International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2014): 5.611

Dose-dependent Inhibition of Singlet Oxidation in Olive Oil by African Nutmeg (*Monodora myristica)* Crude Extract

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Abstract: ***Cleaned, dried and deshelled African Nutmeg* Monodora myristica *was ground and extracted with methanol. Doses of 0.0g, 0.5g, 1.0g, 1.5g, 2.0g of the* Monodora myristica *crude extract were added to each of five sets of reaction mixtures, prepared by dissolving 2g of olive oil in 10% methanol/dichloromethane to which 5mg of methylene blue was added as photosensitizer. Each reaction mixture, except its corresponding control, was subjected to singlet oxidation for a total of 10hours, removing 10ml aliquots every 2 hours to determine its Peroxide Value(PV) using the International Diary Federation(IDF) method. Samples of the reaction mixtures were also analyzed using the Fourier Transform Infrared Spectrophotometer. The peroxide value of the reaction mixtures containing 0.0g, 0.5g, 1.0g, 1.5g, and 2.0g amounts of the crude* Monodora myristica *increased from 9.56Meq/Kg before photoxidation (i.e. control) to 9.77Meq/Kg, 9.56Meq/Kg, 9.71Meq/Kg, 9.67Meq/Kg, and 9.67Meq/Kg respectively after 2hours of photoxidation. Further oxidation did not show any significant increase in PV of the reaction mixtures. The FTIR Spectra showed that the plant extract protected the C=C double bond whose absorption band was at 732cm^{-1}. This protection was optimal in the reaction mixture with 0.5g of the extract. However, the reaction mixture with the 1.5g dose of extract had pronounced peaks of both the C=C double bond group at 732cm^{-1} and C=O group at 1022.27cm^{-1}.***

Keywords: Olive oil**,** Singlet Oxidation, *Monodora myristica*, Peroxide Value, FT-IR analysis

1. Introduction

Oxidation of oils has been a major concern to food chemists and manufacturers alike because of the major role it plays by formation of toxic compounds, which not only cause off-flavors in foods but have also been implicated in inflammation, early aging, cataract, Parkinson dementia, rheumatism, muscular dystrophy, genetic disorders, arthritis, cardiovascular diseases, carcinogenesis and autoimmune diseases[1]. Synthetic antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) would have been viable additives to inhibit oxidation in oils but recent reports reveal that they can be toxic, hence the bid for their replacement with natural ones, mainly from plant materials[2] .

Monodora myristica

Figure 1: African Nutmeg (*Monodora myristica*)

Monodora myristica, also known as African Nutmeg, is a plant that is found in the tropical forests of West Africa. Its edible seeds, embedded in white sweet-smelling pulp, are economically and medicinally important [3].

Medicinally, *Monodora myristica* seeds have been found useful as a stomachic and stimulant. It is also used in the treatment of toothache, dysentery, headache, sores caused by guinea worm, constipation, etc [3][4]. Economically, the seeds are crushed and used as insecticides and also as a popular condiment in Nigerian cuisines [3].

Seed extracts of *Monodora myristica* are known to contain important pharmacological compounds like flavonoids, alkaloids, vitamin A and E as well as many important phytochemicals [3].

Qualitative analysis is used to determine biological active compounds that contribute to flavour, colour and other characteristics of plant materials.

Olive Oil

Olive oil is an edible vegetable oil obtained from olives (*Olea europaea*). Olive oil is composed of 14% saturated fat and 73% monosaturated fat [5]. This makes it highly susceptible to oxidation when compared to other vegetable oils. However, virgin olive oil is known to be rich in vitamins and minerals due to its non-chemical mode of extraction and contains polyphenols which have antioxidative properties, thus making it a very healthy for consumption [5].

