

ASSESSMENT OF THE PHYTOCHEMICAL CONSTITUENTS AND
ANTIOXIDANT PROPERTIES OF OILS FROM DURA AND TENERA
VARIETIES OF *Elaeis guineensis* FRUITS

ABSTRACT

There have been acclaimed medicinal benefits of palm oil and palm kernel oil by rural dwellers in the southeastern part of Nigeria. The study investigated the phytochemical constituents and antioxidant properties of the different varieties of the oil using standard methods. The oil samples include: **DPO** (*dura* palm oil), **TPO** (*tenera* palm oil), **NDPKO** (*dura* native palm kernel oil), **NTPKO** (native *tenera* palm kernel oil), **HDPKO** (*dura* palm kernel oil of hexane extract) and **HTPKO** (*tenera* palm kernel oil of hexane extract). Results obtained revealed the presence of steroids, glycosides, terpenoids, triterpenes and phenolics in all the oil samples whereas saponins, flavonoids and coumarins were only detected in DPO, TPO and NDPKO. The result of the quantitative phytochemical analysis of the oil samples showed that NDPKO statistically had the highest contents of terpenoids (1.77 ± 0.14 mg/kg) and triterpenes (288.50 ± 23.30 mg/100g). Saponins, flavonoids and coumarins were highest in DPO with values corresponding to 1.00 ± 0.001 mg/kg, 308.30 ± 1.81 mg/100g and 43.98 ± 0.13 µg/100g respectively. Furthermore, NTPKO was found to have the highest content of steroids (150.40 ± 0.24 mg/100g) while TPO had the highest content of glycosides (9.01 ± 0.07 mg/100g) and phenolics (45.04 ± 0.12 mg/100g) at $p < 0.05$. TPO had the highest content of β -carotene (2.06 ± 0.00 mg/100ml) and vitamin A (105.60 ± 0.15 IU/100ml) but had the lowest vitamin E content (3.73 ± 0.30 µg/ml) whereas HTPKO had the highest ($p < 0.05$) content of vitamin E (4.67 ± 0.03 µg/ml) among the oil samples. NTPKO had the highest FRAP (780.00 ± 1.82 µg/100g) while DPO had the least ($p < 0.05$) FRAP (601.30 ± 0.91 µg/100g). The oil samples exhibited a concentration-dependent increase ($p < 0.05$) in percentage inhibition of DPPH free radical showing an antioxidant capacity of about 70-80%. The results obtained from the physicochemical and antioxidant properties of the oil samples showed significant positive effects thus justifying their ethnomedical use.

Keywords: antioxidant, dura, tenera, palm oil, palm kernel oil, phytochemicals

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1.0 INTRODUCTION

Elaeis guineensis (oil palm) also known as has been described as one of the best trees given by God to man in the tropical region for survival and solving related needs and as such described as the 'tree of life' (Okolo *et al.*, 2019). The oil palm belongs to the palm family araceae and is of three varieties, namely: dura, pisifera and tenera. It produces two types of vegetable oil which are palm oil derived from the fruit mesocarp and palm kernel oil from the seed (Ekwenye and Ijeomah, 2005) with successful production of the two edible oils in dura and tenera varieties since pisifera has a shellless nature.

The numerous importance of oil palm cannot be complete without its roles in terms of food and nutritional values, traditional medicine and human health of the people as most rural dwellers in Nigeria use the oils in the management and treatment of several diseases such as fever and headaches (Ekwenye and Ijeomah, 2005), ulcers and wounds (Okwu, 2004), intestinal disorder, skin diseases and convulsion in children (Okoye, 2018).

Palm oil and palm kernel oil though regarded as saturated fats are rich sources of beneficial nutrients such as carotenoids, vitamins A and E, phenols, flavonoids which are known to possess loads of health benefits such as anti-inflammatory, antioxidant and anti-carcinogenic properties. (Sommerburg *et al.*, 2015).

Phytochemicals are plant components known to have protective and disease preventive attributes considered to be beneficial to human health. They include: flavonoids, saponins, alkaloids, tannins, phenolics, etc. (Ajuru, 2017). For instance, alkaloids is said to have a wide range of pharmacological properties including antimalarial, antiasthma, anticancer properties and production of steroidal hormones (Kittakoop *et al.* 2014). Flavonoids have been reported to

possess antioxidant, anti-inflammatory, anti allergic, anti carcinogenic, anti microbial, hepatoprotective and anti viral properties. (Okwu and Emenike, 2006). Plants that contain phenol could be used as anti inflammatory, immune enhancers and hormone modulators (Okwu and Omodamino, 2005). Also, saponins are known to be active as expectorant and are very useful in the treatment of upper respiratory tract inflammations; they also have anti-diabetic, anti-fungal properties and anti carcinogenic properties (Jimoh and Oladji, 2005). Terpenoids have been found to be helpful in the prevention and therapeutic treatment of several cancer diseases, including mammary, skin, lung, fore-stomach, colon, pancreatic and prostate carcinomas (Malik and Khan, 2017).

Since palm oil and palm kernel possess important constituents, it is therefore imperative to assess their phytochemical and antioxidant properties owing to their already attributed medicinal benefits.

2.0 MATERIALS AND METHODS

2.1 Collection and authentication of experimental samples

The different bunches of palm fruits were sourced from a land located at The Glory of God Nursery and Primary School, Aladimma, Owerri in Imo State. The palm fruits were of the dura and tenera varieties. Authentication of the samples was done by Prof. F.N. Mbagwu, a taxonomist at the Department of Plant Science and Biotechnology of Imo State University, Owerri. The apparatus and reagents used were of analytical grade.

Methods of extraction

The extraction method for palm oil was as described by Owolarafe *et al.* (2002). The palm fruits were separated from their bunches, washed, sterilized and pulp softened by boiling in a large pot for about one hour and then crushed in a mortar to separate the pulp from the nuts. The oils were separated from the pulp by immersing the latter in water. The whole mass was then stirred and the crude palm oil skimmed off. The fibers then were sifted out of water and finally the nuts were collected and separated from the remaining fibers. The oil obtained was boiled in smaller vessels where any fiber still present sank to the bottom. The oil was decanted into a sterilized reagent bottle and used for the stipulated tests without further purifications.

