in soap making [32]. Therefore, TKS, TKL and TSD oils are not recommended for soap making. Nevertheless, the saponification index of TKL (198.72 ± 4.51 mg of KOH/g) approaches that of conventional oils such as soybean (189-195), peanut (187-196) and cottonseed (189-198) [42]. While the saponification indices of TKS (208.63 ± 1.54 mg KOH/g) and TSD (216.78 ± 2.51 mg KOH/g) were comparable to that of palm oil (190-209).

The iodine value of the oils studied varied from one collection site to another. The highest value (73.86 ± 2.13 g of I_2 /100g) was observed in TSD tubers, and the lowest value was observed in tubers in TKS tubers (54.64 ± 3.40 g of I_2 /100 g of oil). The iodine numbers of TKS, TLK and TSD oils between 54.64 ± 3.40 and 73.86 ± 2.13 g of I_2 /100g of oil are close

to the iodine numbers of the oil of *C. esculentus* from Nigeria (57.30-83.3 g of I₂/100g of oil) rich in monounsaturated fatty acids [9; 44]. So, these oils would be rich in monounsaturated fatty acids. The iodine value is the most useful constant because it highlights the important division of vegetable oils into drying, semi -drying, and non- drying oils. Thus, the oil is said to be non-drying if I₂<95; semi -drying if 95≤ I₂< 130 and drying if 130 ≤ I₂< 200 [45]. With regard to these values, the oils of the TKS, TLK and TSD tubers are non-drying.

Whatever the sample (TKS, TLK and TSD), the ester indices were of the same order and all slightly lower than their saponification index with the exception of TSD (Table III). This means that the TKS and TLK samples contained a moderate amount of free acids.

The calorific value of oils from TKS, TLK and TSD tubers (39422.96, 39768.88 and 39058.50 KJ/Kg, respectively) is more than 3500 KJ/Kg. This would mean that these oils could be used as a bio-lubricant for engines.

3.4 Fatty acid composition of *Cyperus esculentus* oil

The analysis of fatty acid and unsaponifiables composition is one of the important ways to determine the nutritional value of edible vegetable oils. Fatty acids, especially unsaturated and polyunsaturated, are of great importance for the normal functioning of the human body [13; 46]. Acids such as oleic, linoleic, linolenic belong to the group of biologically active vitamin like compounds. Many unsaturated fatty acids are essential, ie are not synthesized in the human body and must therefore be provided by food. Currently, many methods are used for the analysis of fatty acids, the most common of which is GC/MS [47; 48; 49]. Thus, the fatty acid composition of TKS, TLK and TSD tuber oils determined by GC/MS is listed in Table IV. This analysis revealed the presence of several saturated and unsaturated fatty acids, mainly monounsaturated fatty acids whose values are 69, 84; 92.14 and 86.68% respectively for TKS, TLK and TSD. TLK oil contained the highest value (92.14%), while TKS oil (69.84 %) had the lowest. The main unsaturated fatty acids identified were oleic acid, petroselinic acid, linoleic acid, and 2,6-di-O-palmitoyl L-ascorbic acid. Oleic acid is the most abundant fatty acid with a content of 59.14 to 82.83%. This is consistent with literature data that oils from the tubers of *C. esculentus* from different countries contain more oleic acid from 50.85 to 77.20% [8; 9;11; 50; 51]. This fatty acid was found in all the oils with the highest values observed in those of the tubers harvested at Lelekaha (TLK: 82.83%) and Sokala Sobara (TSD: 77.86%) and the lowest values observed in that of Klokakaha (TKS: 59.14%) However, petroselinic acid was only found in TKS (10.15%). This compound is known for its anti-inflammatory [52] and antimicrobial [53; 54] activity in various food, cosmetic and pharmaceutical formulations. Also, in the tubers of *C. esculentus*, 2,6-di-O-palmitoyl-L-ascorbic acid was found for the first time in significant quantities (8.84% in TLK and 8.58% in TSD). Its presence can improve the quality of the oil, since vitamin C esters (palmitate or stearate) are powerful antioxidants, often used as food additives, and also as a source of vitamin C. They confer certain functional properties in cosmetology [55] and in pharmacy [56] to stabilize oil-in-water emulsions. In addition, the data in Table IV show the absence of linoleic acid in TLK while this was observed in TKS (8.96%) and TSD (4.47%), which is comparable to literature data (4–9.35%) [8 ; 37 ; 44].

