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Antimicrobial and Antioxidant Activities of Palm Kernel Oils Extracted from Varieties Dura and Tenera of Oil Palm (*Elaeis guineensis* Jacq.) and the Relationship to Their Chemical Composition

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Abstract

The investigation of the antimicrobial and antioxidant activities of palm kernel oils extracted from varieties Dura (oil D) and Tenera (oil T) of oil palm (*Elaeis guineensis* Jacq.) and the relationship to their chemical composition were carried out in this study. The results obtained show that oil D had the greatest antimicrobial activity. The analysis of gas chromatographic coupled to mass spectrometry (GC-MS) showed that this oil D contained in addition to the lauric acid, the undecylenic acid which both possess antimicrobial activity. This undecylenic acid was not identified in oil T. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and the Fe^{3+} reduction test (FRAP) used to evaluate the antioxidant activity of the palm kernel oils had showed that oil T had the greatest antioxidant activity with a concentration inhibiting 50% of the reaction (IC50%) of 750 mg/L. It was also noted that, this oil T had also the highest contents in polyphenols, α-tocopherol and sterols with predominance of β-sitosterol. Moreover, the β-sitosterol is known to possess a regulatory role of the immune system.

The findings of this study provide thus, useful information which may help customers to

make the best choice in the consumption of these oils.

Keywords: Oil palm varieties, Palm kernel oil, Antioxidant activity, Antimicrobial activity, Chemical composition.

1. Introduction

Food and food additives consumed by people are important to improve their immune system which helps them fight against illness (Pegel, 1997) mainly in periods such as this current period of COVID-19 pandemic. Vegetable oils belong to these useful food additives. Indeed, vegetable oils are an important source of nutrients (presence of essential fatty acids, fat-soluble vitamins, polyphenols, phytosterols) which give them a special role in the diets of populations (Lecerf, 2011). They are extracted from oleaginous plants, which have most of the time, traditionally been used for seasoning and preserving the sanitary quality of foods through the oils they produce (Tchiégang, 2004). In addition to the preservation of food quality, vegetable oils are used in pharmaceutical, cosmetic and oil industry fields (Aubret & Huard 2003). All these properties of vegetable oils are due to their chemical components. Indeed, oil extracts of sulfur-rich vegetables may have potent antimicrobial activity, and could be used in food preparation to get the synergistic effect of the oils and vegetables (Islam et al., 2014). Edible oils contain a number of phenolic compounds, which contribute to oxidative stability, and may serve as antioxidants to reduce the stress of oxidation on human health (Dilini, 2013). Other studies have also shown that phenolic acids and flavonoids possess multifunctional properties and beneficial effects on human health, among which polyphenols have attracted great attention (Bouras et al., 2015). Among the vegetable oils used for several purposes since many years ago, there is palm kernel oil derived from the kernel of the fruit of oil palm (*Elaeis guineensis* Jacq.). This kernel of the fruit is the most important quantity of residues obtained after the extraction of palm oil from the pulp of the fruit of oil palm (Pickard, 2005). The palm kernel cake has been shown especially suitable for feeding ruminants because of its relatively important fibers content (Pickard, 2005). Many studies have shown that palm kernel cake is majorly commercialized as ingredients of beef and dairy feed (Ravber et al., 2015). The oil extracted from the palm kernel is used in folk medicine for the treatment of skin irritations and mycosis. It is also used for seasoning the dishes and as a food preservative in Ivory Coast where this oil is abundant and affordable affordable (Yapi & Kouadio, 2019). This palm kernel oil is an appreciable source of saturated fatty acids but contains also monounsaturated and polyunsaturated fatty acids (Agboola, et al., 2015). Moreover, the palm kernel oil with its richness in lauric acid which possesses antimicrobial and antiviral properties can be recommended for the diet of people living with HIV AIDS (Anonyme, 2010; Anonyme, 2012). Moreover, further investigations must be carried out in order to confirm this information. However, despite these characteristics and these uses, there is a lack of studies on the biological activities (antimicrobial and antioxidant activities) of palm kernel oil and also on the relationship between these biological activities and the mixture major and minor components of the existing types of palm kernel oil.

Thus, in this study, the antimicrobial and antioxidant activities of palm kernel oils extracted from varieties Dura and Tenera of oil palm which were the ones produced and consumed in Côte d'Ivoire were investigated and the relationship to their chemical composition was evaluated in order to identify the variety from which the oil extracted was more suitable for

human diet.

2. Material and Methods

2.1 Material

The biological material used is the palm kernel oil extracted from the kernel of the fruits of two varieties (Dura and Tenera) of oil palm (*Elaeis guineensis* Jacq.). These two varieties are the ones which are produced and consumed in Côte d'Ivoire. For this research, the fruits of these varieties of oil palm were bought on the markets in Abidjan (Côte d'Ivoire). Bacterial strains from the microbial collection of the Bacteriology and Virology laboratory of the Pasteur Institute, Abidjan, Côte d'Ivoire were used. Fungal strains which are frequently isolated in various food products sold on markets in Côte d'Ivoire were also used (Table 1). These fungal strains were from the Biochemistry and Food Sciences laboratory of the UFR Biosciences of University Félix Houphouet-Boigny.

Table 1. Microbial strains tested

Gram negative bacteria	**Gram bacteria positive**	**Fungi**
Escherichia coli (ATCC25922)		*Aspergillus niger*
Pseudomonas aeruginosa (ATCC27853)		*Aspergillus fumigatus*
Salmonella typhi (CHU)	*Staphylococcus aureus* (ATCC25923)	*Aspergillus nidulans*
Enterobacter cloaceae (ATCC23355)		*Aspergillus carbonarius*
Klebsiella oxytoca (ATCC31899)		*Aspergillus flavus*

2.2 Methods

2.2.1 Oil Extraction

Oil extraction was carried out by using the Soxhlet (250 mL - Allihn – LENZ) method with hexane as a solvent which gives the best oil yield with solid sample according to Mohd-Setapar et al. (2004). For the process of extraction, the method used was that described by Yapi and Kouadio (2019). At the end of this extraction process, the oil was recovered by evaporating off the solvent using rotary evaporator where it was heated at 70 °C until the solvent finally evaporated and leaving behind the extracted oil. All experiments were conducted in triplicate. The oil obtained was stored at 4 °C until analysis.

The total fat content (FA) is given by the following formula:

FA (Total fat content) = P2-P1

Oil yield = (FA/P0) × 100

Oil yield = [(P2-P1) / P0] × 100

Where: P0: mass (g) of the test sample; P1: mass (g) of the empty flask

P2: mass (g) of the flask and the total fat extracted.

2.2.2 Determination of the Biochemical composition of Oils Extracted

2.2.2.1 Determination of Fatty Acids Composition by Gas Chromatography Coupled to Mass Spectrometry (GC/MS)

The analysis started with the conversion of the oil into fatty acid triméthyl silyl ester. This esterification was conducted following the procedure described by Kloos et al. (2014).

For this silylation, a quantity of 5 mL of oil sample was mixed with 40 mL of n-hexane and 20 mL of methanol in a separator funnel. The mixture is stirred vigorously for 5 minutes and then left to stand until the appearance of the interface between these two immiscible solvents. The methanolic fraction is recovered and then treated again with 2 x 40 mL of n-hexane. The extract is evaporated to dryness under a nitrogen jet and then recovered with 500 µL of dichloromethane and derivatized at 60 °C for 15 minutes after addition of 200 µL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). A quantity of 1µl of this extract was then injected for analysis by GC-MS. This analysis by GC-MS was carried out using an apparatus of the PerkinElmer brand, model Clarus 680GC 600C MS. It is provided with a Restek Rtx-5ms column 30 m long, with an internal diameter of 0.25 mm and a film thickness with a stationary phase of 0.25 µm. The helium was used as the carrier gas at a fixed flow rate of 1 mL / min. The oven temperature program was 70 °C for 2 minutes, then a gradient of 5 °C / min was applied up to 300 °C. The latter temperature was maintained for 12 minutes for a total analysis time of 60 minutes. The injector temperature was set at 280 °C. The injection was carried out in split mode with a ratio of 1:50. The mass spectrometer was set to electronic impact mode with an ionization source temperature of 200 °C, electron energy of 70 eV and a scanning speed of 200 scans / min.