The native palm kernel oil thermal extraction as described by Gbasauzor *et al.* (2012) was employed; the palm nuts obtained as a by-product in palm oil production were cracked to obtain the kernels. These were washed, dried and heated in a pot until the oil appeared and extracted from the kernels. It was then decanted from the pot into a sterilized reagent bottle and used for the stipulated tests without further purifications as in palm oil extraction.

Another extraction of palm kernel oil was done with a soxhlet extractor according to the method described by Ogburubi *et al.* (2009) using n-hexane (boiling point of 40°C- 60°C) for about six hours. The oil was obtained after the solvent was removed under reduced temperature and pressure and refluxing at 70°C so as to eliminate the solvent remaining in the oil after extraction. The extracted palm kernel oil was kept for subsequent characterization.

Qualitative analysis of oil samples

Qualitative phytochemical analyses of the oil samples were carried out using the method described by Odebiyi and Sofowora (1978).

- a) **Alkaloids:** one centimeter cubed of 1% HCl was added to 3cm³ of the extract in a test tube. The mixture was heated for 20 minutes, cooled and filtered. Then, two drops of Wagner's reagent was added to 1cm³ of the filtrate. A reddish brown precipitate indicated the presence of alkaloids.
- b) **Tannins:** one centimeter cubed of freshly prepared 10% KOH was added to 1cm³ of the extracts. A dirty white precipitate indicates the presence of tannins.
- c) **Phenolics:** two drops of 5% FeCl₃ was added to 1cm³ of the extract in a test tube. A greenish precipitate indicated the presence of phenolics.
- d) **Glycosides:** ten centimeter cubed of 50% H₂SO₄ was added to 1cm³ of the extract, the mixture was heated in boiling water for 15 minutes. Then 10cm³ of Fehling's solution was added and the mixture boiled. A brick red precipitate indicated the presence of glycosides.
- e) **Saponins:** two centimeter cubed of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicates the presence of saponins.
- f) **Flavonoids:** one centimeter cubed of 10% NaOH was added to 3cm³ of the extracts. A yellow colouration indicated the presence of flavonoids.
- g) **Steroids:** five drops of concentrated H₂SO₄ was added slowly down the side of a tube containing 1cm³ of the extracts (salkowski test). Red colouration indicated the presence of steroids.

- h) **Phlobatannins:** to 1cm³ of the extracts, 10cm³ of 1% HCl was added. A red precipitate indicated the presence of phlobatannins.
- i) **Triterpenes:** five drops of acetic anhydride was added to 1cm³ of the extract. A drop of concentrated H₂SO₄ was then added and the mixture steamed for 1 hour and neutralized with 10% NaOH followed by the addition of 2ml of chloroform. A blue green colour indicated the presence of triterpenes.
- j) **Coumarins:** the extract was treated with a solution of dimethylamino-benzaldehyde (5% ethanol) and then acidified by bubbling gaseous hydrochloric acid. The orange color indicated a positive test.
- k) **Terpenoids:** five ml of aqueous extract of the sample was mixed with 2ml of CHCl₃ in a test tube and 3 ml of conc. H₂SO₄ was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed if terpenoids constituent was present.
- l) **Anthocyanins:** the presence of anthocyanins was demonstrated by adding 2 ml of the extract to 2 ml of 2 N HCl. The appearance of a pink-red color that turned purplish blue after addition of ammonia indicated the presence of anthocyanins.

QUANTITATIVE PHYTOCHEMICAL SCREENING

(a) Phenolic Content

The phenolic content of sample was measured according to the method of Makkar *et al.* (1997). The aliquots of the extracts were placed in test tubes and made up to the volume of 1 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tubes. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbances were

recorded at 725 nm against the reagent blank. Using Gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 µg/ml. using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/100g of extract.

(b) Flavonoid Content

Flavonoid content was estimated using aluminium chloride colorimetric assay (Pontis *et al.*, 2014). 1ml of extract and standard solution of Quercetin (500 µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% NaNO₂ was added. After 5 minutes, 0.3 ml of 10% AlCl₃ was added. At 6th min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of the flower was expressed as percentage of Quercetin equivalent per 100 g of fresh mass.

(c) Saponins

The spectrophotometric method of Brunner (1984) was used for the analysis of saponins. Briefly, 1g of the sample was weighed into a 250ml beaker and 100 ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100 ml beaker containing 20ml of 40% saturated solution of MgCO₃. The resulting mixture was again filtered to obtain a clear colourless solution. 1 ml of the colourless filtrate was pipette into a 50 ml volumetric flask and 2 ml of 5% FeCl₃ solution was added and made up to the marked level with distilled water. This was then allowed to stand for 30 minutes for a blood red colour to develop. A range of 0-10ppm saponins standard was prepared from saponins stock solution. The standard

solutions were treated similarly with 2 ml of 5% FeCl₃ solution as earlier described. The absorbance of the samples as well as standard saponin solutions was read after colour development using a Jenway V6300 spectrophotometer at wavelength of 380nm. Percentage saponin was calculated using the formula:

$$\% \text{ saponin} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10,000}$$

(d) Glycoside Content

Glycoside content was estimated according to the method described by Ameen *et al.* (2021). Ten milliliters of the sample was dispensed into a 250 ml conical flask. 50 ml Chloroform was added and shaken on a Vortex Mixer for 1 hour. The mixture was filtered into a conical flask. 10ml pyridine and 2ml of 2% sodium nitroprusside were added and shaken thoroughly for 10 minutes. 3ml of 20% NaOH was later added to develop a brownish yellow colour.

Glycoside standard of concentration ranging from 0-5 mg/ml were prepared from 100mg/ml stock glycoside standard. The series of standards 0-5 mg/ml were treated similarly like the sample above.