Table IV: Results of GC/MS fatty acid analysis of *Cyperus esculentus* tuber oil

Compositions	Samples		
	MG TKS	MG TLK	MG TSD

	TR (min)	%	TR (min)	%	TR (min)	%
Butyl acetate					2.891	0.36
myristic acid , C14:0	9,532	0.13			9,538	0.05
palmitoleic acid , C16:1 ω-6	10,427	0.29	10,432	0.11	10,431	0.11
palmitic acid , C16:0	10,533	15.75	10,534	6.60	10,529	6.29
pentadecyl acid , C15:0	10,695	1.08				
2,6-Di-O-palmitoyl of L-ascorbic acid			10,762	8.84	10,726	8.58
(Z)-Heptadec-10-enoic acid 1	10,879	0.07			10,883	0.04
margaric acid , C17:0	10,974	0.08	10,987	0.04		
14-Methylhexadecanoic acid					10,983	0.03
linoleic acid , C18:2 ω-6	11,299	8.96			11,301	4.47
stearic acid C18:0	11,423	3.33	11,428	1.00	11,424	1.55
petroselinic acid C 18:1 ω- 12	11,518	10.15				
oleic acid , C18:1 ω-9	11,354	59.14	11,352	82.83	11,570	77.8
						6
gondoic acid, C20:1, ω- 9	12,145	0.19	12,159	0.07		
18-methylnonadecanoic acid	12,238	0.42	12,247	0.13		
nonadecylic acid , C19:0	11,696	0.30				
paullinic acid, C20:1, ω- 7					12,154	0.09
behenic acid , C22:0	13,062	0.05				
Cis -vaccenic, C18:1, ω- 7			12,313	0.06		
petroselinic acid, C18:1, ω- 12			12,393	0.23		
arachidic acid, C:20			12,507	0.09		
lignocérique, acid , C24:0					14,145	0.05
Saturated fatty acids		21.14		7.86		8.33

Monounsaturated fatty acids	69.84	92.14	86.6
			8
Polyunsaturated fatty acids	8.96	0	4.47
Total unsaturated fatty acid	78.8	92.14	91.1
			5

TKS: tubers from Klolékaha department of Sinematiali ; TLK: tubers from Lèlèkaha department of Korhogo; TSD tubers from Sokala Sobara department of Dabakala

Moreover, saturated fatty acids were identified with contents ranging from 7.86 to 21.14% with the highest value observed in TKS. The main saturated fatty acids identified were palmitic, stearic and pentadecyl. Palmitic and stearic acids were found in the oil of all samples, but pentadecyl acid (1.08%) was only found in TKS. Palmitic (15.75%) and stearic (3.33%) acid contents in TKS were the highest. They are approximately equal to 12.96-15.4% reported in the literature for palmitic acid [8; 9; 37] and 3.0-3.3% for stearic acid [44]. On the other hand, their content of 6.29-6.60% (palmitic acid) and 1-1.55% (stearic acid) were very low in the TLK and TSD samples. The variability in fatty acid composition of oils from one harvest location to another may be due in part to climatic or environmental factors [13].

Thus, the content in oleic acid (59.14-82.83%) found in this study shows similarity with olive oil [13]. This fatty acid will prevent the deposition of cholesterol in the vessels (antiatherogenic) and lower the level of lipids in the blood (hypocholesterolemic). We also note the presence of linoleic acid in TKS and TSD, an essential fatty acid of the ω -6 type, which only enters the body with food and plays a structural role as the main component of membrane phospholipids [2]. From the above, it can be said that the presence of the polyunsaturated acids (oleic, linoleic and petroselinic acid) in the oils of *C. esculentus* is indicative of its high nutritional potential and, therefore, of its benefits for human health.