Singlet Oxidation in Oils

Chemical reactions occur in oils during storage and processing. Oxidation reaction is the most common of such reactions. Oxidation reactions in oils may proceed through one of the following mechanisms: autoxidation, thermal oxidation, singlet oxidation, lipoxygenase – related oxidation [6]. Singlet oxidation is a process whereby photosensitizer molecules are irradiated by visible light to excite them. The excited sensitizer molecules collide with the ground state

triplet oxygen to convert it to singlet oxygen [4]. This singlet oxygen produced is by far more reactive than the ground state oxygen. It can react with the double bonds in the oils through [2 + 2] cycloaddition and the „ene" photochemical reaction pathways to form dioxetanes and hydroperoxides respectively. These can decompose thermally to produce carbonyl compounds which are responsible for rancidity in deteriorated oils. Unlike other oils, olive oil naturally contains certain polyphenols as well as chlorophyll which can act as photosensitizers to initiate photochemical oxidation of the oil on exposure to visible light [7].

Oxidative Deterioration Parameters of Oils

The hydroperoxides primarily formed may further decompose to yield secondary oxidation products, which include ketones, aldehydes, alcohols, hydrocarbons and volatile organic compounds. Measurement of these compounds constitutes the basis for determination of deterioration levels in lipids. Lipid hydroperoxides have been known to oxidize ferrous ion Fe^{2+} to ferric ion Fe^{3+} in acid medium. The ferric ion formed is further complexed by xylenol orange or thiocyanate to form a ferric thiocynate red complex that shows strong absorption at 470nm [7]. By measurement of the absorbance, the Peroxide Value of the lipid can be quantified, hence the extent of lipid deterioration estimated.

The objectives of this work is to obtain crude extract from *Monodora myristica* plant material, screen the extract quantitatively and qualitatively for phytochemicals present and determine if there is a relationship between the different doses of the crude plant extract in the inhibition of singlet oxidation in olive oil.

Materials

Monodora myristica seeds(African Nutmeg), distilled water, Extra Virgin Olive oil, methanol, dichloromethane, methylene blue, iron II sulphate, iron III chloride, Barium chloride, carbon tetrachloride, ammonia, sodium hydroxide, ethanol, hydrochloric acid, sodium sulphate, pyridine and sulphuric acid. All reagents were of analytical grade.

Method

Sample Collection

The plant material (African Nutmegs) was bought from Mile 3 Market in Port Harcourt, Rivers State. Commercial bottled olive oil was bought from a supermarket in Port Harcourt, Nigeria.

Sample Preparation

The African Nutmeg seeds were deshelled, cleaned, oven dried at 60°C and ground to powder using a handmill.

Extraction

The seeds powder was extracted with methanol solvent by batch extraction in a 2:5 solvent (ml) to plant material ratio by weight (g). The seeds powder was immersed in methanol for 72 hours with periodical stirring. The solvent was then filtered off the plant material using a whatsman No. 1 filter paper. The extraction was done twice on each batch to obtain optimal yield. The resultant crude extract was evaporated to constant weight using a thermostated water bath at 60°C.
The percentage yield of extract was calculated thus:

$$\%\text{Yield} = \frac{\text{Weight of Crude Extract}}{\text{Weight of Ground Plant Material}} \times \frac{100}{1}$$

The concentrated extract was stored in the freezer (4°C to -18°C) for further analysis.

Qualitative Analysis of Phytochemicals in Plant Extract

Qualitative phytochemical screening of the plant extract was carried out by the method of Harborne[8].

Quantification by GC-FID

The analysis was performed on a BUCK M910 Gas Chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m × 250µm × 0.15µm) was used. The injector temperature was 280°C with splitless injection of 2µl of sample and a linear velocity of $30cms^{-1}$. Helium 5.0pa.s was the carrier gas with a flow rate of 40 $mlmin^{-1}$. The oven operated initially at 200°C, was heated to 330°C at a rate of 3°C $min^{-1}$and was kept at this temperature for 5min. The detector operated at a temperature of 320°C.

Irradiation

5 sets of reaction mixtures were prepared by dissolving 2g of olive oil in volume in 10% methanol/dichloromethane to which 5mg of methylene blue was added as photosensitizer. The African Nutmeg crude extract was added to each of the 5 reaction mixtures in doses of 0.0g, 0.5g, 1.0g, 1.5g and 2.0g. Oxygen was bubbled into these reaction mixtures in an impinger, irradiating externally with a 200 watts photo-flood light while cooling externally in an ice bath. Another 5 sets of the reaction mixtures were prepared and stored in the freezer (4^0C to -18^0C) to act as controls for the oxidation reactions. Each of the reaction mixtures (except the controls) was thus photo-oxidized for a total of 10 hours, removing 10ml aliquots of the reaction mixture every 2 hours to determine its peroxide value (PV).