The absorbances of sample as well as standards were read on a spectronic 21D Digital spectrophotometer at a wavelength of 510nm. Percentage Glycoside was calculated using the formula:

$$\% \text{ Glycoside} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10,000}$$

(e) Steroid Content

The steroid content was estimated using the procedures described by Ameen *et al.* (2021). A portion (0.05g) of the sample extract was weighed into a 100ml beaker. 20ml of chloroform-methanol (2:1) mixture was added to dissolve the extract upon shaking for 30 minutes on a shaker and filtered. 1ml of the filtrate was transferred into a 30 ml test tube and 5 ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37°C-40°C for 90 minutes. It was cooled to room temperature and 10 ml of petroleum ether added followed by the addition of 5 ml distilled water. This was evaporated to dryness on the water bath. 6 ml of Liebermann Buchard reagent was added to the residue in dry bottle and absorbance taken at a wavelength of 620 nm on a spectronic 21D digital spectrophotometer. Standard steroids of concentration of 0-4 mg/ml were prepared from 100 mg/ml stock steroid solution and treated similarly like the sample as above.

% steroid was calculated using the formula:

$$\% \text{ steroid} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10,000}$$

(f) Triterpene Content

Estimation of the triterpene content was as described by Babatunde *et al.* (2019). A portion (0.50 g) of the sample was weighed into a 50 ml conical flask and 20ml of 2:1 chloroform-methanol mixture was added, shaken thoroughly and allowed to stand for 15 minutes. The supernatant obtained was discarded, and the precipitate was re-washed with another 20ml chloroform-methanol mixture for re-centrifugation. The resultant precipitate was dissolved in 40ml of 10% Sodium Dodecyl Sulphate (SDS) solution. 1ml of 0.01M ferric chloride solution was added to the above at 30 seconds intervals; shaken well, and allowed to stand for 30 minutes. Standard

triterpenes of concentration range 0-5mg/ml were prepared from 100mg/l stock triterpenes solution from sigma-Aldrich chemicals, U.S.A. The absorbances of sample as well as that of standard concentrations of triterpenes were read on a digital spectrophotometer at a wavelength of 510nm.

The percentage of triterpenes was calculated using the formula:

$$= \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10,000}$$

(g) Terpenoid Content

The terpenoid content was estimated using the method described by Malik *et al.* (2017). The extract (1g) was weighed into 50 ml of ethanol and filtered. To the filtrate (2.5 ml), 2.5 ml of 5% aqueous phosphomolybdic acid solution was added and 2.5 ml of concentrated H₂SO₄ was gradually added and mixed. The mixture was left to stand for 30 min and then made up to 12.5 ml with ethanol. The absorbance was taken at 700 nm and the percentage of the terpenoids was calculated using the formula:

$$\frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10,000}$$

(h) Coumarin content

The coumarin content was estimated using the method as described by Ameen *et al.* (2021). A portion (0.5 ml) of 5N NaOH was added to the solution of 1 ml of the extract (0.5g in 1 ml of methanol). The mixture was heated at 80°C for 5 minutes. After cooling, 0.75 ml of 5N H₂SO₄ was added and mixed thoroughly, then, 0.25g of anhydrous NaHCO₃ was also added and transferred to the extractor and made up to 50 ml with petroleum ether for 3 hours. About 20 ml

of water was added to the petroleum ether extract and the petroleum ether carefully made to evaporate while in a water bath at 50°C-55°C. The aqueous solution was transferred to a volumetric flask and made up to 50 ml with continuous mixing. 25 ml of aqueous solution was pipette into a flask and 1% Na₂CO₃ solution was added and heated in a water bath at 85⁰C for 15 minutes. On cooling, 5 ml of the diazonium solution was added and allowed to stand for 2 hours. The absorbance at 540nm against reagent blank was used.

$$\% \text{ Coumarin content} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10,000}$$

Determination of concentration of β-carotene

Determination of the concentration of β-carotene by spectrophotometer as described by Dauquan *et al.* (2011) was used. β -carotene content in the samples were analysed by Ultraviolet-Visible (UV-vis) spectrophotometer at 446 nm using MPOB test method. The sample was homogenized and weighed to the nearest ±0.0001 g into a 25 mL volumetric flask. The sample was dissolved with n-hexane and diluted to the mark. The solution was transferred into a quartz cuvette and the absorbance was measured at 446 nm against n-hexane. The β-carotene content of different vegetable oils is defined and calculated as β-carotene in parts per milliom (ppm).

The calculation was as follows:

$$\beta\text{-carotene} = [V \times 383 \times (A_s - A_b)] / (100 \times W)$$

Where: V = the volume used for analysis, 383 = the extinction coefficient for β-carotene, A_s = the absorbance of the sample, A_b = the cuvette error, W = the weight of the sample in g, 100= conversion factor.

Determination of Vitamin A

The HPLC method was used as described by Gimeno *et al.* (2000). Oil samples were warmed to 30°C and mixed thoroughly. Samples were diluted in isopropanol at a ratio of 1:10 (oil: isopropanol; v:v) and the diluted oil sample (20 µL) was injected without prior saponification and analyzed on a reverse-phase column. The solvent system consisted of methanol (A) and ethyl acetate (B), which were used as the eluent at a flow rate of 0.5 mL/minute. Each analytical run consisted of the following gradient profile: 0 – 3 min – 0 % B; 3 – 3.1 min – 50 % B; 3.1 – 10 min – 50 % B; 10 – 10.1 min – 0 % B; 3.0 min 0 % B; 3.1 min 50 % B; 10.0 min 50 % B; 10.1 min 0 % B; and 15.0 min rinsing time. The column temperature was 40 °C. Absorption of vitamin A esters (palmitate) was identified by comparison of retention times to the external standard (retinyl palmitate) by use of a photodiode array detector at a wavelength of 325 nm. The exact concentration of the external standard retinyl palmitate was measured on a weekly basis using a UV-VIS spectrophotometer. The detection limit for retinyl palmitate is 50 µg RE/kg. To ensure validity of the results obtained by this reference method, the results of a subset (n = 30) of fortified palm oil samples (range: 0 – 30 mg RE/kg) were compared with those obtained by a third laboratory, the Swiss Vitamin Institute (Epalinges, Switzerland; external validation), using saponification, followed by retinol analysis using an HPLC method.