3.5. Composition of *Cyperus esculentus* oil unsaponifiables

According to El-Naggar (2016) [8], unsaponifiables play a decisive role in the stability of oils. **Table V** data present the compounds identified in the unsaponifiable fraction in TKS, TLK and TSD tuber oil. Most of the components identified are hydrocarbons (41.21; 21.32 and 50.8% in TKS, TLK and TSD, respectively). Stigmastane-3,5-diene, the main identified hydrocarbon (37.12 and 34.24% for TKS and TSD) is formed by dehydration of β -sitosterol [57]. Indeed, this dehydration may be due to the high temperatures during GC analysis and the reducing environment in the chromatographic system. It should be noted that this high hydrocarbon content is higher than that of tuber oil from Nigeria (20.57%) [50], but lower than that of *C. esculentus* from Egypt (77.795 %) [58]. In addition, the presence of phytosterols also manifests in the unsaponifiable fractions studied (30.64; 44.03 and 22.28% in TKS, TLK and TSD, respectively), in which β -sitosterol acetate predominates in TLK (31.33%) and (3 β)-3-acetyloxycholest-5-en-24-one present in high rate (8.18, 6.68 and 7.60% in TKS, TLK and TSD, respectively). Only the TKS sample contained tocopherol (0.48%) and terpene esters (0.23%). It should be noted that tocopherol (vitamin E) also plays an important role in maintaining good health of human organism [8]. The vitamin E content recorded in this study is higher than the content (0.016-0.018%) found for tubers from Turkey [33].

Table V: Results of GC/MS analysis of the unsaponifiable fraction of *Cyperus esculentus* tuber oil

Compositions	Samples					
	Ins TKS		Ins TLK		Ins TSD	
	TR (min)	%	TR (min)	%	TR (min)	%
(E)-4-oxopen-2-en-2-yl acetate					5.006	2.28
2-Ethylhexyl acetate	5.98	0.24				
3-acetylpentane-2,4-dione			6.477	1.53	7,473	4.75
(2,6-bis (1,1- dimethylethyl)- 4-methyl) phenol (polyphenol)	8.370	1.02			8.370	0.65
4a,8-dimethyl-2-(prop-1-en-2- yl)- 1,2,3,4,4a,5,6,8a-octahydronaphthalene (hydrocarbon)					8.399	0.64
3,8-Dimethyl-4-(1- methylethylidene)- 2,4,6,7,8,8a-hexahydro-5(1H)-azulenone (Aromadendrane sesquiterpenoids)			9.149	0.43		
2,6-Di-O-palmitoyl of L-ascorbic acid					10.671	0.76
Hexadecanoic acid (C16:0)	10.687	3.00	10.677	1.56		
2-O-acetoxytetradecane	11.321	1.05	11.321	1.76	11,320	2.68
(Z) octadec-9-enoic acid	11.501	20.47	11,485	16.31	11,477	10.44
Octadecanoic acid (C18:0)	11.964	0.52	11.565	1.10	11,564	0.86
8-Hydroxy-2-methyl-7-O- trifluoroacetyloctadecane (polyphenol)	12.060	0.37	12.059	1.38	12.059	2.27
Farnesyl acetate (terpene)	12.096	0.23				
Eicosane (hydrocarbon)	12.513	0.32	12.123	3.06	12.123	5.83
Octadecanoic acid (C18:0))			12,550	0.70		

Docosanoic acid (behenic acid, C 22:0)	12.549	0.86				
Eicosanoic acid (C20:0)			13.444	0.72	13.446	0.64
Hexatriacontane (hydrocarbon)	13.394	1.04	13.394	18.26	13,940	7.32
4,4'-thiobis [2-(1,1-dimethyl) -5-methyl] phenol (polypheneol)	14.648	0.41				
Squalene (terpene-hydrocarbon)	14.720	0.61			14.717	1.26
Tetratetracontane (hydrocarbon)	12.929	1.58	12.956	6.58	16.362	1.51
β-tocopherol (Vitamin E)	17.427	0.48				
1-Bromotriacontane (C ₃₀ H ₆₁ Br)			17.573	2.58		
1-Octadecanesulphonyl chloride (C ₁₈ H ₃₇ ClO ₂ S)					17.582	1.59
(3β)-3-acetyloxycholest-5-en-24-one (phytosterol)	21.836	8.18	21.775	6.68	21,769	7.60
(3β, 22Z) -3-O-acetylstigmas-5,22-diene (phytosterol)	22.403	9.90	22.317	6.02	22.307	9.85
(3β)-3-O-acetylstigmas-5-ene (β-Sitosterol acetate) (phytosterol)			23.829	31.33		
Stigmastan-3,5-diene (hydrocarbon)	24.027	37.12			23.814	34.24
3-(acetyloxy)-17-hydroxypregn-5-en-20- one (phytosterol)	24.268	2.07				
(3β)-9,19-cyclolanost-24-en-3-ol (phytosterol)	25.187	3.35				
(3β, 5α)-3-O-acetylstigmast-7-ene (phytosterol)	25.491	3.90			25.596	3.71
(3β, 5α) -3-O-acetylstigmas-7,16,25- triene (phytosterol)	25.905	1.28			25.856	1.12

