COMPARATIVE FATTY ACIDS PROFILING AND ANTIOXIDANT POTENTIAL OF PAWPAW AND WATERMELON SEED OILS

Banji Adaramola[1]* and Onigbinde Adebayo[2]

[1,2]Department of Basic Science, Chemistry Unit

Babcock University, Ilishan, Remo, Ogun State, Nigeria

ABSTRACT

Seed oils of watermelon, unripe and ripe pawpaw were extracted and analyzed for their fatty acid composition using GC/EIMS, phenolic content and antioxidant potential using free radical scavenging effect on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical. Watermelon seed oil has eight fatty acids ranging from C_{16} – C_{21}, unripe pawpaw seed oil has nine fatty acids also ranging from C_{16} – C_{21} and ripe pawpaw seed oil has twelve fatty acids ranging from C_{15}-C_{27}. The major chemical component identified in both watermelon seed oil (49.41%) and unripe pawpaw seed oils (39.07%) was 11-Octadecenoic acid methyl ester while 14-Octadecenoic acid methyl ester (40.25%) was the major component identified in ripe pawpaw seed oil. Only watermelon seed oil contained an essential fatty acid; Linoleic acid (17.23%) in contrast to previous literatures values (60%). The fatty acids present in these oils are mixtures of saturated and unsaturated homologues. Ripe pawpaw seed oil shows more chemical compounds than the unripe pawpaw seed oil may be due to ripening . The phenolic contents of the oils ranged from 1.41-1.55 mgGAE/g. The three oils showed significant DDPH free radical scavenging potential. The order of inhibition are; watermelon > ripe pawpaw > unripe pawpaw seed oil. The IC_{50} values of these oils were are, 36 mg/mL, 44 mg/mL and 56 mg/mL for watermelon, ripe pawpaw and unripe pawpaw seed oil respectively. This study however did not show a positive correlation between the phenolic contents and antioxidant activity. We recommend further studies into the use of these oils for treating various specific diseases.

*Tel.: +2348039446793; E-mail address: feyimicheal37@gmail.com or milliangelo@yahoo.com

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INTRODUCTION

Edible oils from plant sources have over the years become an integral part of human diets. They are usually obtained from various parts of plants such as leaves, seeds, nuts, flowers, peels and fruits. Besides their use as foods, many of these phyto-oils have become relevant in soap, cream and biodiesel productions as well as in pharmaceuticals. Many of these oils are rich in protein, micro and macro minerals, essential and non-essential fatty acids and even antioxidants; some of which make them suitable for foods and other industrial uses. However, the fatty acids composition of vegetable and seed oils is one of the factors considered in determining their stability, usability either for food, soap making, biodiesel production and many other industrial and pharmaceutical uses; with each of the fatty acids offering certain distinct advantages. For example, the presence of certain monoglyceride, or fatty-ester types in edible oil makes it suitable for foam production while many seed oils with specific gravity ranging from 0.87 to about 0.90 meet the requirement for an oil to be used as a biodiesel [1, 2, 3]. Likewise, unsaturated fatty acids in diets have been found helpful by acting as cholesterol-lowering

agents. They are however susceptible to oxidation and may produce products that contribute to arteriosclerosis and carcinogenesis [4, 5]. Although fruit seeds account for considerable part of the total weight of fruits, most times they are disposed off as wastes and termed useless. However, few researches [6, 7] have identified the prospects of some of these so-called wastes as important sources of nutrients and bioactive substances.
Pawpaw and watermelon are good examples of such fruits whose seeds could be explored for various uses instead of the norm of disposing them off as mere wastes. An average oil content of 32-36% for watermelon, ripe pawpaw and unripe pawpaw seeds have been reported [8]; making them good economic seeds for industrial or edible oil production. They concluded that extracting oils from these seeds could reduce the amount of agricultural wastes being generated as well as serve as additional source of income for farmers and fruits' processors. Ripe and unripe pawpaw and watermelon seed oils have been reported to be rich sources of both micro and macro minerals which include phosphorus, sodium, copper and magnesium [8].

Papaya is a store house of nutrients. It is a rich source of threes strong antioxidant viz; vitamin C, vitamin A and vitamin E and minerals such as magnesium and potassium. It has also been reportedly rich in the B vitamin pantothenic acid and folate and fiber and it is used to treat all types of digestive abdominal disorders, dyspepsia, hyperacidity, dysentery and constipation, digestion of proteins, premature aging and wounds [9]. The papaya seeds are edible and have a sharp, spicy taste. They are sometimes grounded and used as a substitute for black pepper. The papaya seeds have more potent medicinal values than the flesh. They possess antibacterial properties and effective against E.coli, Salmonella and Staphylococcus infections, can eliminate intestinal parasites, detoxify the liver, act as a skin irritant to lower fever, treat piles and typhoid, and are used as anti-helminthic and anti-amoebic properties. Papaya has many phenolic compounds which may scavenge free radicals.