Determination of Concentration of Vitamin E

The HPLC analysis as described by Dauquan *et al.*, (2011) was used. The concentration of vitamin E in the vegetable oils was determined by HPLC (Hewlett Packard HP1100, FLD). The HPLC analysis was performed using YMC column 150×6 mm I.D. The mobile phase used was composed of 0.5% isopropylalcohol/hexane and the flow rate was 1 ml/min. Total runtime for each standard and sample was 40 minutes. The injection volume was 20 µL. Detection was

performed using a fluorescence detector at excitation 295 nm and emission 330. All standards were obtained from the Malaysian Palm Oil Board (MPOB).

The standard concentration was 40 ppm for each component. Quantification of vitamin E was done by using the following formula:

$$\chi \text{ ppm} = V_s/W_s \times A_s/A_{std} \times V_{Istd}/V_{Is} \times C_{std}$$

Where: V_s = Volume of sample, W_s = Weight of sample, A_s = Area of sample, A_{std} = Area of standard, V_{Istd} = Volume of standard injected, V_{Is} = Volume of sample injected, C_{std} = Concentration of standard.

Antioxidant Tests

(a) Ferric reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out previously as describe by Benzie and Strain (1996). The mechanism of this method is based on the reduction of ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+} -TPTZ) to its ferrous form (Fe^{2+} -TPTZ) in the presence of antioxidants. The FRAP reagent contained 20mM $FeCl_3 \cdot 6H_2O$, 10 mM TPTZ (2,4,6-tripyridyl-s triazine) solution in 40 mM HCl and 0.3 M acetate buffer, pH 3.6. It was prepared freshly and incubated at 37°C for 10 minutes. The FRAP reagent was mixed in the ratio of 1: 1: 10. Aliquot of 100 μ l sample was mixed with 2.9 ml of FRAP reagent. The absorbance of the reaction mixture was measured spectrophotometrically at 593nm after incubation at room temperature for 1 hour. Trolox (1000 μ M) was used for calibration curve and the results were expressed as μ M of Trolox equivalents per mg fresh weight.

(b) DPPH Free Radical Scavenging Assay

The antioxidant activity was carried out through evaluation of free radical scavenging effect on 1,1 -diphenyl-2-picrylhydrazyl (DPPH). The determination was based on the method described by Yamaguchi *et al.* (1998). An aliquot (600 µl) of the sample was added to 4.5 ml of 0.1mM DPPH ethanolic solution. The mixture was then thoroughly vortex and incubated for 20 minutes in dark condition at room temperature. The absorbance was measured at 517 nm against a blank of ethanol. Results were then expressed as percentage of inhibition of the DPPH radical.

Percentage of inhibition of DPPH radical was calculated according to the following equation:

% inhibition of DPPH = [(Abs control – Abs sample) / Abs control] x 100. Where Abs control was the absorbance of DPPH without sample.

Statistical analysis:

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS). Results were presented as mean ± SD. One way Analysis of Variance (ANOVA) and Tukey's Multiple Comparison test were used were appropriate to compare the means. Values for which $p < 0.05$ were considered statistically significant.

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RESULTS AND DISCUSSION

Qualitative Phytochemical Analyses of the Oil Samples from *Elaeis Guineensis* Species

Fruits.

The qualitative phytochemical analyses (table 1) indicated that all the study samples contained steroids, glycosides, terpenoids, triterpenes and phenolics but were devoid of tannins, anthocyanin, amino acids, alkaloids and phlobatanins. Furthermore, it showed the presence of saponins, flavonoids and coumarins in DPO, TPO and NDPKO and their absence in NTPKO, HDPKO and HTPKO.

Quantitative Phytochemical Analyses of the Oil Samples from *Elaeis Guineensis* Species

Fruits

The result of the quantitative phytochemical and chemical analyses of the oil samples showed that NDPKO statistically had the highest contents of terpenoids and triterpenes. Saponins, flavonoids and coumarins were highest in DPO with values 1.00 ± 0.001 mg/kg, 308.30 ± 1.81 mg/100g and 43.98 ± 0.13 µg/100g. Furthermore, NTPKO was found to have the highest content of steroids (150.40 ± 0.24 mg/100g) while TPO had the highest content of glycosides (9.01 ± 0.07 mg/100g) and phenolics (45.04 ± 0.12 mg/100g). It can also be seen that terpenoid contents of DPO and NDPKO had no significant difference ($p > 0.05$). Also, saponin, steroids and coumarin contents of DPO, TPO and NDPKO all varied significantly ($p < 0.05$). Flavonoid contents of NDPKO differed ($p > 0.05$). In addition, triterpenes content of DPO and HDPKO were not significantly different whereas the other study samples had significant differences among them at $p < 0.05$.

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TABLE 1: Qualitative Phytochemical and Chemical Analyses of Oil Samples of *Elaeis guineensis* species fruits

Phytochemicals	DPO	TPO	NDPKO	NTPKO	HDPKO	HTPKO
Saponins	+	+	+	-	-	-
Tannins	-	-	-	-	-	-
Flavonoids	+	+	+	-	-	-
Anthocyanins	-	-	-	-	-	-
Steroids	+	+	+	+	+	+
Coumarins	+	+	+	-	-	-
Glycosides	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+
Triterpenes	+	+	+	+	+	+
Phlobatanin	-	-	-	-	-	-
Phenolics	+	+	+	+	+	+

KEY + = present; - = Absent, **DPO** = *dura* palm oil, **TPO** = *tenera* palm oil, **NDPKO** = *dura* native palm kernel oil, **NTPKO** = native *tenera* palm kernel oil, **HDPKO** = *dura* palm kernel oil of hexane extract, **HTPKO** = *tenera* palm kernel oil of hexane extract (*tenera* variety).