(6 α , 16 α) -6-methyl-16-(1-methylethyl) pregn - 4 -ene-3,20-dione (phytosterol)	27,440	1.96	
Totals	100	99.7	100
Tocopherol	0.48		
Ester terpenes	0.23		
Hydrocarbons	41.21	21.32	50.8
Phenolic compounds	1.8	1.38	2.92
Phytosterols	30.64	44.03	22.28

TKS: tubers from Klolékaha department of Sinematiali; TLK: tubers from Lèlèkaha department of Korhogo; TSD tubers from Sokala Sobara department of Dabakala

On the other hand, it is lower than the content reported (5.39%) for tubers from Nigeria. [50] According to the work of Tao et al. (2012) [59], the presence of β -sitosterol acetate may confer *C. esculentus* oil from bacterial properties. As for the phenolic compounds, they were found in notable quantity (1.8; 1.38 and 2.92% in TKS, TLK and TSD, respectively), which is higher than those of *C. esculentus* from Turkey (0.20-0.27%) [33].

4. CONCLUSION

During this study, the physico-chemical characteristics, the composition of fatty acids and unsaponifiables of the oil of tubers of *Cyperus esculentus* from Côte d'Ivoire have been determined. The values of the physico-chemical parameters show that this oil as a whole has physical and physico-chemical properties that meet the standards of edible oils. It can be an excellent source of oil nutritious due to its richness in omega-9, unsaturated fatty acids (oleic and linoleic). Its consumption may reduce the risk of cardiovascular disease by lowering total cholesterol and LDL ("bad" cholesterol) levels in the blood.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

REFERENCES

- 1 Kapseu C., Tchiégang C., Dandjouma A., Parmentier M. Optimisation de l'extraction de l'huile par pressage des amandes de Ricinodendron heudelotii Pierre ex Pax. Journal of Food Engineering. 2005; 68(1):79-87
- 2 Guesnet P., Alessandri J-M., Astorg P., Pifferi F., Laviolle M. Les rôles physiologiques majeurs exercés par les acides gras polyinsaturés (AGPI). Oil seeds and fats. Crops and Lipids. 2005; 12(5-6): 333-343. doi.org/10.1051/ocl.2005.0333.French.
- 3 Lecerf J.-M. Les huiles végétales. Phytothérapie. 2010; 8(2): 75–76 doi:10.1007/s10298-010-0542-4. French.
- 4 Lunn J., Theobald H. The health effects of dietary unsaturated fatty acids. Nutrition Bulletin. 2006; 31 (3): 178–224.
- 5 Lecerf J.-M. Les huiles vegetales: particularités et utilités. Médecine Des Maladies Métaboliques. 2011 ; 5(3) : 257–262. French.
doi:10.1016/s1957-2557(11)702371
- 6 Addy E. O.D., Eteshola, E. Nutritive value of a mixture of tiger nut tubers (*Cyperus esculentus* L.) and boabab seeds (*Adansonia digitata* L.). Journal of the Science of Food and Agriculture. 1984; 35(4): 437-440. doi.org/10.1002/jsfa.2740350412
- 7 Sánchez-Zapata E., Fernández-López J., Angel Pérez-Alvarez J. Tiger Nut (*Cyperus esculentus*) Commercialization: Health Aspects, Composition, Properties, and Food Applications. Comprehensive Reviews in Food Science and Food Safety. 2012; 11(4): 366–377 doi:10.1111/j.1541-4337.2012. 00190.x
- 8 El-Naggar E. A. Physicochemical Characteristics of Tiger Nut Tuber (*Cyperus esculentus* L.) Oil. Middle East Journal of Applied Sciences. 2016; 6 (4): 1003-1011
- 9 Aremu M. O., Ibrahim H., Aremu O. S. Lipid composition of Black Variety of Raw and Boiled Tigernut (*Cyperus esculentus* L.) Grown in North-East Nigeria. Pakistan Journal of Nutrition. 2016; 15(5) :427-438 ISSN 1680-5194
10. Adel A. A. M., Awad A. M., Mohamed H. H., Iryna S. Chemical composition, physicochemical properties and fatty acid profile of Tiger Nut (*Cyperus esculentus* L) seed