Determination of Peroxide Value

The determination of peroxide values in the irradiated and non-irradiated oil (ie. control) samples were carried out using the modified International Diary Federation standard method [9], a procedure based on the oxidation of Fe(II) to Fe(III) by hydroperoxide contents of oils. The Fe (III) is reacted with ammonium thiocyanate to form a complex which can be determined spectrophotometrically. The peroxide value (PV) is calculated using the formula:

$$\text{PV (Mequiv Peroxide/Kg of Sample)} = \frac{A_{sm} - A_{bl}}{55.84 \times 2 \times m \times W_{sm}}$$

Where:
A_{sm} = absorbance of the sample at 470 nm
A_{bl} = absorbance of the blank at 470nm
m = slope of the Fe (III) calibration plot
55.84 = atomic weight of Fe
2 = factor to convert mequiv of Fe to mequiv of Peroxide
W_{sm} = sample weight in grams

A. Calibration of Spectrophotometer Using Fe (III)
A working 1040µg/ml solution of Fe (III) was prepared by dissolving 0.1040g of $FeCl_3$ in 100ml of distilled water with 1% HCl. The solution was diluted with distilled water to a set of Fe (III) concentrations in the range 0 - 100µg/ml by successive dilutions of the working solution. 0.1ml of saturated ammonium thiocyanate solution was added to 1ml each of the different concentrations and left to stand for 10mins before its absorbance was measured at 470nm.

B. Oxidation of Fe (II) to Fe (III)
Fe (II) stock solution was prepared by mixing a solution of 0.4g of $BaCl_2.2H_2O$ in 50ml distilled water with a solution of 0.5g of $FeSO_4.7H_2O$ in 50ml distilled water. Concentrated hydrochloric acid (2ml) was added and the resulting solution was filtered and stored under cover. The Fe (II) solution was prepared freshly and used within 12hours. 1 ml of each of the reaction mixtures, which contains 0.01 - 0.05g of oil was introduced to a test tube to which 0.1ml of stock Fe (II) solution was added.

C. Complexation with Ammonium Thiocyanate
0.1ml of saturated ammonium thiocyanate solution was added to the reaction mixture in B and mixed for 5mins to ensure the complete formation of a reddish Fe(III)-thiocyanate complex. The complex formed was poured into a 1cm cuvette and absorbance measured at 470nm using a Surgispec SM – 23D spectrophotometer. The absorbance of the reagent blank was also measured.

IR Analysis
The IR analysis of the Olive Oil in Reaction Mixtures was performed using FTIR SYSTEM, IRAffinity_1S, SCHIMADZU. The method used was by direct application of the samples on cell.

2. Results and Discussion

Extraction
Table. 1. shows the percentage yield (13.55%) of *Myristica monodora* crude extract using methanol as a solvent.

Table 1: Percentage yield of Crude Extract

Plant Material	African Nutmeg
Weight of Ground plant Material(g)	220.00
Weight of Extract(g)	29.80
Yield(%)	13.55

Qualitative Analysis of Phytochemicals
The results of the qualitative analysis on the plant material shown in Table 2 indicates the presence of Alkaloid, Saponin and Steroids in moderate amount while Flavonoid, Cardiac Glycoside, Phenol, Anthracene Glycoside and Glycoside were detected in low quantity. Tanin and Cyanogenic Glycoside were absent.

Table 2: Qualitative Analysis of Phytochemicals in Crude Extract

Phytochemicals	Presence
Alkaloid	++
Flavonoid	+
Tannin	-
Saponin	++
Cardiac glycoside	+
Phenol	+
Steroids	++
Anthracene glycoside	+
Glycoside	+
Cyanogenic glycoside	-

Key: ++ = Moderately Present, + = Slightly Present, - = Absent

Quantitative Analysis of Phytochemicals
The results of the quantitative analysis of the *Myristica mondora* crude extract showed that catechin, rutin, kaempferol and lunamarine phytochemicals occur in very high percentages (see Table 3). Catechin, Rutin and kaempferol are flavonoids which are known to inhibit lipid peroxidation and lipoxygenases [1]. Lunamarine is a quinolone alkaloid, which has shown some in vitro anticancer activity [10]. The presence of these phytochemicals can inhibit singlet oxidation. Phenolics have also been identified as chemical quenchers of singlet oxygen [11].