2.2.2.2 Determination of Sterols Content

A quantity of 3 g of oil and 1 mL of internal standard (5 α-Cholestane: 2 mg / mL of chloroform) were subjected to saponification for extraction of the unsaponifiable fraction according to the method of AFNOR NF T60-205 (1984). This unsaponifiable fraction was derived by silylation according to the method described by Grandgirard and Gordelet (1998). Indeed, a quantity of 400 µL of Tri-Silyl (200 µL of CH_2Cl_2 and 200 µL of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) were added to the unsaponifiable fraction extracted. The whole was brought to a hot water bath (60 °C for 45 min) to form the trimethyl silyl ether derivatives (TMS). The excess of Tri-Silyl was evaporated under nitrogen flow and the sample was diluted in 10 ml of hexane. The TMS sterols were analyzed by gas chromatography coupled to a mass spectrometer (Perkin Elmer brand device, model Clarus 680GC 600C MS). The helium used as carrier gas was adjusted at a speed of 0.9 mL / s. The programming of the oven temperature was 70 - 280 °C for 42 min (5 °C / min) then 280 - 310 °C for 10 min (3 ° C / min). The injector temperature was set at 250 °C and the detector temperature at 280 °C. The injection was carried out in split mode (with flow divider). The mass spectrometer parameters for the electronic impact mode were: the

ionization source temperature (230 °C), the electron energy (70 eV), the scanning speed (50 scans / s) and the acquisition speed (10,000 m.a.u. / s).

The identification of the peaks was based on the comparison of the mass spectra with those of the Wiley library, namely, HPCHEM, Wiley, 275, 6th edition and also with those of the literature. In some cases, a comparison of the retention times and their fragments with those of the TMS ether derivatives, prepared from standard, was carried out. The quantities of the various sterols of the oils studied were calculated as follows:

Sterol × (mg/100 g of oil) = (Ax × ms × 100) / As × m

The content of each single sterol is expressed in milligrams per 100 grams of oil.

With:

Ax: peak x area of the sterol,

As: peak area of the 5α-cholestane,

ms: mass of added 5α-cholestane (mg)

m: mass of the test portion (g).

The percentage of each sterol is given by the relation: % sterol x = (Ax / A) x100

With Ax: area of the peak x

A: sum of the areas of all the peaks.

2.2.2.3 Determination of the α-tocopherol Content

The α-tocopherol was determined because this compound is the most active form of vitamin E and it has the greatest biological value in the body. This assay was carried out according to ISO 14565 (2000). Indeed, a quantity of 25 g of oil was dissolved in 100 mL of petroleum ether. A quantity of 0.15 g of vitamin C and 0.1 g of hydroquinone were added to the mixture obtained.

The whole was homogenized using a vortex and then 3 mL of sodium sulfide prepared in a glycerol / water mixture were added to it. Subsequently, the solution obtained was heated at 70 °C for 45 min and then transferred into a separator funnel. The unsaponifiable matter was extracted 3 times with 100 mL of petroleum ether. The ethereal phases were combined and washed until neutral with distilled water. The petroleum ether was removed by heating in a water bath at 75 °C. The residue obtained was added to 25 mL of methanol and then filtered under vacuum in vials and 0.1 g of the standard (α-tocopherol) was prepared under the same conditions. The analysis of the α-tocopherol was carried out by a gas chromatograph equipped with a mass spectrometer with methanol as the elution solvent. The automatic injection made it possible to inject 1 µL of sample in split mode (with flow divider). The flow divider injectors make it possible to inject very small volume which does not saturate the column. The split corresponds to a ratio between the part actually injected into the column and that directed towards the outside of the device. The elution rate was 0.25 mL / min. Detection was carried out at 292 nm using a UV detector. The peak corresponding to the α-tocopherol content of the oil samples was identified by comparison of its retention time with that of the α-tocopherol standard. The α-tocopherol contents were determined using Emporio brand software.

2.2.2.4 Determination of Polyphenols Content

In this case, as the palm kernel oil is derived from the kernel which is the residues obtained after the extraction of palm oil from the pulp of the fruit of oil palm, we decided to demine the total polyphenolic content. This total polyphenolic content was evaluated according to the Folin-Ciocalteu method described by Albano and Miguel (2011). For this analysis, two (2) g of oil were dissolved in 5 mL of hexane in an Erlenmeyer flask, and then 5 mL of a methanol / water solution (60/40, v / v) were added. The methanolic phase was recovered using a separatory funnel. The operation was repeated 3 times to extract more polyphenols. The methanolic phases were collected and then concentrated. To 50 µL of the residue obtained, 1.95 ml of distilled water, 0.5 mL of Folin-Ciocalteu reagent diluted to 1/10 (v / v) were added and then the whole was incubated for 3 minutes at 1 shelter from light. Subsequently, 0.4 mL of sodium carbonate (Na2CO3) at 75 g / L was added. The whole was incubated for 40 min in the dark. The determination of total phenols was carried out using a UV Visible spectrophotometer by measuring the absorbance of phenolic solutions at 760 nm. The tests were carried out in triplicate for each oil sample. The content of total polyphenols was determined using a Gallic acid calibration line performed at different concentrations (20 µg / mL; 40 µg / mL; 60 µg / mL; 80 µg / mL and 100 µg / mL). The total polyphenol content of the oil samples (Q), expressed in milligrams of Gallic acid equivalent per gram of oil (mg EAG / g of oil) was calculated according to the formula below:

$Q = (V \times C \times d) / m$

With V: final volume of the extract (mL),

C: concentration of the extract obtained with the calibration curve (mg / mL),

d: dilution,

m: mass of oil in the test sample (g).

2.2.3 Evaluation of the Biological Activities of Oils Extracted

2.2.3.1 Antibacterial Activity

2.2.3.1.1 Preparation of the Tested Species

The preparation of the tested species was done according to the method of [CLSI, (1999). A quantity of 1 mL of each strain previously stored in glycerol 15% at -20 °C was thawed in 9 ml of liquid Mueller-Hinton medium. The obtained suspension was firstly incubated at 30 °C for 8 h. In a second step, 1 ml each of the microbial suspension obtained after 8 h of incubation was put in 9 ml of Mueller-Hinton. The whole was incubated at 30 °C overnight. The absorbance of this second culture was measured with a spectrophotometer at 630 nm. The optical density was adjusted at 0.6 by diluting (1/10) and the microbial suspension was used for the inhibition of bacteria growth.

2.2.3.1.2 Effect of Oils on Bacterial Growth

To facilitate the miscibility of palm kernel oils (oil D and oil T) in the culture medium, Tween 80 was previously added to these oils at the amount of 90% oil for 10% tween 80 (Sofiane, 2009). Each mixture of oil obtained was added to the liquid medium of Mueller-Hinton agar (v/v) to obtain mediums with different concentrations of 5%, 10%, 15%, 20%, 25% and 30%. Medium without oil was also used as positive control. Each medium was put into a Petri dish

and after solidification, 10 µL of each bacteria suspension were sprayed onto the medium. The incubation was made at 30 °C for 72 hours. The experiment was carried out three times. As the bacteria tested were pathogens, we have decided to express the results obtained in terms of growth (+) or absence of growth (-).

2.2.3.2 Antifungal Activity

2.2.3.2.1 Preparation of the Tested Strains

The fungal strains were sprayed onto the Czapeck Yeast Extract Agar (CYA) for 3 days. After this incubation time, the different suspensions of spores were then prepared by scraping the conidiospores into 10 mL of sterilized distillate water and filtered onto sterilized Miracloth (Filter composed of rayon polyester with a pore size of 22 µm and an acrylic binder). The conidia concentration of each strain was determined by counting them in a hemacytometer and appropriate dilution was made to obtain a concentration of 106 spores / mL. These suspensions of 106 spores / mL were used for the tests of inhibition of fungal growth (CLSI, 1999).

