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Research Article

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High-Performance Liquid Chromatography Screening of Flavonoids in *Monodora Myristica* which Prevent Oxidation of Palm Fruit Emulsion

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Abstract

The oil stabilizing property of Monodora myristica extract was evaluated to ascertain its potential in inhibiting palm fruit oil emulsion. Monodora myristica was extracted with absolute methanol. Crude palm fruit emulsion was also prepared according to standard procedures. Oxidation of palm fruit emulsion in the presence or absence of 0.5 % Monodora myristica extract was carried out. Vitamin E served as reference antioxidant. The phytochemical analysis of Monodora myristica extract showed 2.49± 0.77mg GAE/g extract of phenol and 0.95 ± 0.34 mg QE/g extract of flavonoid. The high-performance liquid chromatography of flavonoid revealed many flavonoids, flavan-3-ol, catechin, gallocatechin and kaempferol. The peroxide value of Monodora myristica at 0 days and 7th day was 9.42± 0.17 meq O_2/kg oil and 13.50 ± 1.0 meq O_2/kg oil. In the oxidation experiment the value of thiobarbitanic acid expressed as malondialdehyde was higher at 0 day and 7th day which was 27.4 ± 1.71mg MDA/kg and 38.16 ±1.92 mg MDA/kg respectively. Conjugated diene and triene values increases in oxidation group but decreases in the Monodora myristica and vitamin E stabilizing groups as depicted. The GC analysis of fatty acid profile also revealed both saturated and unsaturated fatty acids. In conclusion the extract of Monodora myristica can be used in stabilizing oil during processing.

Keywords: Palm Fruit, Oxidation, Peroxide Value, GC-MS, HPLC, *Monodora myristica*

1. Introduction

Nowadays consumers of oil and related products are increasingly aware of the link between food and health. Therefore most consumers are interested in functional foods. These functional foods are also therapies that can fight sedentary related diseases [1]. Palm fruit is essential for man and animals' diet. Man can process it into palm oil, it can also be processed and used for frying, cooking and for preserving other food products [2]. Crude palm fruits extract or palm fruit emulsion is obtained from the fruits of *elaeis guinensis* It has orange red colour, it contains saturated and unsaturated fatty acid like: Palmitic acid, oleic acid, stearic acid and linoleic acid [3,4]. It is also a common ingredient for the production of margarine, also used in the cosmetic industry like soap, cream lotions and shampoos [3]. Palm fruit emulsion can be oxidized to produce ketones, aldehydes, alcohol. These products can therefore affect the appearance of the emulsion, quality, palatability and texture [5]. Oxidation can be prevented by anti-oxidant compounds like butylated hydroxyl anisole and butylated hydroxyl toluene which are all synthetic anti-oxidants. They have the ability to extend the shelf life of Pharmaceutical and food products [6].

There is a large awareness for the use of natural anti-oxidant especially from plants or animals due to certain toxicity posed by synthetic anti-oxidant [7]. Also some synthetic antioxidants are banned in some lands, due to there health related risks *Monodora myristica* belongs to the Annonaceae family, it is commonly known as African nutmeg or calabash nutmeg, it flourishes in Africa and the Caribbean areas [8-10]. It is used in the repulsion of insects [11]. It is a spice for African cooking, enhancement of flavor, aroma to many foods' preparation [9,12].The fruits and seeds of *Monodora myristica* are effective as stimulants and the management of certain African ailments like sores, pains and eye disorders [13]. The pleasant aroma from *Monodora myristica* makes it important in cooking [14,15]. Many scientific studies on *Monodora myristica* showed that extract from seeds are antibacterial, antioxidant, antifungal [10,16-18]. The therapeutic potential of *Monodora myristica* is due to the presence of bioactives [19]. *Monodora mrystica* possesses anti-inflammatory and anti-microbial properties. It also contain Eugenol and myristica which act as anti-oxidant by scavenging free radicals into [20,21]. *Monodora myristica* seeds are highly medicinal and are also used in African dishes. Several plant active compounds, such as phenols, saponin, alkaloids, glycosides, flavonoids are abundant in Monodora myristica seeds [22,23]. These active substances are secondary metabolites, and scientific studies has proven them to havemany positive health effects, such as antioxidant, antimicrobial, anti-diabetic, hepatoprotective, antibacterial, antifungal, diuretic, antispasmodic, and antihypertensive properties [24,25]. Therefore, the aim of this study was to improve the stability and shelf life of palm fruit oil extract using *Monodora myristica* seed extracts as natural antioxidants.