Table 3: Quantitative Analysis of African Nutmeg Crude Extract

Phytochemicals	Weight(µg/g)	Percentage (%)
Catechin	95.3420	38.5390
Rutin	78.1504	31.5890
Kaempferol	39.0818	15.7980
Lunamarine	17.8497	7.2150
Phenol	7.0567	2.8520
Ribalidine	3.7052	1.4978
Anthocyanin	3.5553	1.4370
Sapogenin	1.6521	0.6678
Oxalate	0.8495	0.3433
Phytate	0.1468	0.0590
Spartein	0.0024	0.0009
Total Composition	247.3917	100

Calibration of Spectrophotometer Using Fe (III)
The calibration curve used for the determination of Fe (III) in the oxidized oil samples is shown in Figure 2. The R^2 (Coefficient of multiple determination for multiple regression) value of the curve is 0.960, which is within the acceptable range.

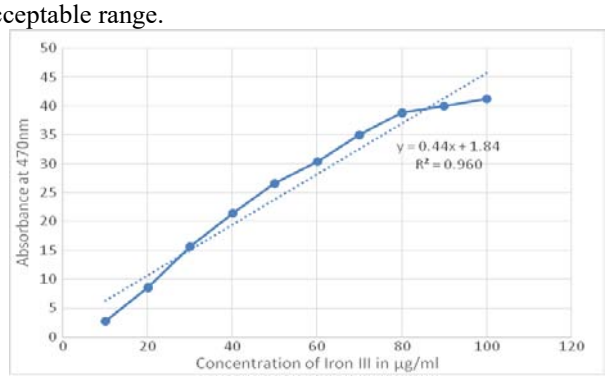

Figure 2: Calibration Curve for the Determination of Fe(III)

Determination of Peroxide Value

Figure 3 is the calibration curve that was used to translate Fe (III) absorbance readings to PV. It was derived by substituting the Fe(III) absorbance readings into the PV equation. It shows that PV is directly proportional to the absorbance of Fe (III) – thiocyanate complex formed. This relationship is what the IDF method is based on.

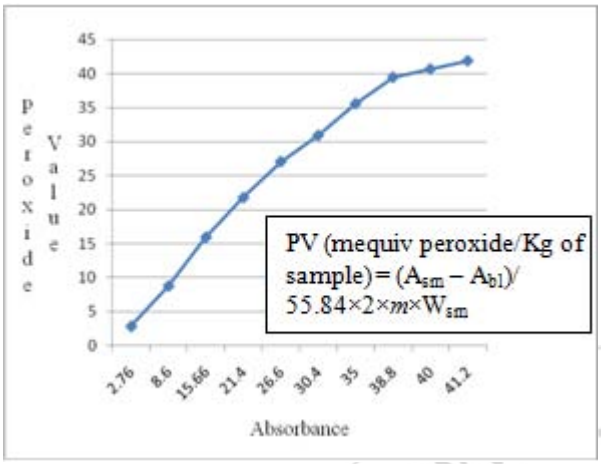

Figure 3: Calibration Curve for Determination of Peroxide Value

Derived Peroxide Values of Olive Oil with Different Doses of *Monodora myristica* Crude Extract

Table 4. shows that methanol extracts of African nutmeg has singlet oxidation inhibitory properties as the introduction of this plant extract reduced the peroxide value of Olive oil relative to the olive oil exposed without the plant extract. It has been demonstrated that methanol extracts of some plant materials (red pepper, onion bulb, and carrot) had properties attributable to singlet oxygen quenching [12].