2.2.3.2.2 Effect of oils on Fungal Growth

To facilitate the miscibility of palm kernel oils in the culture medium, Tween 80 was previously added to these oils at the amount of 90% oil for 10% tween 80 (Sofiane, 2009).

Each mixture of oil obtained was added to the medium yeast extract agar (CYA) to obtain mediums with different contents of 15%, 10%, 15%, 20%, 25% and 30%. Each medium was put into a Petri dish and after solidification, 10 µL of the *Aspergillus* conidia suspension were put aseptically in the centre of this medium. The medium without palm kernel oil was also inoculated. For each oil content in the medium, three Petri dishes were used. All the inoculated mediums were incubated at 30°C. The growth rate was determined by measuring the diameter of the colony after 7 day of incubation according to the method of (Pitt, 1988). The tests were done in triplicate. The measurement of the diameter was made using a graduated ruler.

2.2.3.3 Evaluation of the Antioxidant Activity of Palm Kernel Oils

Two methods were chosen for their ease of implementation and their reliability for evaluating the antioxidant activity of oils. There was the DPPH test (1, 1-Diphenyl -2-picrylhydrazyle) and that of FRAP (Ferric reducing antioxidant power). The choice also resided in the simplicity of implementation of these methods and their good reproducibility.

2.2.3.3.1 DPPH Test

For this antioxidant test, the samples were prepared by dissolving oils in absolute methanol. For both palm kernel oils, the solutions were prepared in methanol to have different concentrations of the order of mg/L (100, 250, 500, 750 and 1000 mg/L). The measurement of the anti-radical activity of the oils was carried out by the test with 1.1 diphenyl-2-picrylhydrazyl (DPPH) according to the method described by Parejo et al., (2000). The absorbance was read using a spectrophotometer at 517 nm against a blank (methanol solution) using a UV-VIS type spectrophotometer of the BioMérieux brand. Vitamin C (ascorbic acid), which is the reference substance, was prepared under the same conditions and then used as a standard. The percentage inhibition (I %) of the free radical DPPH is

calculated according to the following formula:

$$\% \text{ Inhibition: } I\ (\%) = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \times 100$$

Where:

A sample: absorbance of the solution when the sample has been added

ADPPH: absorbance of the DPPH solution.

The IC50% (inhibitory concentration) value was determined as the inhibition of a concentration necessary to decrease the DPPH radical concentration by 50% and expressed in mg/mL.

Thus, a lower IC50% value indicates higher DPPH radical scavenging activity. All measurements were performed in triplicate.

2.2.3.3.2 FRAP Test

The reducing activity of iron in oils was determined according to the method based on the reduction of Fe3+ in the K3Fe (CN) 6 complex to Fe2+ (Yildirim et al., 2001). The absorbance of the reaction medium was then read at 700 nm against an identically prepared blank by replacing palm kernel oil with distilled water which made it possible to calibrate the device (UV-VIS spectrophotometer). The positive control was represented by a solution of ascorbic acid (a standard antioxidant), and its absorbance was measured under the same conditions as the samples.

2.2.4 Statistical Analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) version 16. Quantitative data were presented as means ±SD. The independent sample- ANOVA with post-hoc (LSD) test was used to analyse the mean difference. Probability values (P) of less than 0.05 were regarded as statistically significant.

3. Results and Discussion

3.1 The Fat Content

The extraction of the fat content showed that the palm kernel oil yields obtained for these two varieties were around 50% (Table 2). This value fell in that obtained by Yapi and Kouadio (2019) and also in that indicated by the Codex Alimentarius (2015). It is also noted that the oil yields obtained were similar to that obtained by Pickard (2005), but fell below the range of 75-80% found by Asuquon (2008). This could be explained by the variety of fruits of oil palm used by Asuquo (2008) which unfortunately was not specified.

Table 2. Fat content

Quality parameters	Samples		
	Oil extracted from variety Dura (Oil D)	Oil extracted from variety Tenera (Oil T)	Standards Codex Alimentarius Adopted in 1999 and amended in 2005-2015
Fat content (g)	4.939 ± 0.1[a]	4.987 ± 0.1[a]	-
Oil yield (%)	49.39 ± 0.1[a]	49.87 ± 0.1[a]	50

Values are means ± S.D (n=3)

Means in each row followed by different letters are significantly different ($p<0.05$).

3.2 Biochemical Composition of the Palm Kernel Oils

3.2.1 Fatty Acids Composition

Fatty acids are the major compounds identified in these oils. This identification showed that both oils contained 17 types of fatty acids (Figures 1 and Figure 2). It was also noted that, the oils analysed had in common 16 fatty acids (Table 3). Beside these fatty acids, undecylenic acid was identified only in oil D, while in oil T, heptanoic acid was found (Table 2). The undecylenic acid, which has been identified only in oil D, is known to be a potent fungicide that is very well tolerated (Dongmei et al., 2016). This undecylenic acid is also a precursor in the manufacture of many pharmaceutical products, personal hygiene products, cosmetics and perfumes (Kohlpaintner et al., 2008).

The determination of the total fatty acids content showed that the total unsaturated fatty acids was 20.2% ± 0.14 and 18% ± 0.52 respectively for oil D and oil T. For the total saturated fatty acids, the values obtained were 79.8% ± 1.2 and 82% ± 1.5 respectively for oil D and oil T (Figure 3). Among these saturated fatty acids, lauric acid was the predominant fatty acid with level of 50.69% ± 0.22 and 50.63% ± 0.19 respectively for oil D and oil T (Table 2). Similar results were obtained by Orsavova et al., (2015) with coconut oil. This lauric acid could decrease the ratio of total HDL cholesterol (Orsavova et al., 2015). Moreover, it has the greatest antimicrobial activity of all medium chain aliphatic fatty acids according to Ruzin and Novick (2000).

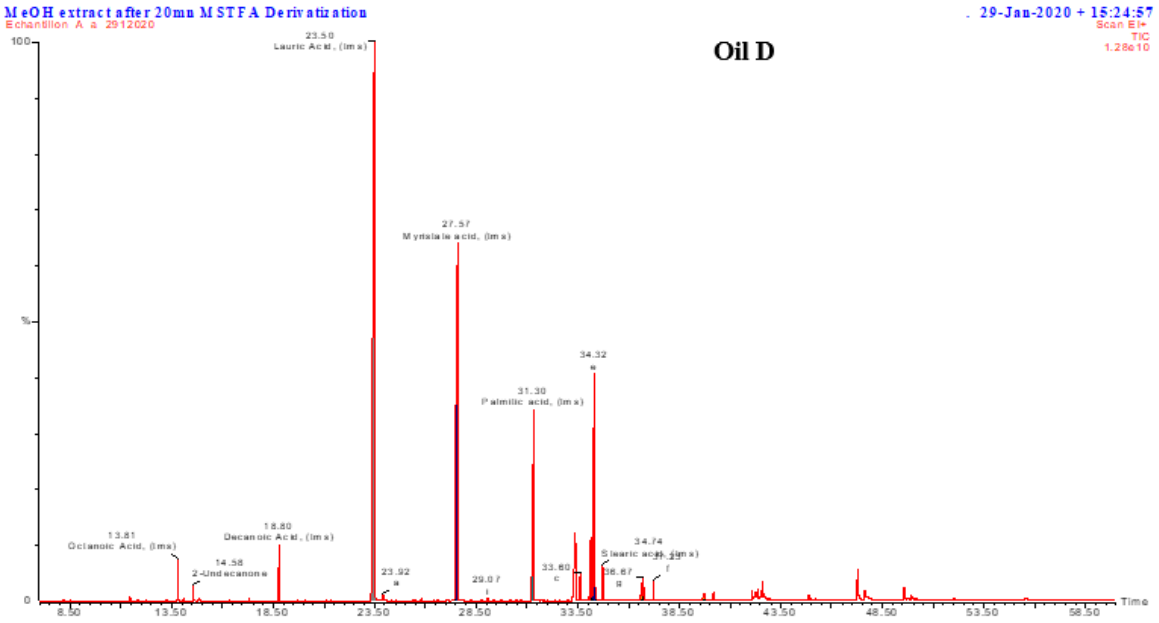

Figure 1. Chromatogram of oil D analyzed by gas chromatography coupled to mass spectrometry (GC-MS)

Fatty acid peaks on the chromatogram:

1. Caproic acid; 2. Octanoic acid; 3. Nonanoic acid; 4. Decanoic acid; 5. Undecanoic acid; 6. Lauric acid; 7. n-Tridecanoic acid; 8. Undecylenic acid; 9. Myristic acid; 10. n-Pentanoic acid; 11. Palmitic acid; 12. Heptadecanoic acid; 13. Trans-9-octadecenoic acid; 14. Oleic acid; 15. Stearic acid; 16. Linoleic acid; 17. Arachidic acid.