2. Materials and Methods

2.1 Chemicals

Methanol, cyclohexane, chloroform, xylenol orange, iron(ii) chloride, iron(iii) chloride, dimethyl sulfoxide (DMSO), thiobarbituric acid, trichloroacetic acid, hydrochloric acid, potassium acetate, aluminium chloride, quercetin, gallic acid, sodium carbonate, Folin-Ciocalteau reagent.

2.2 Palm Fruit and *Monodora myristica*

Both palm fruits and *Monodora myristica* were obtained in sufficient quantities at Tombia market, Yenagoa Bayelsa state. The palm fruit as well as *Monodora myristica* were identified in Pharmacognosy Department, Faculty of Pharmacy, Niger Delta University, Bayelsa state. Voucher numbers were obtained.

2.3 Preparation of Monodora myristica Extract

Monodora myristica was grounded into powder afterwards 200 g of *Monodora myristica* powder was soaked in 0.5 L of methanol with occasional shaking for 3 days. The extract was filtered through Whatman No. 4 filter paper and was later concentrated into a light brownish paste. This was then stored at 40 C for future use.

2.4 Total Flavonoid Content

The extract of *Monodora myristica* was subjected to flavonoid content analysis spectrophotometrically according to the method of Ahmed et al., [26]. Extract (1mg/mg) and quercetin solutions 10 – 200 µg/ml were mixed with 10% Aluminum chloride 200µl and 100µl of 1M potassium acetate in tubes. All tubes were kept at 37°C for 30 minutes, Absorbance was determined at 520nm. Flavonoid content was expressed as mg QE/g of extract.

2.5 Total Phenol Content

Monodora myristica extract was determined according to the method reported by Ahmed *et al*., [25]. *Monodora myristica* dissolved in methanol (1mg/ml) and 10-20 µg/ml solutions of gallic acid as reference phenolic compound are mixed with 1ml of Folin- Ciocalteu reagent and were incubated for 5 min. Thereafter 10ml of sodium bicarbonate 7% was added and the volume raised to 25ml using distilled water. Solutions were incubated for 1.5 h at 37°C. Absorbance was read at 725nm. Total phenol in *Monodora myristica* was expressed as mg GAE/g extract.

2.6 High Performance Liquid Chromatography of Flavonoids in *Monodora Myristica*

Flavonoids from *Monodora myristica* were extracted with methanol, hexane hot water according to standard procedure. Flavonoid analysis was performed on a BUCK M910 HPLC equipped with a RESTEK 15 meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280^{o}C with splitless injection of 2µL of sample and velocity of 30cms^{-1}, methanol 5.0pas was the carrier gas with a flow rate of 40mlmin^{-1}. The oven operated initially at 2000C was heated to 330^{0}C at a rate of 3^{0}C min^{-1} and was kept at this temperature for 5min. the detector operated at a temperature of 3200C. Bioactive compound were determined by the ratio between the area and mass of internal standard and the area of the identified (Dolchy Analyticals).

2.7 Extraction of Palm Fruits

Exactly 100g of palm fruit were added to 500ml of boiling water. Cooking was carried out for 45minutes. The crude palm fruit oil was extracted by pounding the fruits until the oil and water emulsion comes out. The palm kernels and chaff were discarded but the oil and water was used for oxidation [3].

2.8 Preparation of Stock Solution of *Monodora Myristica* and Vitamin E

Exactly 0.5g of *Monodora myristica* or vitamin E was weighed and dissolved in 50ml of DMSO, to make a 1% stock solution. From the stock 0.5% of vitamin E or *Monodora myristica* was made.