Table 2: Quantitative Phytochemical contents of oil samples of *Elaeis guineensis* species fruits

Phytochemical	Samples					
	DPO	TPO	NDPKO	NTPKO	HDPKO	HTPKO
Terpenoids (mg/kg)	1.75 ± 0.00 ^a	1.53 ± 0.00 ^b	1.77 ± 0.14 ^a	1.69 ± 0.00 ^{ab}	1.70 ± 0.00 ^{ab}	1.02 ± 0.00 ^c
Saponin (mg/kg)	1.00 ± 0.001 ^a	0.90 ± 0.00 ^b	0.88 ± 0.00 ^c	-	-	-
Steroids (mg/100g)	101.40 ± 0.24 ^a	141.50 ± 0.24 ^b	124.10 ± 0.35 ^c	150.40 ± 0.24 ^d	114.10 ± 0.12 ^e	127.10 ± 0.35 ^f
Flavonoids (mg/100g)	308.30 ± 1.81 ^a	175.20 ± 2.54 ^b	146.30 ± 0.91 ^c	-	-	-
Glycosides (mg/100g)	8.99 ± 0.04 ^a	9.01 ± 0.07 ^a	8.91 ± 0.03 ^a	8.70 ± 0.02 ^b	8.70 ± 0.04 ^b	8.94 ± 0.03 ^a
Coumarins (µg/100g)	43.98 ± 0.13 ^a	43.02 ± 0.03 ^b	42.28 ± 0.24 ^c	-	-	-
Triterpenes (mg/100g)	285.90 ± 0.35 ^a	247.90 ± 0.71 ^b	288.50 ± 23.33 ^{ab}	275.20 ± 0.24 ^{ab}	276.90 ± 0.12 ^a	163.40 ± 0.12 ^c
Phenolics (mg/100g)	44.77 ± 0.38 ^a	45.04 ± 0.12 ^a	42.59 ± 0.18 ^b	39.31 ± 0.12 ^c	34.23 ± 0.02 ^d	41.03 ± 0.04 ^e

*Values are mean \pm standard deviation of duplicate determinations. Values with different superscript letter per row are statistically different ($p < 0.05$). **KEY:** **DPO** = *dura* palm oil, **TPO** = *tenera* palm oil, **NDPKO** = *dura* native palm kernel oil, **NTPKO** = native *tenera* palm kernel oil, **HDPKO** = *dura* palm kernel oil of hexane extract, **HTPKO** = *tenera* palm kernel oil of hexane extract.

β -carotene, vitamins A and E Contents of the Oil Samples from *Elaeis guineensis* species

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fruits.

The results from table 3 showed that TPO had the highest β -carotene content (2.06 ± 0.00 mg/100ml) while HDPKO had the least content (1.74 ± 0.02 mg/100ml) of it. There was no significant decrease ($p > 0.05$) between DPO and TPO, NDPKO and NTPKO as well as between HDPKO and HTPKO. TPO had the highest content of vitamin A (105.60 ± 0.15 iU/100ml) followed by DPO, NTPKO, NDPKO, HTPKO and then HDPKO. No significant difference ($p > 0.05$) was observed between vitamin A content of DPO and TPO. Vitamin E content was found to be highest in HTPKO (4.67 ± 0.03 μ g/ml) and lowest in TPO (3.73 ± 0.30 μ g/ml). Also, HTPKO was seen to have a significantly higher ($p < 0.05$) vitamin E content than TPO, NDPKO and NTPKO.

Table 3: β -carotene, Vitamins A and E Contents of Oil Samples of *Elaeis Guineensis* species fruits

Parameters	DPO	TPO	NDPKO	NTPKO	HDPKO	HTPKO	RDA (FNBIM 2011)
B-carotene (mg/100ml)	2.03 \pm 0.01 ^a	2.06 \pm 0.003 ^a	1.94 \pm 0.02 ^b	1.98 \pm 0.02 ^b	1.74 \pm 0.02 ^c	1.75 \pm 0.03 ^c	4.0mg/day' >4.4mg/day''
Vit. A (iU/100ml)	104.20 \pm 0.17 ^{ab}	105.60 \pm 0.15 ^a	99.86 \pm 1.09 ^c	101.80 \pm 0.06 ^{bc}	89.22 \pm 1.00 ^d	89.82 \pm 0.16 ^d	400 μ g/day' 700 μ g/day'' 900 μ g/day'''
Vit E (μ g/ml)	4.46 \pm 0.13 ^{ab}	3.73 \pm 0.30 ^a	3.75 \pm 0.20 ^a	3.67 \pm 0.08 ^a	4.28 \pm 0.33 ^{ab}	4.67 \pm 0.03 ^b	4 μ g/day' 15 μ g/day'' 15 μ g/day'''

*Values are mean \pm standard deviation of duplicate determinations. Values with different superscript letter per row are statistically different ($p < 0.05$). Recommended daily allowances (RDA) (FNBIM-Food and Nutrition Board Institute of Medicine, National Academy, 2011).

KEY: 'Children, ''women, '''men. **DPO** = *dura* palm oil, **TPO** = *tenera* palm oil, **NDPKO** = *dura* native palm kernel oil, **NTPKO** = native *tenera* palm kernel oil, **HDPKO** = *dura* palm kernel oil of hexane extract, **HTPKO** = *tenera* palm kernel oil of hexane extract.

Antioxidant Tests

Comment [h8]: Prefix number

(a) The ferric reducing antioxidant power (FRAP) of oil samples from *Elaeis guineensis* species fruits.

Results from table 4 showed that the FRAP of all the samples varied significantly ($p < 0.05$). However, the values were significantly lower than that for the standard gallic acid ($1677.00 \pm 8.21 \mu\text{g}/100\text{g}$) which has a very high FRAP. Glaringly, DPO ($601.30 \pm 0.91 \mu\text{g}/100\text{g}$) had the lowest FRAP while NTPKO ($780.00 \pm 1.82 \mu\text{g}/100\text{g}$) had the highest FRAP, with TPO, NDPKO, HDPKO and HTPKO having $627.40 \pm 2.28 \mu\text{g}/100\text{g}$, $763.20 \pm 0.91 \mu\text{g}/100\text{g}$, $688.10 \pm 2.28 \mu\text{g}/100\text{g}$ and $718.70 \pm 0.91 \mu\text{g}/100\text{g}$, respectively.