oil as affected by different preparation methods. *International Food Research Journal*.2015; 22(5): 1931-1938

11. Yeboah S. O., Mitei Y. C., Ngila J. C, Wessjohann L., Schmidt J. Compositional and structural studies of the oils from two edible seeds: Tiger nut, *Cyperus esculentus*, and asiato, *Pachira insignis*, from Ghana. *Food Research International*.2012; 14: 259-266.doi.org/10.1016/j.foodres.2011.06.036

12. Imam T.S., Aliyu F.G., Umar H.F. Preliminary phytochemical screening, elemental and proximate composition of two varieties of *Cyperus esculentus* (Tiger nut) *Nigerian Journal of Basic & Applied Sciences*.2013; 2(4):247-251.

13 Ezeh O., Gordon M. H., Niranjan K. Tiger nut oil (*Cyperus esculentus* L.): A review of its composition and physico-chemical properties. *European Journal of Lipid Science and Technology*.2014; 116 (7): 783-794.

14 El Shebini S.M., Moaty M.I.A., Tapoza S.T., Hanna L.M., Mohamed H.I., Raslan H.M. Short-term effect of (*Cyperus esculentus*) supplement on body weight, insulin sensitivity and serum lipoproteins in Egyptian obese patients. *International Journal of Academic Research*.2011; 3:539–544.

15 Ban-koffi L., Nemlin G. J., Lefevre S., Kamenan A. Caractérisation physico-chimique et potentialités thérapeutiques du pois sucré (*Cyperus esculentus* L. (Cyperaceae)). *Agronomie Africaine*.2005; 17 (1): 63-71

16 Codina-Torrella I., Guamis B., Trujillo A. J. Characterization and comparison of tigernuts (*Cyperus esculentus* L.) from different geographical origin: Physico-chemical characteristics and protein fractionation. *Industrial Crops and Products*.2015; 65: 406-414.

17 E Joh R. A., Djomdi, Ndjouenkeu R Caractéristiques du tigernut (*Cyperus esculentus*) les tubes et leur performance dans la production d'une boisson lactée. *Journal of Food Processing and Preservation*.2006; 30: 145–163.

[18]. Bamba S., Mamyrbekova B. J. A., Virieux D., Kabran G. R. M., Pirat J. L., Békro Y. A. Analysis of a Rutaceae fat matter from Côte d'Ivoire. *Der chemical Sinica*.2015; 6 (4): 47-50.

19. AFNOR (Association française de normalisation). Recueil des normes françaises des corps gras, graines oléagineuses, produits dérivés. 3ième édition.1984; 459 p.French.

20. AOAC (1981). (Association of Official Analytical Chemists). *Official Methods of Analysis*, 13th ed, Washington DC.

21 Bamba S. Contribution à la valorisation de l'huile des amandes d'Afraegle paniculata (rutaceae) de Côte d'Ivoire. Thèse de doctorat, Université NANGUI ABROGOUA, Côte d'Ivoire.2016); 128p.French

22 Batel W., Grael M., Meyer G.J., Moller R., Schoedder F. Pflanzenole für die kraftstoff-und energieversorgung. *Grundlagen der landtechnik*.1980; 30: 40-51