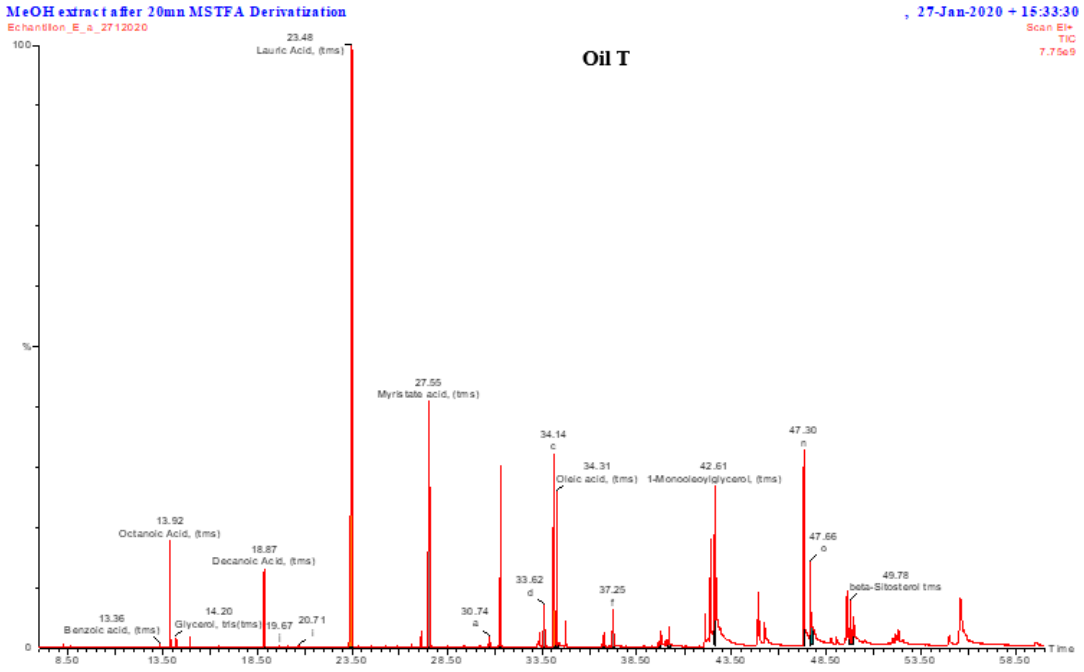

Figure 2. Chromatogram of oil T analyzed by gas chromatography coupled to mass spectrometry (GC-MS)

Fatty acid peaks on the chromatogram:

1. Caproic acid; 2. Heptanoic acid; 3. Octanoic acid; 4. Nonanoic acid; 5. Decanoic acid; 6. Undecanoic acid; 7. Lauric acid; 8. n-Tridecanoic acid; 9. Myristic acid; 10 n-Pentanoic acid; 11. Palmitic acid; 12. Heptadecanoic acid; 13. Trans-9-octadecenoic acid; 14. Oleic acid; 15. Stearic acid; 16. Linoleic acid; 17. Arachidic acid.

Table 3. Fatty acids composition of palm kernel oils analyzed by gas chromatography coupled to mass spectrometry (GC-MS)

	Oil D	Oil T
Fatty acids	Content (%)	Content (%)
Caproic acid (C6:0), (tms)	0.03 ± 0.001[a]	0.1 ± 0.003[b]
Heptanoic acid (C7:0), (tms)	Abs	0.02 ± 0.01
Octanoic acid (C8:0), (tms)	1.03 ± 0.11[a]	4.53 ± 0.11[b]
Nonanoic acid (C9:0), (tms)	0.03 ± 0.01[a]	0.05 ± 0.01[a]
Decanoic acid (C10:0), (tms)	1.49 ± 0.21[a]	3.36 ± 0.18[a]
Undecanoic acid (C11:0), (tms)	0.02 ± 0.01[a]	0.04 ± 0.01[a]
Lauric acid (C12:0), (tms)	50.69 ± 0.22[a]	50.63 ± 0.19[a]
n-Tridecanoic acid (C13:0), (tms)	0.04 ± 0.01[a]	0.04 ± 0.01[a]
Undecylenic acid (C11:1 [cis - 1]), (tms)	0.01 ± 0.001[a]	Abs
Myristic acid (C14:0), (tms)	18.56 ± 0.24[a]	12.70 ± 0.17[b]
n-Pentanoic acid (C5:0), (tms)	0.01 ± 0.001[a]	0.08 ± 0.004[b]
Palmitic acid (C16:0), (tms)	6.70 ± 0.21[a]	9.05 ± 0.21[b]
Heptadecanoic acid (C17:0), (tms)	0.01 ± 0.001[a]	0.02 ± 0.001[a]
Trans-9-octadecenoic acid (C18: 1 [trans-9]), (tms)	7.27 ± 0.21[a]	4.61 ± 0.18[b]
Oleic acid (C18: 1 [cis-9]), (tms)	10.36 ± 0.1[a]	10.69 ± 0.2[a]
Stearic acid (C18:0), (tms)	1.14 ± 0.02[a]	1.33 ± 0.11[a]
Linoleic acid (C18: 2 [cis-9,12]), (tms)	2.56 ± 0.2[a]	2.70 ± 0.3[a]

Arachidic acid (C20:0), (tms)	0.04 ± 0.01[a]	0.04 ± 0.01[a]

Values are means ± S.D (n=3)

Means in each row followed by different letters are significantly different ($p<0.05$).