(b) Percentage inhibition of DPPH (1, 1 – diphenyl- 2- picrylhydrazyl) by the oil samples from *Elaeis guineensis* species fruits.

Table 5 showed the antioxidant activity (% inhibition of DPPH) of the oil samples and the standard BTH (Benzo-thiadiazole- 7-carbothioic acid S-methyl ester). At the highest DPPH concentration of 50mg/ml, NDPKO ($105.70 \pm 0.04\%$) was observed to have the highest antioxidant activity but lowest in HTPKO ($74.28 \pm 0.75\%$) though all the study samples had significantly lower antioxidant activity than the standard BTH ($120.00 \pm 0.81\%$) at that concentration. At the lowest DPPH concentration of 10mg/ml, NDPKO ($88.26 \pm 0.11\%$) had the highest antioxidant activity while HTPKO ($68.87 \pm 0.21\%$) had the lowest activity. furthermore, it can be observed that a decrease in the concentration of DPPH resulted to a significant decrease in the antioxidant activity of all the oil samples.

Table 4: The Ferric Reducing Antioxidant Power (FRAP) of Oil Samples of *Elaeis guineensis* species fruits

Parameter	Sample						Gallic acid (standard)
	DPO	TPO	NDPKO	NTPKO	HDPKO	HTPKO	
FRAP	601.30	627.40	763.20 ±	780.00 ±	688.10 ±	718.70 ±	1677.00 ±
(µg/100g)	± 0.91 ^a	± 2.28 ^b	0.91 ^c	1.82 ^d	2.28 ^e	0.91 ^f	8.21 ^g

*Values are mean ± standard deviation of duplicate determinations. Values with different superscript letter per row are statistically different (p<0.05).**KEY:** **DPO** = *dura* palm oil, **TPO** = *tenera* palm oil, **NDPKO** = *dura* native palm kernel oil, **NTPKO** = *tenera* native palm kernel oil, **HDPKO** = *dura* palm kernel oil of hexane extract, **HTPKO** = *tenera* palm kernel oil of hexane extract.

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Table 5: Percentage inhibition of DPPH at 50 – 10 mg/ml by oil samples of *Elaeis guineensis* species fruits

DPPH concentration	Sample (% inhibition)						BHT (standard)
	DPO	TPO	NDPKO	NTPKO	HDPKO	HTPKO	
50mg/ml	99.81 ± 0.99 ^{ab}	100.60 ± 0.05 ^a	105.70 ± 0.04 ^c	101.40 ± 0.30 ^a	97.58 ± 0.20 ^b	74.28 ± 0.75 ^d	120.00 ± 0.81 ^e
	89.45 ± 0.30 ^a	85.02 ± 0.91 ^b	93.11 ± 0.17 ^c	89.60 ± 0.09 ^a	84.93 ± 0.78 ^b	73.28 ± 1.25 ^d	96.73 ± 1.24 ^e
40mg/ml	86.33 ± 0.45 ^a	86.61 ± 0.28 ^a	93.49 ± 0.28 ^b	85.16 ± 0.40 ^a	79.39 ± 0.20 ^c	72.66 ± 0.98 ^d	88.85 ± 0.13 ^e
	85.67 ± 0.23 ^a	86.79 ± 0.71 ^{ab}	90.84 ± 0.04 ^b	85.03 ± 0.83 ^a	77.25 ± 0.44 ^c	71.51 ± 0.58 ^d	86.25 ± 2.76 ^a
30mg/ml	80.11 ± 0.83 ^{ab}	78.20 ± 0.45 ^{ac}	88.26 ± 0.11 ^d	82.63 ± 1.32 ^b	74.74 ± 1.20 ^e	68.87 ± 0.21 ^f	83.47 ± 0.22 ^c
20mg/ml							
10mg/ml							

*values are mean ± standard deviation of duplicate determinations. Values with different superscript letter per row are statistically different (p<0.05).**KEY:** DPO = *dura* palm oil, TPO = *tenera* palm oil, NDPKO = Native *dura* palm kernel oil, NTPKO = *tenera* native palm kernel oil (*tenera* variety), HDPKO = *dura* palm kernel oil of hexane extract, HTPKO = *tenera* palm kernel oil of hexane extract, BHT= Butylated hydroxytoluene

DISCUSSION

Comment [h9]: Discuss by providing subheadings as per results and Prefix numbers

The absence of alkaloids, tannins and phlobatannins in all the samples (table 1) may be as a result of their tendency to partition away from the oil due to their lipid insoluble nature. Also, being water soluble, they may have been lost in the waste water used for palm oil extraction or leached away during soaking of the kernel seeds.

The presence of terpenoids in all the oil samples are similar to the report of Njoku *et al.* (2010) for beniseed oil. The values of terpenoids for the oil samples were close with NDPKO having the highest concentration and HTPKO having the lowest (table 2) which may be due to the sundrying of the seeds before oil extraction. Their presence accounts for their use as anti-diabetic agents as reported by Ramesh *et al.* (2005). Terpenoids have also been reported to be a heart-friendly phytochemical constituent which helps to reduce diastolic blood pressure. Terpenoids are also known to lower the sugar level in the blood (Hawkins and Erlich, 2006). Triterpenes are described to present different properties such as anti-tumoral, cardio-protective, anti-inflammatory and antioxidant activity and could be useful compounds for the prevention of multiple diseases related to cell oxidative damage (Quesada *et al.*, 2013). These may be some of the reasons DPO is used in the management of fever and headaches (Ekwenye and Ijeomah, 2005). However, the high concentrations of triterpenes in the oil samples is of great concern as higher concentrations of triterpenes may lead to cytotoxic or biphasic effects (Quesada *et al.*, 2013).