2.9 Oxidation of Palm Oil Emulsion

Oxidation of crude palm fruit oil emulsion was heated in the absent, presence of vitamin E or *Monodora myristica* extract at 0.5 % concentration. The crude palm fruit oil emulsion was heated to 60^{0} C in a water bath except the control before addition of 0.5 % vitamin E or *Monodora myristica* for 7 days. (1)The control: no heating (2) oxidation: heated to 60^{0} C without antioxidant (3) oxidation + 0.5 % *Monodora myristica* and (4) oxidation + 0.5% vitamin E based on the method of Azman et al., [27].

2.10 Assay of Peroxide Value

A weighed amount of control, oxidation, oxidation + *Monodora myristica* and oxidation + Vitamin E were dissolved in 9.9ml of chloroform in methanol in the ratio 7:3 volume/volume. Thereafter methanol orange 50 µL was added to 50 µL of iron (ii) chloride solutions. The mixed solution was incubated at room temperature for 10 mins. The mixture was later centrifuged and the absorbance of the supernatant red at 560nm. A standard curve of iron (iii) chloride was constructed at concentration of 5µg/ml - 20µg/ml. Peroxide value was expressed as milliequivalent active oxygen per kg [28].

2.11 Conjugated Diene/Triene

Conjugated diene and triene was determined in control, oxidation, oxidation + *Monodora myristca* and oxidation + vitamin E at 0 days and 7 days according to the method of Abdalla and Roozen, [29]. Fifty milligram of samples were weighed and mixed with 5ml of cyclohexane and the CD and CT was determined using a UV spectrophotometer at 234 and 270nm respectively.

2.12 Measurements of Thiobarbituric Acid Reactive Substance

The method of Buege and Aust was applied [30]. The control, oxidation, oxidation + *Monodora myristica* and oxidation + vitamin E, one ml each was mixed with 5ml of TBA reagent. TBA reagent contained 0.375% thiobarbituric acid, 15% of trichloroacetic acid and 0.24 M HCl. The solutions were vortexed and heated in water bath at 95°C for 10 min. It was later cooled and centrifuged at 3000rpm for 20mims. The absorbance was measured at 532nm. The values were calculated and expressed as mg MDA/kg.

2.13 Gas Chromatography Analysis of Fatty Acid Profile in Palm Fruit Emulsion

Ten milliliters of palm oil emulsion was mixed with sodium sulphate in a 250 ml beaker, fatty acids were then extracted with 100 ml of n-hexane for 24 hrs. The dried crude extract 5 mg was transferred into 10 ml centrifuge tube then 2 ml of water was added, 2 ml of 5% (w/v) methanolic sodium methoxide solution and 2 ml of hexane and neutralizing solution. The tubes were kept for 3 min before centrifuging at 1,750 rpm for 5 mins. About 200 µL of supernatant was transferred into 10 ml flask and then diluted to the mark with hexane, thereafter 1 µL was injected into the gas chromatography column [31].

2.14 Statistical Analysis

The results are mean ± SEM, n = 3. The data was processed using SPSS version 17.0 New York, USA. Significant values are considered at $p < 0.05$.

3. Results

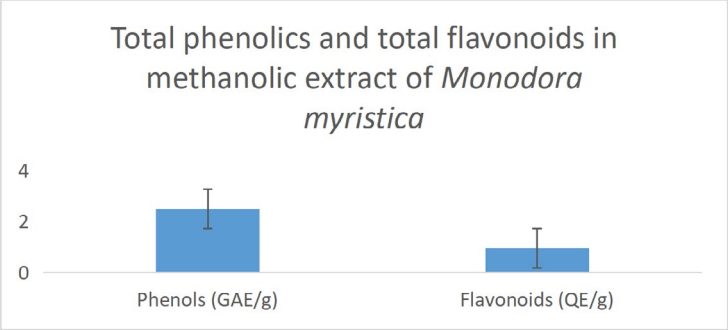

Figure 1: Total Phenolic and Flavonoid Content in Methanolic Extract of *Monodora Myristica*