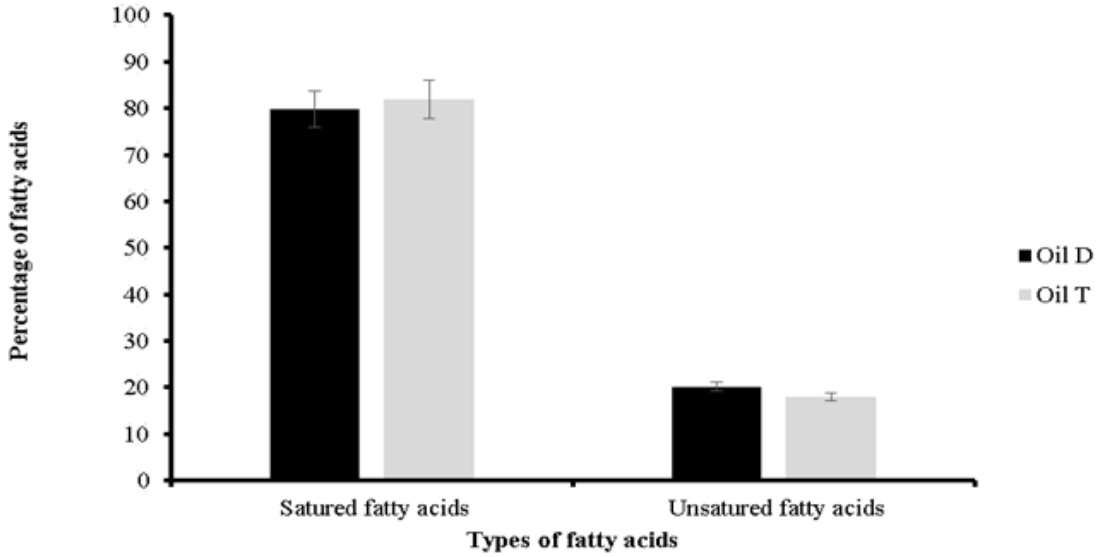

Figure 3. Total fatty acids contents

3.2.2 Sterols Composition

Among the minor components identified, the total sterols content of oil T was significantly higher than that of oil D ($P<0.05$). Indeed, the sterols content of oil D was 350 ± 1.01 mg / 100 g of oil, while that of oil T was 4980 ± 1.8 mg / 100 g of oil (Table 4). The value obtained for this oil T is similar to that obtained in olive oil which is an oil rich in sterols (Sofiane, 2009). The identification of the sterols fraction revealed the presence of β-sitosterol, campesterol, and stigmasterol. The β-sitosterol was the predominant sterol in the two types of oil with proportions of 75.09% ± 1.34 and 76.29% ± 1.8 followed by campesterol (13.42% ± 1.06 and 12, 35% ± 0.91) and stigmasterol (11.48% ± 0.8 and 11.36% ± 0.75) for oil D and oil T respectively (Table 5).

By relating the proportion of each sterol to the total contents of sterols in each oil, it is noted that the quantity of β-sitosterol (3799.242 mg / 100 g of oil) in oil T was higher than that of oil D (262.815 mg / 100 g of oil). This β-sitosterol has several beneficial effects on health. Indeed, it possess a regulatory role of key molecules involved in inflammation, the immune response and anti-cancer defences. It is a potential candidate in cancer chemotherapy. Epidemiological evidence has shown that β-sitosterol inhibits the growth of tumours in colon cancer, prostate cancer, breast cancer, and leukaemia. In the case of breast cancer, studies have shown that, β-sitosterol inhibits the growth of tumour cells, while campesterol doesn't possess this action (Moon et al., 2007). That makes this oil T very interesting for human diet. It is also interesting to note that during this study, we didn't find previous studies relating to

the research of sterols in palm kernel oil.

Table 4. Minor components contents of palm kernel oils analyzed

Components	Oil D	Oil T
Polyphenols content (mg EAG/g of oil)	62.3 ± 1.02[a]	94.07 ± 1.16[b]
α-Tocopherol content (mg/100 g of oil)	44.1 ± 0.52[a]	58.9 ± 0.76[b]
Sterols content (mg/100 g of oil)	350 ± 1.01[a]	4980 ± 1.8[b]

Values are means ± S.D (n=3)

Means in each row followed by different letters are significantly different ($p<0.05$).

Table 5. Sterol composition of palm kernel oils analyzed

	Oil D	Oil T
Sterols	(%)	
Campesterol	13.42 ± 1,06[a]	12.35 ± 0.91[a]
Stigmasterol	11.48 ± 0.8[a]	11.36 ± 0.75[a]
β-Sitosterol	75.09 ± 1.34[a]	76.29 ± 1,8[a]

Values are means ± S.D (n=3)

Means in each row followed by different letters are significantly different ($p<0.05$).

3.2.3 α-Tocopherol Content

The α-tocopherol content which is the most active form of vitamin E was also determined in the oils. The analysis showed that the α-tocopherol content of oil T was significantly higher than that of oil D ($P <0.05$). Indeed, the α-tocopherol contents were 44.1 ± 0.52 mg / g of oil and 58.9 ± 0.76 mg / g of oil respectively for oil D and oil T (Table 4). The biological activity of this α-tocopherol is mainly attributed to its ability to act as an antioxidant, thus protecting lipids against peroxidation and mutagenic oxidized nitrogen species, and also allows the stabilization of membrane structures (Takeda et al., 1996). This fact add another interesting characteristic to oil T quality.

3.2.4 Polyphenols Content

The polyphenols content was also determined in the oils extracted. Indeed the total content of polyphenols in each oil was determined using the linear regression equation of the calibration curve plotted for Gallic acid (Figure 4). Oil T had the highest polyphenols content with a value of 94.07 ± 1.16 mg EAG / g oil compared to that of oil D which was 62.3 ± 1.02 mg EAG / g oil (Table 4) ($P <0.05$). As it is known, polyphenols are natural antioxidants which in addition to having a definite interest in the conservation of edible foodstuffs, could be useful

in the prophylaxis and the treatment of diseases in which oxidative stress is implicated (Kouamé et al., 2009).

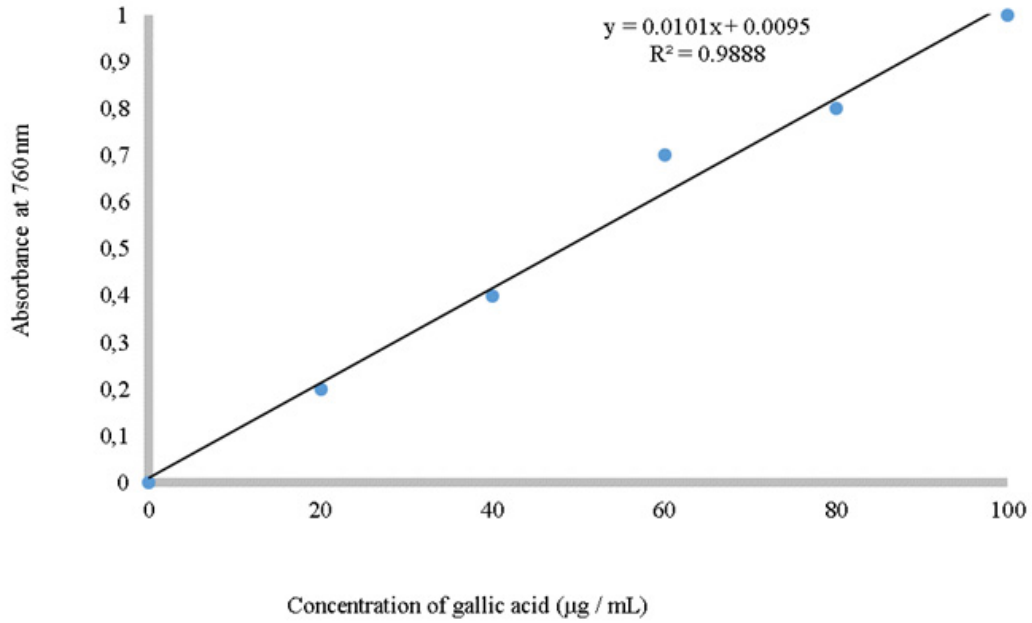

Figure 4. alibration curve of Gallic acid

3.3 Antimicrobial Activities

The results obtained show that among the five Gram negative strains tested, *Salmonella typhi* was the most sensitive (Table 6). Indeed, for this bacterial strain, the absence of growth indicating the minimum concentration inhibitor (MIC) was at 20% of oil in the culture medium for both oils analyzed. For *Escherichia coli* (ATCC25922), this MIC was observed at 20% of oil T in the medium, while it was observed at 25% of oil D in the medium. At this concentration of 25% of oil in the culture medium, the total inhibition of *Pseudomonas aeruginosa* (ATCC27853) was noted with both oils. This bacterial strain is a bacteria with intrinsic resistance to bactericidal agents, in relation to the nature of its outer membrane. It is found to be resistant to a very large number of oils, particularly essential oils (Alloun, 013). Thus, the total inhibition of *Pseudomonas aeruginosa* by these palm kernel oils is a great finding for consumers.

However, the strains of *Enterobacter cloaceae* (ATCC23355) and *Klebsiella oxytoca* (ATCC31899) were resistant to both categories of oil. For the Gram positive strain tested namely, *Staphylococcus aureus* (ATCC25923), the MIC was observed at 25% of oil in the culture medium with oil D, while no inhibitory effect was observed with oil T.