Watermelon seed oil, which reportedly has Linoleic acid (18:2) as the major fatty acid (~64.5%), is used for frying and cooking in some African and Middle Eastern American countries owning to its unique flavor [10]. This is similar to those found in other oils, such as sunflower seed oil, linseed and hempseed oils [11]. The use of oil is determined by many factors intrinsic of its constituents; high percentage of fatty acids in oil had been reported to influence the tendency of such substances to foam [12, 13].

This study was aimed at evaluating the chemical composition and antioxidant potential of pawpaw and watermelon seed oils so as to adjudge their probable use for food, bio-diesel production and most importantly for pharmaceuticals.

MATERIALS AND METHODS

Sample collection, preparation and seed oil extraction

The collection and preparation of pawpaw and watermelon seeds as well as oil extraction from these seeds were carried out according to the method described by [8]. Mature ripe and unripe fruits of *Carica papaya* were obtained from a *Carica papaya* tree within Babcock University premises while mature fruits of *Citrullus lanatus* were purchased from Ilishan market in Ogun state, Nigeria. The fruits were washed with distilled water, cut into two longitudinal halves and their individual seeds were manually removed. The slimy sac-like substance coating

the *Carica papaya* seeds were removed by bursting with the aid of a mortar and pestle, followed by copious washing with distilled water. The clean seeds that resulted were thereafter oven dried at 50ºC for 48 hours and then pulverized with the use of laboratory blender (LEXUS MG-2053 OPTIMA). The pulverized samples were packaged separately in waterproof polyethylene bags and stored at 4ºC until required for analysis.

Solvent extraction was carried out on 50 g of each pulverized sample with soxhlet apparatus for a period of eight hours using n-hexane as the extraction solvent. The extraction solvent was removed *in vacuo* using rotary evaporator (Eyela N-1001) at 40ºC to recover the seed oil. The oil was placed on a water bath at 50ºC for 2hours to ensure complete removal of residual solvent after which it was stored in a glass bottle and the analysis was carried out on the freshly extracted seed oils.

Total phenolic content

The total phenolic content of the oil sample was determined by the spectrophotometric method of [14] with a slightly modification. Solution of the oil sample in hexane containing 10 mg/ml was used in this assay. The reaction mixture was prepared by mixing 0.5 ml of hexane solution of oil, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO3 in a 10ml test tube. Blank was simultaneously prepared by mixing 0.5 ml hexane, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO3. The mixture was agitated for about 2 min and the sample was thereafter incubated in a thermostat (EYELA SB-1000) at 45^0C for 45 mins with constant agitation. The absorbance of the resulting mixture was read on a spectrophotometer (JENWAY 6305) at a λmax of 765 nm. The same procedure was repeated for standard solutions of Gallic acid (2-10 µg/ml, R^2=0.996) from which the calibration curve was obtained. The samples were prepared in triplicate for each analysis. Based on the measured absorbance of the oil sample, the phenolic content of the oil was read from the calibration curve and expressed as mg Gallic Acid Equivalent per gram (mgGAE g-1) of the oil.

DPPH Scavenging Activity

The method of [15] was used to estimate the antioxidant potential of the oils by evaluating their free radical scavenging effect on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical.

1.0 mL of DPPH solution (0.3mM) in methanol was added to 2.5 mL of oil solution of various concentrations (20, 40, 80 and100mg/mL) prepared in methanol and incubated in the dark at room temperature for 30 mins. Gallic acid solution at various concentrations (2, 4, 6, 8 and 10µg/ml) in methanol was similarly treated. The absorbance of the resulting mixture was taken at 518nm on a spectrophotometer (JENWAY 6305) and converted to percentage inhibition using the equation;

$$\% \text{ inhibition of DPPH} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100 \quad \_\_\_\_\_ 1$$

Each concentration was analyzed in triplicate. A control was prepared by adding 2.5ml methanol to 1ml of 0.3mM DPPH solution in methanol. IC50 value which represents the concentration of the compounds that caused 50% inhibition of free radical formation was obtained by interpolation from linear regression analysis [16].