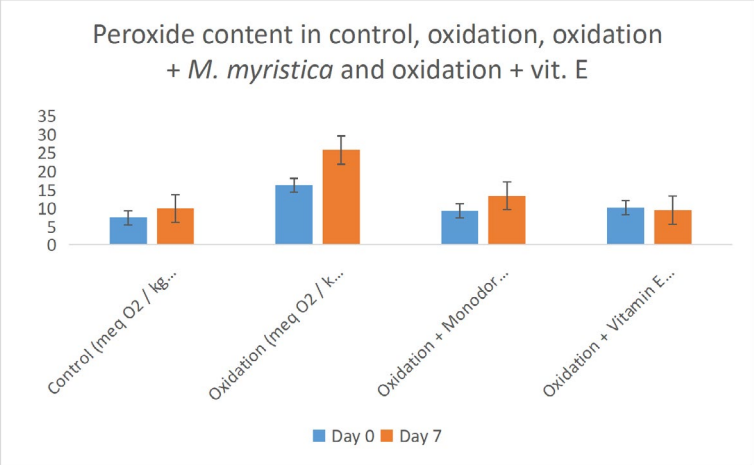

Figure 2: Effect of *Monodora Myristica* on Peroxide Content in Control, Oxidation, Oxidation + *Monodora Myristica* and Oxidation + Vitamin E of Palm Oil Emulsion

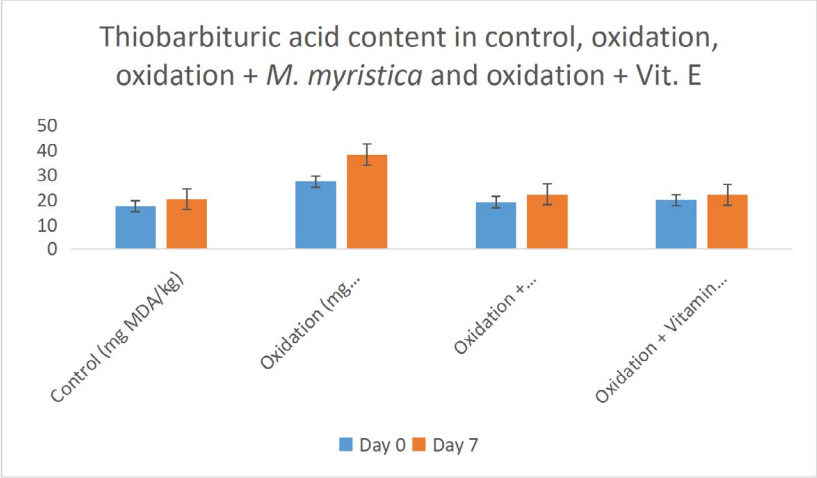

Figure 3: Effect of *Monodora Myristica* on Thiobarbituric Acid Content in Control, Oxidation, Oxidation + *Monodora Myristica* and Oxidation + Vitamin E of Palm Oil Emulsion

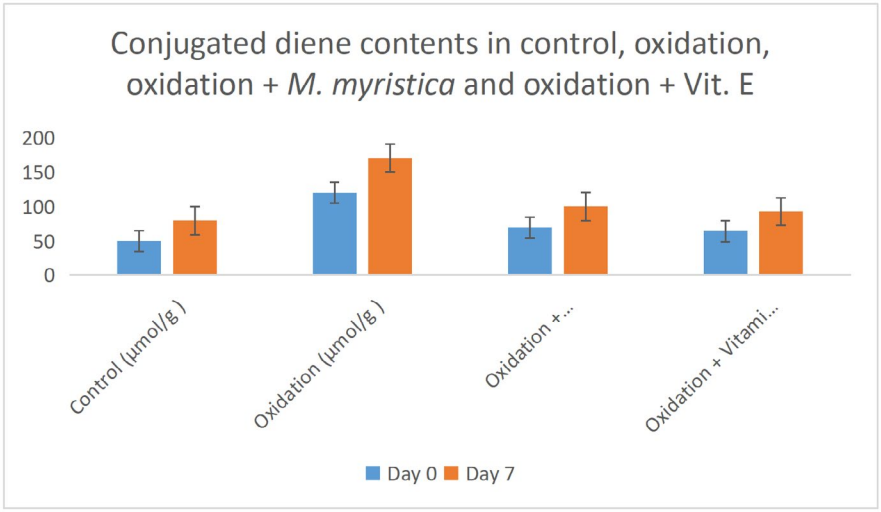

Figure 4: Effect of *Monodora Myristica* on Conjugated Diene Content in Control, Oxidation, Oxidation + *Monodora Myristica* and Oxidation + Vitamin E in Palm Fruit Emulsion.