Table 6. ffect of palm kernel oils on growth of the bacterial strains

	Oil D						Oil T						Tween 80 content in the culture medium
	Oil content in the culture medium												
Bacterial strains in the culture medium	0%	10%	15%	20%	25%	30%	0%	10%	15%	20%	25%	30%	10%
Escherichia coli (ATCC25922)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)
Pseudomonas aeruginosa (ATCC27853)	(+)	(+)	(+)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(-)	(-)	(+)
Enterobacter cloaceae (ATCC23355)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Klebsiella oxytoca (ATCC31899)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Salmonella thyphi	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(+)
Staphylococcus aureus (ATCC25923)	(+)	(+)	(+)	(-)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

Growth: (+)

No growth: (-)

For the antifungal activities, the results obtained with oil D show that, with increases in this oil content in the medium, there was less fungal growth (Figures 5). Indeed, a dose-dependent inhibition of the growth with the increasing of the oil D content in the medium was observed for all the strains of *Aspergillus* tested (Table 7) ($P<0.05$). The total inhibition of growth indicating the MIC was observed at 25% of oil D in the culture medium for *A. niger*, while for *A. nidulans*, and *A. carbonarius*, it was noted at 30% of oil D in the medium. However, with this oil D, the MIC was not determined for *A. fumigatus* and *A. flavus* after 7 days of

incubation although a significant reduction of their growth was observed ($P<0.05$). The results obtained with oil T show that *A. niger* and *A. carbonarius* were the most sensitive to this oil with total inhibition of their growth observed on the culture medium at 30% of oil (Figure 5). The MIC is therefore at this concentration for these strains from the Nigri section. Furthermore, a dose-dependent effect of this oil on the growth of the strains tested was observed except the strain of *A. fumigatus* which was completely resistant to this oil (Table 7). These results suggest that the two categories of oil (oil D and oil T) tested had an inhibitory effect on the bacterial and fungal growth. However, oil D was more active on the bacterial and fungal strains tested. This could be explained by its chemical composition as it had been shown for other types of vegetable oil in previous study (Alloun, 2013). Indeed, oil D contained chemical compounds such as lauric acid, undecylenic acid and trans-9 octadecenoic acid which are known to possess antimicrobial activities (Dongmei et al., 2016; Anonyme, 2012). Among these three fatty acids, the undecylenic acid known to possess a powerful antifungal activity (Dongmei et al., 2016) was not found in oil T. Thus, it is believed that there could be a synergic effect of these three fatty acids. Moreover, as the lauric acid and trans-9 octadecenoic, were also found in oil T, this study has confirmed the antimicrobial activities of these two fatty acids mainly for the lauric acid which has the greatest antimicrobial activity of all medium chain aliphatic fatty acids (Ruzin and Novick, 2000). This shows a great characteristic for oils analyzed. This lauric acid was the predominant fatty acid found in both oils analyzed. Similar results were obtained by Orsavova et al., (2015) with coconut oil. This lauric acid has another great activity. Indeed, it could decrease the ratio of total HDL cholesterol (Orsavova et al., 2015). The undecylenic acid also has another great activity. Indeed, it is a precursor in the manufacture of many pharmaceutical products, personal hygiene products, cosmetics and perfumes (Kohlpaintner et al. 2008). Thus, this oil D could be recommended for the diet of people mainly people living with HIV AIDS as shown in previous studies (Anonyme, 2010; Anonyme, 2012). However, further studies must be carried out in order to confirm this finding.

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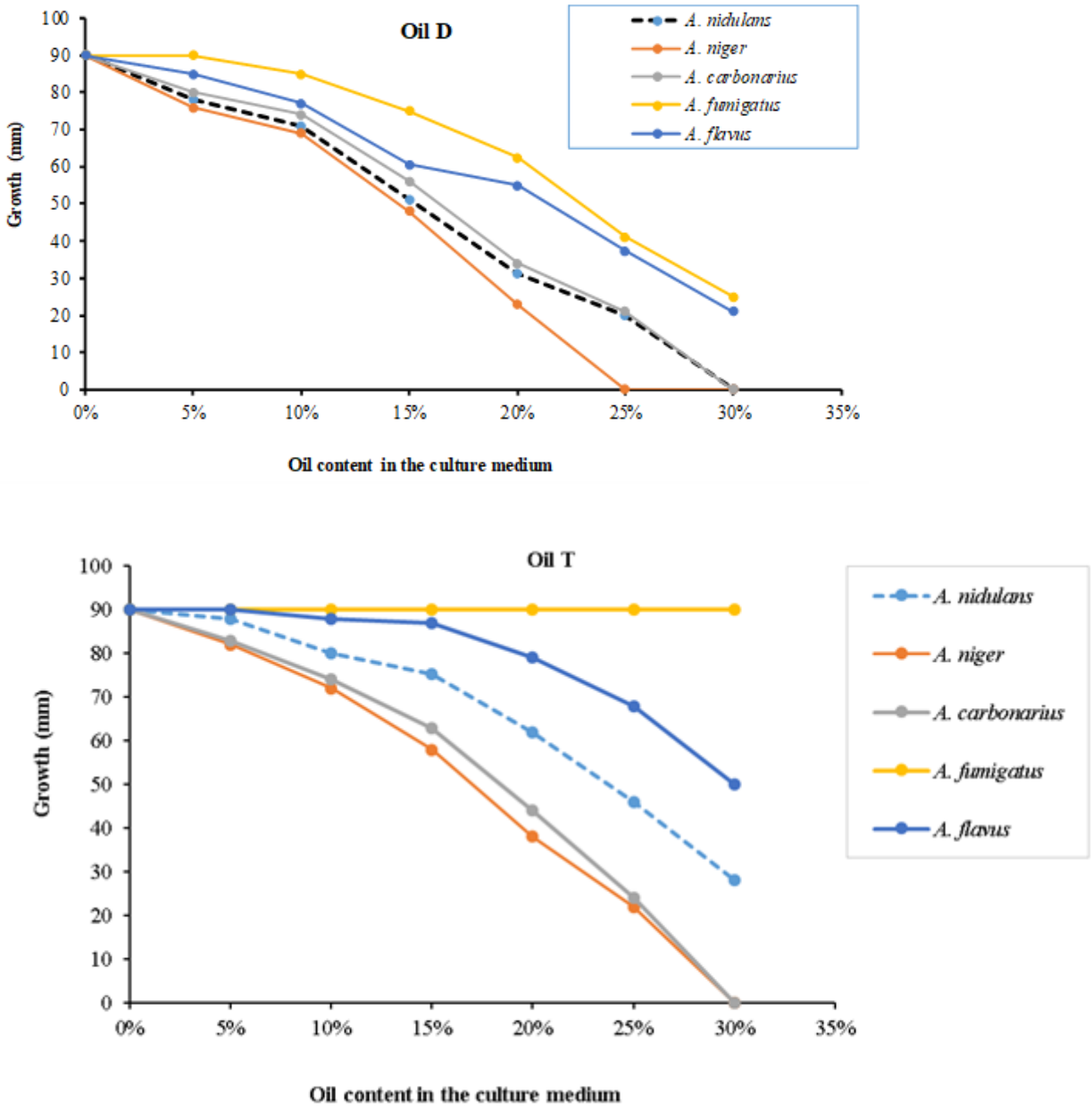

Figure 5. Effect of palm kernel oils on growth of fungal strains

Table 7. Dose dependent effect of oil D and oil T on growth of the fungal strains