The higher concentration of steroids in NTPKO compared to that of DPO may be related to their non-polar nature which obviously favours their increased concentration in the palmkernel oil as they were not leached away. The presence of steroids in the oil samples are also similar to that reported by Njoku *et al.* (2010) for beniseed oil and coconut seed oil reported by sani *et al.*

(2014). The presence of steroids in these oils justifies their use in the pharmaceutical industries due to their importance in the synthesis of sex hormone compounds (Sani *et al.*, 2014), little wonder why the oils are locally used as aphrodisiacs (Irvin, 1985; Sasidharan *et al.*, 2012), steroids also exert anti-inflammatory properties (Schneider and Wolfling, 2004). The glycosides contents of the oil samples as seen in table 1 and 2 appeared very close with TPO having the highest concentration and NTPKO having the lowest concentration. These differences can be attributed to the water insoluble nature of glycosides. Glycosides have been reported to have a pronounced physiological action with cardiac glycosides being the drug of choice for the treatment of congestive heart failure (Sui *et al.*, 1994) and are also known to have laxative, diuretic and antiseptic properties (Chakarborty *et al.*, 1995; Boyce and Christy, 2004). These may be the reason TPO is used in the management of cardiovascular diseases (Ekwenye and Ijeomah, 2005), high blood pressure (Irvin, 1985; Sasidharan *et al.*, 2012) and intestinal disorder and skin diseases (Okoye 2018) respectively. Hence, all the oil samples seen to contain glycosides may possess the aforementioned health benefits. Table 2 also revealed the oil samples to be potential sources of phenolic compounds with TPO having the highest concentration and HDPKO the lowest concentration. Phenolic compounds have been reported to be essential for the prevention of chronic illnesses such as cardiovascular disease, certain types of cancers, neurodegenerative diseases and diabetes (Scalbert *et al.*, 2005). Plants and oils reported to contain phenolics could be used as anti-inflammatory (they possess the ability to block specific enzymes that cause inflammation) compounds, immune enhancers and hormone modulators (Okwu and Omodamino, 2005). They exhibit a wide range of biological physiological properties due to their ability to act as antioxidants, free radical scavengers and chelators of divalent cations (Afanas *et al.*, 1989). Flavonoids are known to be phenolic compounds. The presence of

flavonoids was seen to be highest in DPO and absent in NTPKO, HDPKO and HTPKO probably due to loss of some nutrients during soaking, sundrying and grinding of the kernel seeds for oil extraction. Flavonoids present in DPO, TPO and NDPKO inferred that the oils have biological functions such as anti-oxidation and protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumour (Farquar 1996; Okwu, 2004). These may be the reasons why these oils are used in hastening wound healing (Okwu, 2004; Okoye 2018). They are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and different levels of carcinogenesis (Okwu, 2004). These values were higher when compared to flavonoids in coconut seed oil (0.23 ± 0.08 mg/g) as reported by Sani *et al.* (2014). The presence of saponins was observed to be highest in DPO and lowest in NDPKO which may have been associated with high heat involved in the kernel roasting. Saponins are reported to be used in maintaining high density lipoprotein cholesterol (HDL-C) levels and lowering low density lipoprotein cholesterol (LDL-C) levels as reported by WHO (1992). This justifies their local use in the management of cardiovascular diseases. Saponins aid in reducing cholesterol levels by forming complexes with cholesterol and bile acids which prevents them from being absorbed or reabsorbed, respectively through the small intestine hence lowering the cholesterol levels in the blood and liver (Sani *et al.*, 2014). Furthermore, Chavali and Campbell (1987) reported that saponins are used as adjuvants in vaccines and oral intakes since they are known to stimulate antibody productions, inhibit viruses and induce the immediate response of lymphocytes (white blood cells that fight infections) that have been used to help in managing retroviral infections. Also, they have been reported to be useful in the treatment of upper respiratory tract inflammation which may be the reason palm oil and palm kernel oils are used in the management of cold (Okoye, 2018). Saponins also possess anti-

diabetic, anti-fungal and anti- carcinogenic properties. They carry out immune modulation activities and regulation of cells proliferation as well as the inhibition of the growth of cancer cells (Jimoh and Oladji, 2005). They also have the property of precipitating and coagulating red blood cells (Okwu and Josiah, 2006) which may be why these oils are used in stopping bleeding and treating wounds (Okwu, 2004 ; Okoye, 2018). Coumarins are contained in minute quantities by DPO, TPO and NDPKO probably due to the high temperatures at which the oils were extracted. The presence of coumarins may enable these oils to carry out various pharmacological activities such as oedema modification, faster reabsorption of oedematous fluid which may be why these oils are used to strengthen fontanel of newborns (Okoye, 2018) and treatment of lymphoedema as reported by Casleymith (1993).

Carotenoids act as precursors in the synthesis of vitamin A which is required for good vision (Wattanapenpaiboon and Wahlqvist, 2003). They can also protect against cardiovascular diseases and suppress growth of various cancer cells as well as tumours. They have also been shown to enhance cell to cell communication in exerting their anti-cancer effects (Zhang *et al.*, 1999). Of all the known carotenoids, β -carotene is believed to be the most important in human nutrition (Gurunadh *et al.*, 2005). Table 3 revealed the presence of β -carotene in all the oil samples with TPO having the highest concentration and HDPKO having the lowest concentration. These values were far lower when compared to the β -carotene content of beniseed oil (325mg/100ml) as reported by Njoku *et al.* (2010). This fact can be explained by several factors such as the ripening stage of the fruits, the region of prevalence and to a greater extent the extraction procedure which involved high heat in boiling and oil clarification and roasting of the kernels since it has been reported by Cmolik and Pokorny (2000) that high heat leads to the destruction and loss of β -carotene. Furthermore, soaking and drying of the kernels as well as