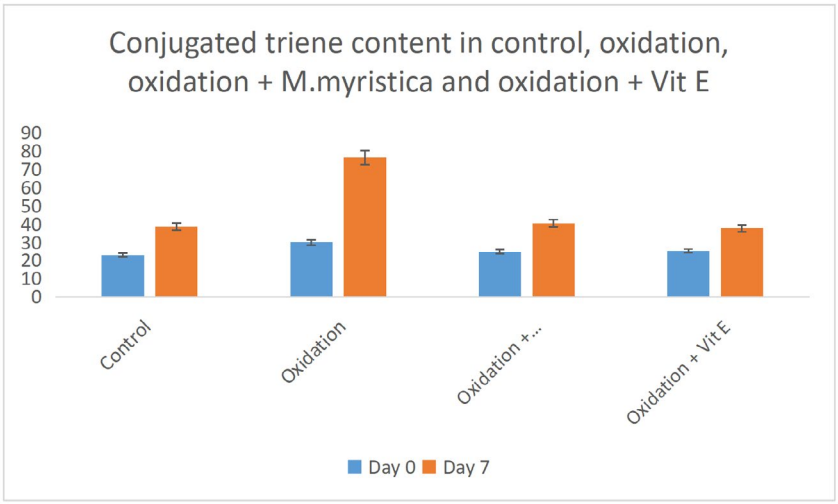

Figure 5: Effect of *Monodora MYristica* on Conjugated Triene Content in Control, Oxidation, Oxidation + *Monodora Myristica* and Oxidation + Vitamin E in Palm Fruit Emulsion

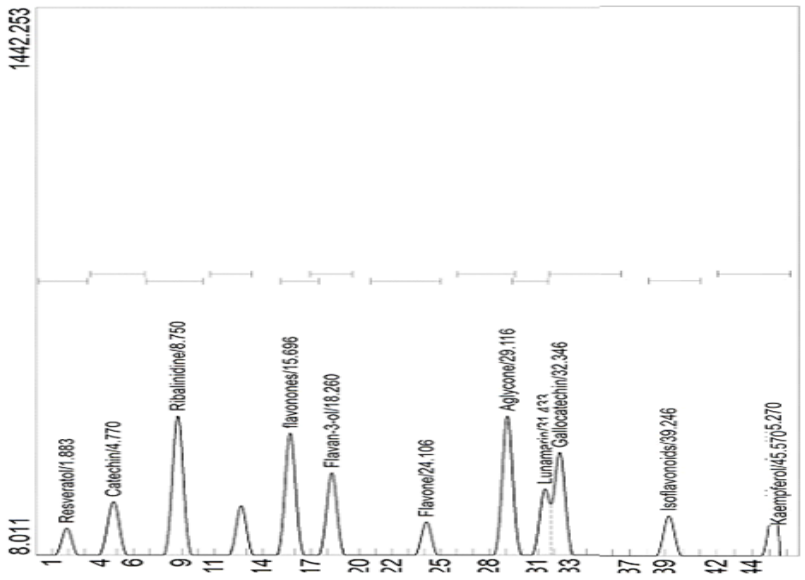

Figure 6: Showing the HPLC Chromatogram of Flavonoids in *Monodora Myristica* Extract