	Homogeneus subsets Tukey HSD	Fungal Growth under influence of oil D (mm)								Fungal Growth under influence of oil T (mm)						
	Palm kernel oil content in the medium	alpha = 0.05								alpha = 0.05						
		N	1	2	3	4	5	6	7	1	2	3	4	5	6	7
	Medium without oil	3	90							90						
	Medium at 5%	3		78 ± 0.146						88 ± 0.12						
	Medium at 10%	3			0.17						80 ± 0.19					
L. nidulan	Medium at 15%	3				0.4						75.33 ± 0.23				
	Medium at 20%	3					31.33 ± 0.4						67 ± 0.22			
	Medium at 25%	3						20 ± 0.25						46 ± 0.17		
	Medium at 30%	3							0.00						28 ± 0.22	
	Significance		1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.74	1.000	1.000	1.000	1.000	1.000	
	Medium without oil	3	90							90						
	Medium at 5%	3		76 ± 0.72							82 ± 0.23					
	Medium at 10%	3			69 ± 0.14							72 ± 0.14				
A. niger	Medium at 15%	3				48 ± 0.17							58 ± 0.11			
	Medium at 20%	3					23 ± 0.2							38 ± 0.2		
	Medium at 25%	3						0.00							22 ± 0.32	
	Medium at 30%	3						0.00								0.00
	Significance		1.000	1.000	1.000	1.000	1.000	0.072		1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Medium without oil	3	90							90						
	Medium at 5%	3		80 ± 0.09							83 ± 0.478					
	Medium at 10%	3			74 ± 0.12							74 ± 0.87				
A. carbon	Medium at 15%	3				56 ± 0.26							63 ± 0.63			
	Medium at 20%	3					34 ± 0.44							44 ± 0.21		
	Medium at 25%	3						21 ± 0.21							24 ± 0.23	
	Medium at 30%	3							0.00							0.00
	Significance		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Medium without oil	3	90							90						
	Medium at 5%	3	90							90						
	Medium at 10%	3		85 ± 0.94						90						
	Medium at 15%	3			75 ± 0.7					90						
A. flavigus	Medium at 20%	3				62.5 ± 0.78				90						
	Medium at 25%	3					41 ± 0.78			90						
	Medium at 30%	3						25 ± 0.35		90						
	Significance		0.968	1.000	1.000	1.000	1.000	1.000		0.81						
	Medium without oil	3	90							90						
	Medium at 5%	3		85 ± 0.246						90						
	Medium at 10%	3			77 ± 0.22					88 ± 0.22						
A. flavus	Medium at 15%	3				60.5 ± 0.2				87 ± 0.14						
	Medium at 20%	3					55 ± 0.1463				79 ± 0.19					
	Medium at 25%	3						37.33 ± 0.12				68 ± 0.18				
	Medium at 30%	3							21 ± 0.49				50 ± 0.22			
	Significance		1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.8	1.000	1.000	1.000			

Means of groups in homogeneous subsets are displayed.

Uses Harmonic Mean Sample size = 3.000

3.3 Antioxidant Activities

The DPPH radical scavenging profiles reveal that oil D and oil T exhibited a dose-dependent radical scavenging activity (Table 8). Indeed, this DPPH radical scavenging increases with the increasing of oils concentration. The concentration inhibiting 50% of the reaction (IC50%) was 750 mg / L for oil T. However, for oil D, this IC50% could not be determined (Table 8). The DPPH radical scavenging activities of these oils were lower than that of the standard

(ascorbic acid) (Table 8). It was also noted that the capacity of reducing the Fe3+ increased with the increasing of the concentration of oil (Table 8). Moreover, oil T exhibited the most activity of reducing Fe^{3+} compared to oil D. The capacity of reducing the Fe3+ of the two types of oil were moreover lower than that of ascorbic acid (Table 8).

These results show that oils analyzed possess antioxidant activity. This is a great characteristic for these oils as it has been shown in previous work for other vegetable oils (Djeddi et al., 2015). However, the greatest antioxidant activity was observed with oil T. This fact could be due to its high α-tocopherol and polyphenols contents observed. Indeed, the α-tocopherol possesses ability to act as an antioxidant, thus protecting lipids against peroxidation and mutagenized oxidized nitrogen species, and also allows the stabilization of membrane structures (Takeda et al., 1996). The polyphenols also are natural antioxidants which in addition to having a definite interest in the conservation of edible foodstuffs could be useful in the prophylaxis and the treatment of diseases in which oxidative stress is implicated (Kouame et al., 2009). The synergetic antioxidant activity of polyphenols and α-tocopherol has been demonstrated in previous work (Oussou et al., 2010). This fact makes oil T extracted from variety Tenera more interesting for human diet.

Table 8. Antioxidant activities and IC_{50} values of palm kernel oils and ascorbic acid

Samples	Samples' concentrations (mg/L)	DPPH radical scavenging activity (%)	Fe^{3+} reduction activity (Absorbance)	DPPH Scavenging Activity (IC_{50})
oil D	50 mg/L	0.4 ± 0.01	0.1 ± 0.001	nd
	100 mg/L	1.08 ± 0.01	0.15 ± 0.001	
	250 mg/L	5.78 ± 0.2	0.2 ± 0.004	
	500 mg/L	17.25 ± 1.02	0.32 ± 0.001	
	750 mg/L	28.5 ± 1.01	0.46 ± 0.002	
	1000 mg/L	36.19 ± 0.95	0.5 ± 0.001	
Oil T	50 mg/L	1.8 ± 0.02	0.7 ± 0.001	750 mg/L
	100 mg/L	4.43 ± 0.08	0.23 ± 0.003	
	250 mg/L	21.16 ± 0.08	0.4 ± 0.001	
	500 mg/L	38.46 ± 0.2	0.48 ± 0.001	
	750 mg/L	50.2 ± 0.12	0.64 ± 0.004	
	1000 mg/L	64.13 ± 0.11	0.79 ± 0.007	
Ascorbic acid	0.5 mg/mL	25 ± 0.02	0.1 ± 0.001	1.5 mg/mL
	1 mg/mL	38.7 ± 0.02	0.2 ± 0.001	
	1.5 mg/mL	50.1 ± 0.02	0.38 ± 0.006	

2 mg/mL	76.5 ± 0.7	0.618 ± 0.002
2.5 mg/mL	88.3 ± 0.8	0.759 ± 0.007
3 mg/mL	98 ± 0.6	0.94 ± 0.004

nd: not determined

4. Conclusions

This study investigated the antimicrobial and antioxidant activities and analysed chemical composition of palm kernel oils extracted from varieties Dura (oil D) and Tenera (oil T) of oil palm in order to establish a link between these biological activities and the chemical compositions identified. Oil D had the greatest antimicrobial activities. The GC-MS analysis showed that this oil D possess more fatty acids known to possess antimicrobial activities. These fatty acids were lauric acid, trans-9-octadecenoic and undecylenic acid. This undecylenic acid was not identified in oil T. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and the test of FRAP (capacity of reducing the Fe^{3+}) showed that oil T had the greatest antioxidant activity.

The chemical analysis showed that this oil T had also the highest contents of total polyphenols, α-tocopherol and sterols with predominance of β-sitosterol. The polyphenols and α-tocopherol are known to possess antioxidant activities.

Thus, in this study, it has been noted a relationship between the biological activities of palm kernel oils and their chemical components. These findings provide useful information for consumers of these oils.

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Competing interest

The authors do not declare any conflict of interest.

References

AFNOR NF T60-205 (1984). (Association Française pour la Normalisation). Recueil des normes françaises: corps gras, graines oléagineuses et produits dérivés (3e édition). AFNOR: Paris.

Agboola, J. B., Abubakre, O. K., Mudiare, E. Adeyemi, M. B., & Hassan, S. B. (2015). Physicochemical characteristics and fatty acids composition of some selected Nigerian vegetable oils for quenching medium. *Br. J. Appl. Sci. Technol, 8*(3), 246-253. https://doi.org/10.9734/BJAST/2015/16177

Albano, S. M., & Miguel, M. G. (2011). Biological activities of extracts of plants grown in Portugal. *Ind. Crops Prod., 33*, 338-343. https://doi.org/10.1016/j.indcrop.2010.11.012

Alloun, K. (2013). Composition chimique et activité antioxydante et antimicrobienne des huiles essentielles l'anth (*Anethum graveolens* L.), de la sauge (*Salvia officinalis* L.). Ecole

Nationnale Supérieure agronomique EL-Harrach –Alger-, 119.