grinding/crushing of the kernels may have led to the leaching and loss of β -carotene respectively. The presence of β -carotene in these oil samples justifies their therapeutic use as β -carotene has long been known to be an efficient quencher of singlet oxygen and as such have protective properties against free radical damage that is believed to be responsible for numerous degenerative diseases such as atherosclerosis, arthritis, carcinogenesis etc (Choo *et al.*, 1991). This justifies the use of TPO in the reduction of fever and headaches (Ekwenye and Ijeomah, 2005). Also, β -carotene plays a vital role in vitamin A biosynthesis as it is cleaved into retinal by the enzyme β , β -carotene-15, 15'-monooxygenase (Gurunadh *et al.*, 2005) and then to retinol. Vitamin A (retinol) is a fat soluble vitamin which was observed in all the oil samples at appreciable amounts with TPO having the highest vitamin A concentration obviously due to the high content of β -carotene. This accounts for their use in several therapeutic ways since Vitamin A has been known to prevent visual impairment such as night blindness and the likes, aids in bone and tooth development, reproduction, cell division, gene expression, regulation of the immune system and an antioxidant that may play a role in the prevention of certain cancers, prevents skin infections and diseases and as well as maternal anaemia (Berdanier *et al.*, 2009). Infants and children have higher vitamin A requirements to promote rapid growth and better immunity to infections. Vitamin A deficiency in children causes visual impairment, blindness, stunting, anemia, respiratory disease, increased risk of infection, and mortality due to common childhood infections such as diarrhea and measles (WHO, 2011). Vitamin E was also found in all the oil samples in appreciable amounts with HTPKO having the highest vitamin E content though all the samples had lower vitamin E content than soyabean oil (958 mg/kg) as reported by Malekbala *et al.* (2015). This may be attributed to the process of extraction as reasonable quantities of vitamins and minerals are lost during the extraction processes as a result of high

temperature, soaking and sundrying. Vitamin E is a micronutrient that affects the oxidative stability and nutritional quality of the oils. It is a fat soluble vitamin comprising of 8 isomers including α , β , γ and δ known as four tocopherols and four tocotrienols. Vitamin E has been reported to help reduce blood cholesterol and arteriosclerotic functions, encompass possible anti-angiogenic functions, exhibit efficient antioxidant activity which justifies the use of HTPKO in the cosmetic industry since they help to reduce oxidative stress a major cause of hair loss thus accelerating hair growth as well as aids in skin elasticity. It also possess anti-cancer and anti-inflammatory effects, aids in the prevention of arthritis and osteoporosis, prevention of skin diseases as well as anti-diabetic and neuroprotective properties (Sen *et al.*, 2000; Sen *et al.*, 2010). Tocotrienols and tocopherols have also been shown to inhibit or delay the oxidative deterioration of cellular membranes making it protective against chronic conditions (Wattanapenpaiboon and Wahlqvist, 2003). On the other hand, malignant cells are very sensitive to tocotrienols. In fact, the more cancerous the cell, the more susceptible it is to the destructive effects of tocotrienol, so very little is required to accomplish its favorable role of cancer cell annihilation (Ebong *et al.*, 1999).

The ferric reducing antioxidant power (FRAP) is a method for evaluating the antioxidant activity of different compounds. Table 4 showed the FRAP of the oil samples with the standard gallic acid having the highest activity (twice the FRAP of the study samples) followed by NTPKO but lowest in DPO. The mechanism of this method is based on the reduction of the ferric form of ferric 2,4,6- tripyridyl-s-triazine complex (Fe^{3+} -TPTZ) to its ferrous form (Fe^{2+} -TPTZ) in the presence of antioxidants (Benzie and Strain, 1996). Generally, the reducing properties are associated with the presence of compounds which exert their actions by breaking the free radical chain through the donation of a hydrogen atom (Gordon, 1990) thus from the result, the samples

exhibited about 40-50% FRAP suggesting that they can scavenge free radicals and therefore act as primary antioxidants.

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity is also a way of determining the antioxidant properties of the oil samples. The principle involves the scavenging of the DPPH radical by hydrogen donating compounds. The reaction between antioxidants and DPPH occurs with the transfer of the most labile hydrogen atom to the radical (Dangles *et al.*, 2000) thus;

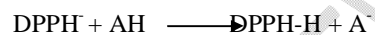


Table 5 showed the DPPH scavenging behaviours of the oil samples to be similar i.e, a decrease in the concentration of the oil samples resulted to a decrease in the DPPH scavenging ability of the samples. This seem to be in contrast with the DPPH scavenging behaviours of the aqueous extract of palm fruit as reported by Ikechukwu *et al.*, (2017). The sample concentration dependent decrease is comparable to that of the standard Butylated hydroxytoluene (BHT), although BHT exhibited a higher DPPH scavenging capacity at concentrations of 50 and 40mg/ml whereas NDPKO exhibited a higher DPPH scavenging capacity at lower concentrations which may be the reason why it is used by local dwellers in regulating body temperature, reducing fever as well in curbing convulsion whereas HTPKO showed the lowest scavenging ability probably due to loss of bioactive compounds during the processes of extraction. The samples exhibited about 70-80% DPPH scavenging abilities thus suggesting that the oil samples can act as electron donors and as such can terminate radical chain reactions. Furthermore, the antioxidant activity demonstrated by these oil samples could be attributed to the

presence of multi-antioxidant components such as β -carotene, flavonoids, phenolics and vitamins A and E (Ikechukwu *et al.*, 2017).

Conclusion

Comment [h10]: Prefix number

The data obtained revealed the presence of terpenoids, terpenes, steroids, glycosides and phenolics in all the oil samples though saponins, flavonoids and coumarins were only contained in DPO, TPO and NDPKO at variable and appreciable amounts. The β -carotene and vitamin A were more in TPO whereas HTPKO had most of vitamin E. The variation in the quantities of these compounds have been generally attributed to temperature, environmental conditions, genetic variations as well as the extraction procedures. However, the oil samples exhibited appreciable antioxidant properties due to the presence of various phytochemicals which have been shown to be beneficial to the human body. The research results therefore seem to support the traditional use of the oils in the management of various diseases.

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Comment [h11]: The references quoted in the text may be provided a numerical not the author et al as per the journal format and accordingly modify in the whole text

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