23 Dahouenon A. E., Djenontin T. S., Codjia D.R.M., Tchobo F.P., Alitonou A.G., Dangou J., Avlessi F., Sohounhloue D.C.K. Morphologie des fruits et quelques caractéristiques physiques et chimiques de l'huile et tourteaux d'*Irvingia gabonensis* (Irvingiaceae). *International Journal of Biological and Chemical Sciences*,2012; 6: 22-65

- 24 Soulier J., Farnier M. Manuel des corps gras, tome 1, Lavoisier, Paris.1992
- 25 Lavoisier. Obtention des corps gras. In Manuel des corps gras. Volume 1. Ed Tec.doc Laisney.1992
26. Benaziza A., Semad D. Oléiculture: Caractérisation De Six Variétés D'olives Introduites Dans Le Sud–Est Algérien, European Scientific Journal.2016; 12(33): 537-553.French.
27. Sanders T.H.Groundnut (peanut) oil, in: Gunstone F. D. (Ed.), Vegetable Oils in Food Technology. Composition, Properties, and Uses, Blackwell Publishing Ltd, Oxford, UK.2002; 231–243.
- 28 Saxhold E., Christensen A.T., Moller A., Hartkopp H.B., Hess Y.K., Hels O.H. Danish. Food Composition Databank, Natural Food Institute, Technical University of Denmark version 7.1. 2009
- 29 Mamyrbékova B.J.A., Bamba S., Akaffou S., Bekro Y.A. Caractéristique de la matière grasse extraite des amandes de *Azizelia Africana* (Fabaceae-caesalpinioideae) de Côte d'Ivoire. Revue Ivoirienne des Sciences et Technologie.2009; 13: 191-198.
- 30 Coşkuner Y., Ercan R., Karababa E. & Nazlıcan A. N. Physical and chemical properties of chufa (*Cyperus esculentus* L) tubers grown in the Çukurova region of Turkey.Journal of the Science of food and Agriculture.2002; 82: 625-631.doi: 10.1002/jsfa.1091
- 31 Bouharmont J., Agronomie moderne: bases physiologiques et agronomiques de la production végétale. Paris (France) Hatier.1995; 544 p.
- 32 Codex Alimentarius,). Norme pour les huiles végétales portant un nom spécifique.Codex-stan 210-1999.2005; 17 p
- 33 Duman E. Some physico-chemical properties, fatty acid compositions, macro-micro minerals and sterol contents of two variety tigernut tubers and oils harvested from East Mediterranean region. Science et technologie alimentaires.2019; ISSN 0101-2061.DOI: <https://doi.org/10.1590/fst.28018>
- 34 Bereau D. Huiles et fractions insaponifiables de huit especes de palmiersAmazoniens. Thèse de doctorat. L'institut National Polytechnique De Toulouse (France). 2001; 152p.French
- 35 Codex Alimentarius. Norme pour les huiles végétales portant un nom spécifique Codex-stan 210-1999.2017; 14 p.
- 36 Tekaya I.B., Hassouna M. Étude de la stabilité oxydative de l'huile d'olive vierge extra tunisienne au cours de son stockage. OCL.2005; 12 (5): 447-454
- 37 Sabah M. S. A., Shaker M., Arafat M. S. A., Fawzia I. M. Nutritional value of Tiger Nut (*Cyperus Esculentus* L.) Tubers and Its Products. Journal of Biological Chemistry and Environmental Sciences.2019; 14(1): 301-318
- [38] Muhammad N., Bamishaiye E., Bamishaiye O., Usman L. & al. (2011). Physicochemical properties and fatty acid composition of *Cyperus esculentus* (Tiger Nut) tuber oil. Bioresearch Bulletin. 2011, 5, 51–54.