Table 4: Derived Peroxide Values of Olive Oil with Different Doses of *Monodora myristica* Extract

Dose of Extract/ Duration of Exposure	*0.0g Extract*	*0.5g Extract*	*1.0g Extract*	*1.5g Extract*	*2.0g Extract*
0 Hours Exposure	9.564665	9.564665	9.564665	9.564665	9.564665
2 Hours Exposure	9.768169	9.564665	9.717293	9.666417	9.666417
4 Hours Exposure	9.971672	9.564665	9.666417	9.768169	9.717293
6 Hours Exposure	9.971672	9.666417	9.717293	9.666417	9.819045
8 Hours Exposure	10.02255	9.462914	9.717293	9.717293	9.768169
10 Hours Exposure	9.971672	9.564665	9.717293	9.717293	9.920796

The result in Table 4 was subjected to a statistical test (analysis of variance; ANOVA) investigating if there is any significant difference between the groups having different doses of the plant extract.

The result of the ANOVA test gave F statistic (7.20) as greater than critical F-value (2.76) at significance level of 0.05. This implies that there is a significant difference between the means of the different groups; hence, the singlet oxygen quenching ability of African Nutmeg in Olive Oil is dose dependent.

The bar chart in Figure 4 gives the general trend of the singlet oxygen quenching effect of the African nutmeg crude extract in Olive Oil. Figure 4 shows that the group with the smallest dose of extract (0.5g) had the maximum inhibition of singlet oxidation in olive oil. This is in agreement with the definition of antioxidants being any substance that delays, prevents oxidation of Lipids, DNA, Proteins, etc, when present at low concentrations[1].

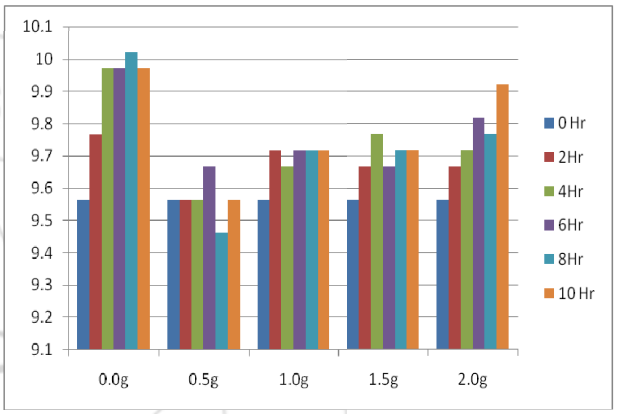

Figure 4: Bar Chart Showing the Peroxide Value of Photoxidized Olive Oil with Different Doses of African Nutmeg Extract

Antioxidants slow down the oxidation rates of foods by quenching singlet oxygen physically or chemically. Physical quenching involves deactivation of singlet oxygen to ground state triplet oxygen by energy transfer on collision. Chemical quenching involves the oxidation of quencher molecules, producing their oxidation products. [11]. The results obtained permit the interpretation that at a low dose (0.5g) of the African nutmeg extract, physical deactivation of singlet oxygen was favored over chemical deactivation, thus, causing the maximum inhibition of singlet oxidation in the various extract doses applied to the oil. However, at plant extract doses higher than 0.5g, chemical quenching of singlet oxygen appears to have been favored, with the oxidation products of the quencher molecules contributing to the relatively higher Peroxide Values of the Olive Oil.

FTIR Analysis

The result of the FTIR analysis carried out on the unexposed oil sample (Figure 5) showed two pronounced peaks, one at 732.95cm^{-1}, the other at 1022.27cm^{-1}. The 732.95 cm^{-1} peak represents - HC = CH – bending (rocking) vibration while the second peak at 1022.27cm^{-1} represents – C – O stretching[13]. For the unexposed oil, these peaks were very strong since olive oil contains 73% monosaturated fats, and the - C – O peak is also intense due to the acyl group found in oils.