Component	RT	Area	Height	Concentration
Resveratrol	1.88	10310.46	79.00	11.38 µg/ml
Catechin	4.77	7981.31	148.70	22.53 µg/ml
Flavonones	15.69	15628.31	330.43	33.44 µg/ml
Flavan-3-ol	18.26	10767.58	226.43	30.48 µg/ml
Flavone	24.10	4624.65	97.99	12.65 µg/ml
Gallocatechin	32.34	13533.58	279.18	48.57 µg/ml
Isoflavonoids	39.24	5364.37	113.52	7.23 µg/ml
Kaempferol	45.27	5218.41	149.34	27.96 µg/ml

Table 1: Showing the Retention Time, Height, Area and Concentration of Flavonoids in *Monodora Myristica* Extract

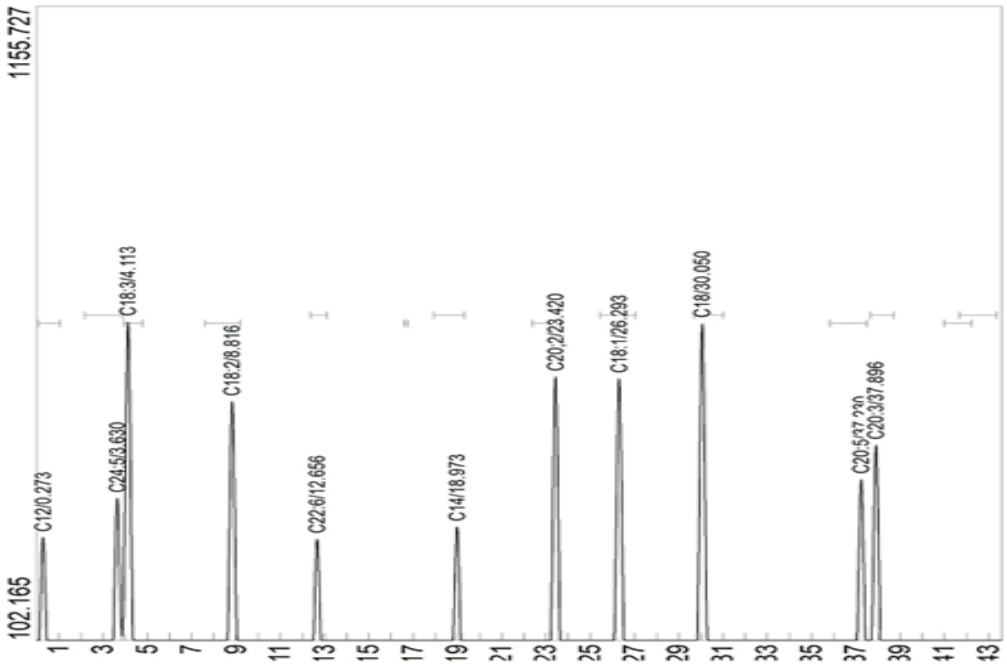

Figure 7: Showing the GC Chromatogram of Fatty Acids in Palm Fruit Emulsion

FFA	RT	Area	Height	Concentration
C12	0.273	4636.95	273.84	19.32 µg/ml
C24:5	3.630	5896.91	339.14	1.29 µg/ml
C18:3	4.113	11295.95	632.31	52.06 µg/ml
C18:2	8.816	8838.26	500.54	17.29 µg/ml
C22:6	12.656	4803.14	272.73	1.05 µg/ml
C14	18.973	5160.56	293.14	0.83 µg/ml
C20:22	23.420	9581.19	542.12	2.10 µg/ml
C18:1	26.293	9544.97	539.00	10.85 µg/ml
C18	30.050	11160.01	630.66	13.48 µg/ml
C20:5	37.230	6529.93	371.00	5.59 µg/ml
C20:3	37.896	7548.70	427.83	1.11 µg/ml
RT: retention time; FFA: free fatty acid				

Table 2: Showing the Retention Time, Height, Area and Concentration of Fatty Acids in Palm Fruit Emulsion

Statistics

All the experimental results were analyzed statistically and were expressed as mean _+ 5.0 (n= 3). The results were also subjected to ANOVA using SPSS version, 6.0.