- 39 FAO/WHO. Report of the 21st session of the Codex Alimentarius Committee on fats and oils. Kola Kinabalu, Malaysia.2009; 16-20
- 40 Noumi G. B., Njoukam Y.M., Njiné C. B., Ngameni E., Kapseu C.). Effets du séchage sur le rendement et la qualité de l'huile extraite de la pulpe de safou. *Tropicultura*,2011; 29 (3): 138.-142
- 41 M'baye B. K., Alouemine S. O., Lô B. B., Bassene E. Etude physico-chimique des huiles consommées en mauritanie. ScienceLib Editions Mersenne.2011; 4 (120101) ISSN 2111-4706
- 42 FAO (Food and Agricultural Organization). Codex Alimentarius Commission. Graisses et huiles végétales, division 11, Version abrégée FAO/WHO. Codex Stan.1981; 20-23.
- 43 Balla, A Baragé M. Analyses physico-chimiques de la pulpe et caractérisation de la fraction lipidique des amandes du fruit du pommier de Cayor (*Neocarya macrophylla* Sabine). *Bulletin de la Recherche Agronomique du Bénin*. 2008; 61 (1).
- 44 Oderinde R.A., Tairu D.A. Evaluation of the Properties of Yellow Nutsedge (*Cyperus esculentus*) Tuber Oil. *Food Chemistry*.1988; 28 (3), 233-237
- 45 Marcusson J. Manuel de laboratoire pour l'industrie des huiles et graisses, Librairie polytechnique CH. Béranger. Paris.1929; 15:179p
- 46 Thomas M. Mobilisation de l'acide arachidonique et sensibilité au peptide β -amyloïde Thèse doctorale de l'Université de Lorraine.2015 ;240 p.
- 47 Alonso L., Fontecha V., Lozada L., Juarez, M. Determination of mixtures in vegetable oils and milk fat by analysis of sterol fraction by gas chromatography. *Journal of American Oil and Chemical Society*.1997; 74: 131-135.
- 48 Jekel A.A., Vaessen H.A.M.G., Schothorst R.CCapillary gas-chromatographic method for determining non-derivatized sterols – some results for duplicate 24 h diet samples collected in 1994. *Journal of Analytical Chemistry*.1998; 360: 595–600.
- 49 Aïssi V.M., Soumanou M.M., Tchobo F.P., Kiki D.). Etude comparative de la qualité des huiles végétales alimentaires raffinées en usage au Bénin. *Bulletin d'Informations de la Société Ouest Africaine de Chimie*.2009; 06: 25-37.
- 50 Oderinde R. A., Tairu A. O. Determination of the triglyceride, phospholipid and unsaponifiable fractions of yellow nutsedge tuber oil.*Food Chemistry* 1992 ; 45: 279-282
- 51 Eteshola E., Oraedu A. C. I.). Fatty acid compositions of tigernut tubers (*Cyperus esculentus* L.), baobab seeds (*Adansonia digitata* L.), and their mixture. *Journal of the American Oil Chemists' Society*.1996; 73: 255–257.
- 52 Alaluf S., Green M.R., Powell J.R., Rogers J.S., Watkinson A., Cain F.W., Hu H.L., Rawlings A.V. Petroselinic Acid and Its Use in Food. U.S. Patent 6,365,175 B1.2002
- 53 Placek L.L. A review on petroselinic acid and its derivatives. *Journal of the American Oil Chemists' Society*.1963; 40, 319–329.

54 Malnoe A., Baur M., Fay L'utilisation de l'acide pétrosélinique pour le traitement des inflammations des tissus superficiels. Patent EP 0 888 773 A1.1999. French

55 Carlotti M.E., Ugazio E., Gastaldi L., Sapino S., Vione D., Fenoglio I., Fubini B. Journal of Photochemistry and Photobiology B.2009; 96(2): 130–135.

56 Moribe K., Maruyama S., Inoue Y., Suzuki T., Fukami T., Tomono K., Higashi K., Tozuka Y., Yamamoto K. International Journal of Pharmaceutics.2010; 387(1-2): 236–243.

57 Cert A., Lanzón A., Carelli A. A., Albi T., Amelotti G.). Formation of stigmasta-3,5-diene in vegetable oils. Food Chemistry.1994; 49(3): 287–29. doi :10.1016/0308-8146(94)90173-2

58 El-Naggar A.E.). Effet biologique de la noix tigrée (*Cyperus esculentus* L.) Huile sur Rats sains et hypercholestérolémiques. Journal syrien de recherche agricole. 2017; 4(3):133-147

59 Tao R., Wang C., Kong Z. Antibacterial Activity of Polyprenols and Other Lipids from Ginkgo biloba L. Leaves. Proceedings of the 2012 International Conference on Applied Biotechnology.2012; 1581-1589

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