Anonyme (2010). La biotechnologie au Canada. www.strategie.org.

Anonyme (2012). Palm Kernel oil Hudson and knight. Co.za. Hudson and Knight. Retrived 12 September.

Aubret, J. M., & Huard M. (2003). Qualité des huiles et acides gras de palme et des mélanges d'huiles acides, caractérisations chimique et biochimique. Cinquièmes Journées de la Recherche Avicole; Travail conduit dans le cadre du GIE EURO NUTRITION (France).

Bouras, M. Chadni, M. Barba, F. J. Grimi, N. Bals, O. Vorobiev, E. (2015). Optimization of microwave-assisted extraction of polyphenols from Quercus bark. *Ind. Crops Prod., 77*, 590-601. https://doi.org/10.1016/j.indcrop.2015.09.018

CLSI (Clinical and laboratory standards institute) (1999). "Methods for determining bactericidal activity of antimicrobial agent: approved guideline", Document M26-A, Wayne, PA: CLSI.

Dilini, B., & Terrence, M. (2013). Antioxidant activity and total phenolic content of sesame (*Sesamum indicum* L.) seed oil. *Trop. Agric. Res, 24*, 296-302. https://doi.org/10.4038/tar.v24i3.8015

Djeddi, S., Yannakopoulou, E., Papadopoulos, K., Skaltsa, H. (2015). Activités anti-radicalaires de l'huile essentielle et des extraits bruts de *Thymus numidicus* Poiret. Algérie. *Afrique Science, 11*(2), 58- 65.

Dongmei, S., Yaxin, Z., Hongxia, Y., Hongjun, F., Yongnian, S. Guixia, L., … Weida, L. (2016). Antifungal effects of undecylenic acid on the biofilm formation of *Candida albicans*. *Int. J. Clinical Pharmacol. Therapeutic*s, 54(05), 343-353. https://doi.org/10.5414/CP202460

Grandgirard, A., & Gordelet, C. (1998). Comparaison de la chromatographie en phase liquide à haute performance et de la chromatographie en phase gazeuse pour l'analyse des oxystérols. *Annalusis Magazine*, 26(3), 55-60.

ISO 14565: (2000). Aliments des animaux—Détermination de la teneur en vitamine A et E.

Islam, K., Rowsni, A. A., Khan, M. Kabir, S. (2014). Antimicrobial activity of ginger (Zingiber officinale) extracts against food-borne pathogenic bacteria. *Int. J. Sci. Environ. Technol*. 3:867–871.

Kohlpaintner, C., Schulte, M., Falbe, J., Lappe, P., & Weber, J. (2008). Aldehydes, Aliphatic. *ULLMANN'S Ullmann's Encycl. Industr. Chem*. https://doi.org/10.1002/14356007.a01_321.pub2

Kloos, D., Gay, E., Lingeman, H., Bracher, F., Müller, C. Mayboroda, O. A., …, Giera, M. (2014). Comprehensive GC–MS analysis of fatty acids and sterols using sequential one-pot silylation: quantification and isotopologue. *Rapid Commun Mass Spectrom, 28*(13), 1507-14. https://doi.org/10.1002/rcm.6923

Kouamé, J., Gnoula, C., Palé, E. Bassolé, H. Guissou, I. P., Simporé, J., & Nikiéma, J. B. (2009). Etude des propriétés cytotoxiques et anti-radicalaires d'extraits de feuilles et de galles de *Guiera senegalensis* JF Gmel (Combretacae). *Science et technique, Sciences de la santé*, 32.

Lecerf, J. M. (2011). Les huiles végétales: Particularités et utilités/ Médecine des maladies

métaboliques, 5(3), 257-262. https://doi.org/10.1016/S1957-2557(11)70237-1

Mohd-Setapar, S. H., Lee, N. Y., & Mohd-Sharif, N. S. (2014). Extraction of rubber (*Hevea brasiliensis*) seed oil using soxhlet method. *Malaysian J. Fundamental Appl. Sci, 10*(1), 1-6. https://doi.org/10.11113/mjfas.v10n1.61

Moon D. O., Lee K. J., Choi, Y. H., & Kim, G. Y. (2007). #-Sitosterol-induced-apoptosis is mediated by the activation of ERK and the downregulation of Akt in MCA-102 murine fibrosarcoma cells. *Int. J. Immunopharmacol*, *7*(8), 1044-1053. https://doi.org/10.1016/j.intimp.2007.03.010

Pitt, J. I. (1988). A laboratory guide to commun Penicillium species, *Tingalpa. Food Sci. Australia*.

Orsavova, J., Misurcova, L., Ambrozova, J. V., Vicha, R., & Mlcek, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *Int. J. Mol. Sci.,* 16, 12871-12890. https://doi.org/10.3390/ijms21082694.

Oussou, K. R., Youlou, S., Kanko, C., Tue Bi, B., Kanko, C., Boti, J. B., … Casanova, J. (2010). Etude Chimique Bio-Guidée de L'huile Essentielle de *Ocimum gratissimum* (Lamiaceae). *Eur. J. Scientific. Res,* 1, 50-59.

Parejo, I., Codina, C., Petrakis, C., Kefalas, P. (2000). Evaluation of scavenging activity assessed by col (II) EDTA-induced luminal chemilunes-cence and DPPH (2, 2-diphényl-1 pycrylhydrazyl) free radical assay. *J. pharmacol. Toxicol. Methods, 44*, 507-512. https://doi.org/10.1016/S1056-8719(01)00110-1

Pegel, K. H. (1997). The importance of sitosterol and sitosterolin in human and animal nutrition. *South Africa J. Sci,.* 93, 263-268.

Pickard, M. D. (2005). By-products utilization. In: Bailey's industrial oil products. Edible Oil and Fat Products: Products and Applications. Shahidi, F. (Ed). *Wiley- Interscience*. 6th Edition. https://doi.org/10.1002/047167849X.bio081

Ravber, M., Knez, Ž., & Škerget, M. (2015). Simultaneous extraction of oil- and watersoluble phase from sunflower seeds with subcritical water. *Food Chem, 166*, 316-323. https://doi.org/10.1016/j.foodchem.2014.06.025

Ruzin, A., & Novick, R. P. (2000). Equivalence of lauric acid and glycerol monolaurate as inhibitors of signal transduction in *Staphylococcus aureus*. *J. Bacteriol*, *182*(9), 2668-2671. https://doi.org/10.1128/JB.182.9.2668-2671.2000

Sofiane, L. (2009). Extraction et caractérisation physicochimique de l'huile de graines de *Moringa oleifera*. Mémoire en vue de l'obtention du diplôme de Magister en Sciences Agronomiques. Ecole Nationale Supérieure Agronomique El-Harrach Département, Technologie Alimentaire.

Takeda, H., Shibuya, T., Yanagawa, K., Kanoh, H., & Takasaki, M. (1996). Simultaneous determination of #-tocopherol and #-tocopherolquinone by High-performance Liquid Chromatography and coulometric detection in the redox mode. *J. Chromatogr. A*., 722(1-2), 287-294. https://doi.org/10.1016/0021-9673(95)00532-3

Tchiégang, C., Ngo, O. M., Dandjouma, A. A., & Kapseu, C. (2004). Qualité et stabilité de

l'huile Ricinodendron heudelotii (Bail.) Pierre ex Pax pendant la conservation à température ambiante. *J. Food Eng, 62*, 69-77. https://doi.org/10.1016/S0260-8774(03)00172-9

Yapi, A. P., & Kouadio, A. I. (2019). Physico-chemical characterization of palm kernel oil extracted from the seeds of two varieties of oil palm (*Elaeis guineensis* Jacq.) for possible use in feed or food. Eur. *J. Nutr. Food Safety, 9*(4), 341-353. https://doi.org/10.9734/ejnfs/2019/v9i430081

Yildirim, A., Mavi, A., & Kara, A. A. (2001). Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *J. Agri. Food Chem, 49*, 4083-4089. https://doi.org/10.1021/jf0103572

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