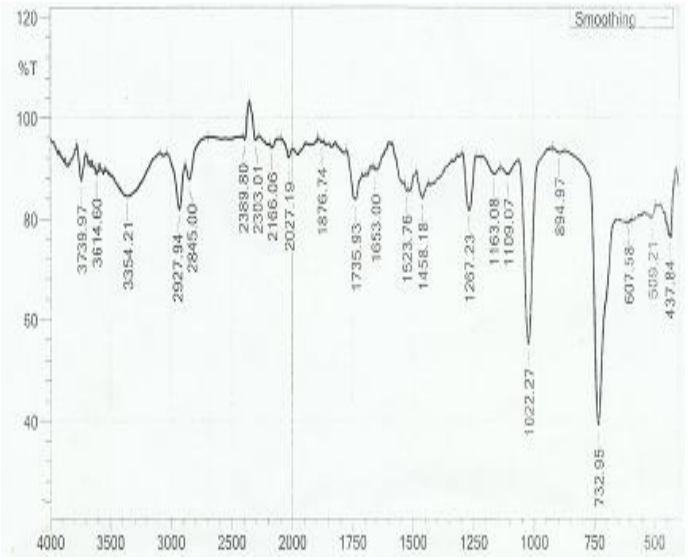

Figure 5: FTIR spectrum of unexposed Olive Oil

The absence of the peak at 732cm^{-1} in the oil that was photoxidized without plant extract (Figure 6) implies that the – HC = CH – bond were not protected.

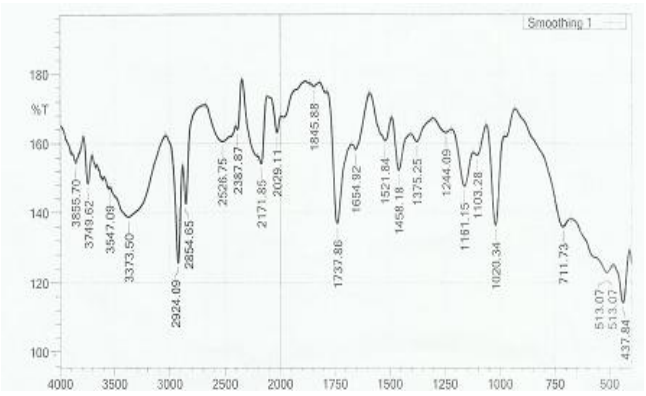

Figure 6: FTIR spectrum of photoxidized Olive Oil without plant extract

In contrast, the addition of 0.5g of *Monodora myristica* extract to the photoxidized Olive Oil appears to have protected the – HC = CH – bond from photoxidation, as evidenced by the intense peak at 734cm^{-1} in Figure 7.