4. Discussion

Synthetic antioxidant like BHA and BHT were widely used especially in the past but of recent it is shown that they possess adverse health effects in this regard *Monodora myristica* extract was experimented as antioxidant that can prevent, improve or mitigate the deleterious effect of free radicals and improve the shelf life of palm fruit emulsion [3,7]. Flavonoids are medicinal bioactive that possess antioxidant activity. Studies abound that showed the importance of flavonoids capable of mitigating oxidative stress and lipid peroxidation link diseases [32]. Free radical, metal ion and enzymes that are contaminants in the food industry can lead to food deterioration especially those that are lipid based during processing and storage [33]. These deterioration reactions by radical, metals and enzymes produce offensive flavours and odors there by decreasing the shelf life of the food and the market value of the food [3]. The total phenols and flavonoids in *Monodora myristica* seed extract were depicted in figure 1 the reports of phytochemical values are similar to the findings of Afolabi et al., [34].

The high performance liquid chromatography of *Monodora myristica* seeds revealed the presence of resveratrol, catechin, flavones, flavan-3-ol, flavone, gallocatechin, isoflavonoids and kaempferol. The highest concentrated flavonoid was gallocatechin 48.57 µg/ml, these flavonoids are responsible for the preventive mechanism involved in the extract of *Monodora myristica* in preventing oxidation of palm oil emulsion, this report is similar to the findings of Fadul et al., [35]. The fatty acid composition of the palm oil indicated that there are more of unsaturated fatty acids than saturated as shown in the GC-MS analysis results this is similar to the reports of Li et al., [4]. They also reported more unsaturated fatty acids in Chinese palm fruit than the palm kernel. The unsaturated fatty acids in this report incuded linolenic acid, arachidonic acid, oleic acid etc. the unsaturated fatty acid of highest concentration was linolenic acid (52.06 µg/ml).

Lipid peroxidation give rise to offensive flavour, colour, texture, and loss of nutritional unsaturated lipids, health risk is on the increase due to the formation of secondary toxic compounds [36]. In the present study the levels of lipid peroxidation increased in the oxidation control of palm fruit emulsion at zero day and day seven, but these levels were decreased when *Monodora myristica* extract was added during oxidation and also when the standard antioxidant vitamin E was added the result is depicted in fig. the report of this findings is in agreement with the work of Kamkara 2010 who also reported the antioxidant effect of *Mentha pulegium* extracts in sunflower oil.

Peroxide value determination measures the amount of peroxides and hydroperoxides in lipid samples. Peroxide determination is a nice indicator of oxidation of oils. In the present study the peroxide values of oxidation of palm fruit oil increases from day zero and day 7 due to the increase in the formation of both hydroperoxide and peroxide. The peroxide value decreases in the experiments that *Monodora myristica* extract was added or vitamin E was added, this suggested that both *Monodora myristica* or vitamin E prevented the oil from oxidation due to their antioxidant and anti-radical effects. Our study is in line with the works of Womeni et al., and Alizadeh et al., Conjugated diene and triene are produced when double and single bond rearranges to form dienes and trienes during the preliminary stages of peroxidation [37,38]. These can be detected through the maxima absorption utilizing a UV spectrophotometer. The rate of CD and CT formation decreased significantly ($p < 0.05$) in the palm fruit emulsion treated with *Monodora myristica* or vitamin E (Figures 5 and 6).

This report is in tandem with the report of Siriwardhana et al., who also reported the preventive ability of *Hizikia Fusiformis* against fish oil and linoleic acid [38]. Oxidized palm oils produced unstable molecules that give rise to: alkens, alcohols, aldehydes, ketones, and acids, which are responsible for unwanted flavour and odour.

Malondialdehyde produced as part of these unwanted molecules was determined in the present study. The results revealed that at day zero and seven the amount of MDA significantly increased as compared to control experiment without oxidation. Therefore, the addition of *Monodora myristica* or vitamin E decreases the amount of MDA to the levels found in the control experiments. This report is in agreement with the works of kamkara et al., and Siriwardhana et al., In conclusion, the experiment has shown *Monodora myristica* can be additive to edible oils [39,40]. Therefore *Monodora myristica* can be a suitable alternative for many syntehetic antioxidants.

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