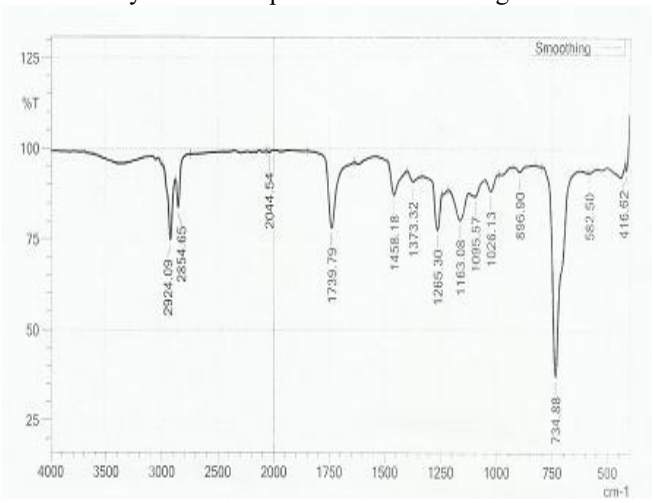

Figure 7: FTIR spectrum of photoxized Olive Oil with 0.5g plant extract

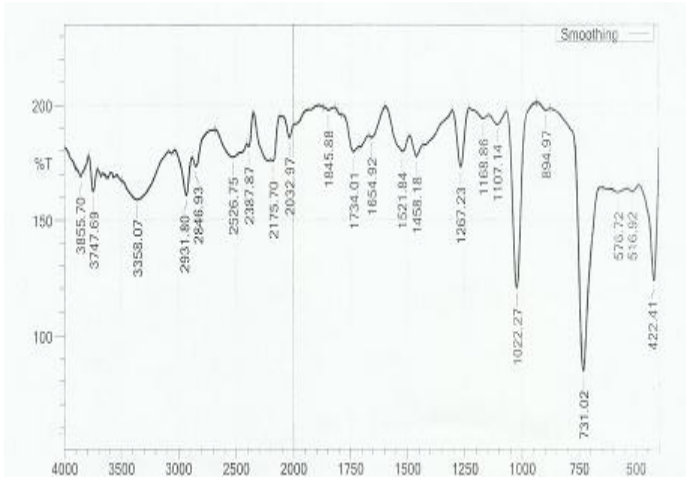

Figure 8: FTIR spectrum of photoxized Olive Oil with 1.5g plant extract

However, at the 1.5g dose of plant extract, the FTIR spectrum (Figure 8) showed pronounced peaks of both the C=C double bond group at 732cm-^{1} and - C- O group at 1022.27cm^{-1}. A plausible explanation of this observation is that at higher doses of the plant extract, oxidation of the antioxidants (quencher) molecules have become more predominant than physical quenching, leading ultimately to the formation of carbonyl compounds.

CONCLUSION

The results obtained in this study are consistent with the suggestion that *Monodora myristica* has singlet oxygen quenching properties in Olive Oil, which is dose-dependent. The study also indicates that the 0.5g dose of the plant extract was the optimal dose. Further studies on the quenching ability of this and other plant extracts are being undertaken.

References

[1] V.K Gupta and S.K Sharma, "Plants as Natural Antioxidants", Natural Product Radiance, 5(4), pp.326 - 334, 2006.
[2] Y.C Wong, C.T Wong, S. O Onyiruka, L. E Akpanisi, "Chemistry and Food" in University Organic Chemistry. Africana – FEP Publishers, Nigeria, 2002.
[3] P. E, Eze-Steven, C. N. Ishiwu, S. C. Udedi, and B.O. Ogeneh, "Evaluation of antioxidant potential of Monodora myristica(African Nutmeg)", International Journal of Current Microbiology and Applied Sciences, 2(11), pp. 373 – 383, 2013.
[4] C. R, Ekeanyanwu, G. I, Ogu, P. U, Nwachukwu, "Biochemical Characteristics of the African Nutmeg, Monodara myristica", Agricultural Journal, 5(5), 303 – 308, 2010.
[5] Diffen, "Olive oil vs Vegetable Oil" diffen.com, para. 5. Available:www.diffen.com/difference/Olive_Oil_vs_vegetable_oil.[accessed:April 21,2016]
[6] Y. W. Park, M. K. Jeong, C. Park and J. Lee, "Distribution of Tracylglycerols and Fatty Acids in Soybean Oil with Thermal Oxidation and Methylene

Blue Photosensitization", Journal of American Oil Chemical Society, 88, pp. 373 – 380, 2011.
[7] F. Shahidi and Y. Zhong, Bailey"s Industrial Oil and Fat Products, John Wiley& Sons, Canada, 2005.
[8] J. B. Harbone, Phytochemical Methods, A guide to Modern Techniques of Plant Analysis, Second Editon, Chapman and Hall, London (pp.54 -84), 1998.
[9] D. Hornero-Mendez, A. Perez-Galvez and M. I. Minguez-Mosquera, "A Rapid Spectrophotometric Method for the Determination of Peroxide Value in Food Lipids with High Carotenoid Content", Journal of American Oil Chemical Society, 78(11), 2001.
[10] V.K Gupta and S.K Sharma, "Plants as Natural Antioxidants", Natural Product Radiance, 5(4), pp.326 - 334, 2006.
[11] K.A. Manu and G. Kuttan, "Punarnavine induces apoptosis in B16 – 10 melanoma cells by inhibiting NF – kappaB Signaling". Asian Pacific Journal of Cancer Prevention. 10(6), pp. 1031 – 1037, 2009.
[12] E. Choe and D.B. Min, "Mechanisms of antioxidants in the oxidation of foods" Comprehensive Reviews in Food Science and Food Safety. 8, pp 345 – 358, 2009.
[13] S. S. Angaye, G.K. Fekarurhobo and N.J. Maduelosi, "Inhibition of Singlet Oxidation in Some Edible Vegetable Oils by Plant Extracts". Discourse Journal of Agriculture and Food Sciences. 2 (4), pp.100 – 106, 2014.
[14] M. D. Guillen and N. Cabo, " Infrared Spectrocopy in the Study of Edible Oils and Fats",Journal of Science and Agriculture, 75, pp 1-11, 1997.