Fatty Acids' Content

The fatty acids methyl esters of the seed oils were prepared using the method described by [17]. A mixture of about 1ml of oil with 5 ml 3% sulphuric acid in absolute methanol and 2 ml of benzene was made in a test tube and heated for 90 minutes at 90 ^{o}C. After cooling, about two (2) drops of distilled water were added to achieve phase separation and the resulting methyl esters were extracted 3 times with 2 ml hexane each time. The organic phase containing the methyl esters was separated, and filtered through anhydrous sodium sulphate. After cooling, the chemical composition of the methylated oil was determined with a gas chromatograph (GCMS-QP2010Plus).

RESULTS AND DISCUSSION

Fatty Acid composition of watermelon, ripe and unripe pawpaw seed oils

The results of the fatty acid compositions of seed oils of watermelon, unripe pawpaw and ripe pawpaw are represented in Tables 1, 2 and 3 respectively. The GC/EIMS chromatogram of the seed oils are also shown in Fig 1. The results showed the presence of eight fatty acids ranging from C_{16} – C_{21} in watermelon seed oil, nine fatty acids also ranging from C_{16} – C_{21} in unripe pawpaw seed oil and twelve fatty acids ranging from C_{15}-C_{27} in the ripe pawpaw seed oil. The major chemical component identified in both watermelon seed oil (49.41%) and unripe pawpaw seed oils (39.07%) was 11-Octadecenoic acid, methyl ester while 14-Octadecenoic acid, methyl ester (40.25%) was the major component identified in ripe pawpaw seed oil. The least occurring compounds were Arachidic acid methyl ester (0.85%), Palmitoleic acid methyl ester (0.45%) and11-Octadecenoic acid, methyl ester (0.45%) in watermelon, unripe and ripe pawpaw seed oils respectively. Meanwhile, in contrast to several authors [5, 18] who have reported Linoleic acid as the major component (at about 60%) of watermelon seed oil, the results of this present study showed 11-Octadecenoic acid as it major component while Linoleic acid occurred at a concentration of 17.23%. This disparity may be due to variation in species, age, maturity or level of ripening, soil and many other areas of variation. The three oils contains a mixture of saturated and unsaturated fatty acids but more of saturated fatty acids hence might not be that suitable for long term consumption but might find suitable use in soap making [8]. Three (3) fatty acids namely Palmitic acid, Stearic acid and Arachidic acids were found common in each of the three (3) oils. However, only watermelon seed oil contained an essential fatty acid; Linoleic acid. It also contained Ricinoleic acid which was absent in the two (2) pawpaw seed oils. Palmitoleic acid, Oleic acid and Cyclopropaneoctanoic acid, 2-hexyl- methyl ester were found at almost the concentrations in both ripe pawpaw seed oil (0.48%, 23.37%, 1.09%) and unripe pawpaw seed oil (0.45%, 25.09%, 0.99%) but were absent in watermelon seed oil. Myristic acid (0.33%) and Hexacosanoic acid (0.50%) were found in ripe pawpaw seed oil but absent in both ripe pawpaw seed and watermelon seed oils. The presence of more chemical compounds in the ripe pawpaw seed oil than the unripe pawpaw seed oil may imply that ripening of the fruit has some effects on the number and concentration of constituents, especially fatty acids present in its seed oil. However, there were fewer other chemicals apart from the fatty acids in the seed oils and these included; 2-Ethyl-2-Hexenal present in the three (3) oils, an unknown compound with a molecular formular of $C_{18}H_{31}O_2$ which is suspected to be fragment of a higher molecular weight fatty acid in the unripe pawpaw seed

oil and Geijerone present in the ripe pawpaw seed oil.

Table 1: Fatty Acid Chemical composition of

Watermelon Seed Oil

Peak #	Retention Time	Component	% Composition
1	4.092	2-Ethyl-2-hexenal ($C_8H_{14}O$)	1.20
2	15.457	Pentadecanoic acid, 14-methyl, methyl ester ($C_{17}H_{34}O_2$)	14.07
3	15.925	Palmitic acid ($C_{16}H_{32}O_2$)	3.14
4	17.213	11-Octadecenoic acid, methyl ester($C_{19}H_{36}O_2$)	49.41
5	17.392	Stearic acid methyl ester ($C_{19}H_{38}O_2$)	10.30
6	17.602	Linoleic acid, methyl ester ($C_{19}H_{34}O_2$)	17.23
7	17.799	Stearic acid ($C_{18}H_{36}O_2$)	2.92
8	18.878	Ricinoleic acid, methyl ester. ($C_{19}H_{36}O_3$)	0.88
9	19.134	Arachidic acid methyl ester ($C_{21}H_{42}O_2$)	0.85

Table 2: Fatty Acid Chemical composition of Unripe Pawpaw Seed Oil

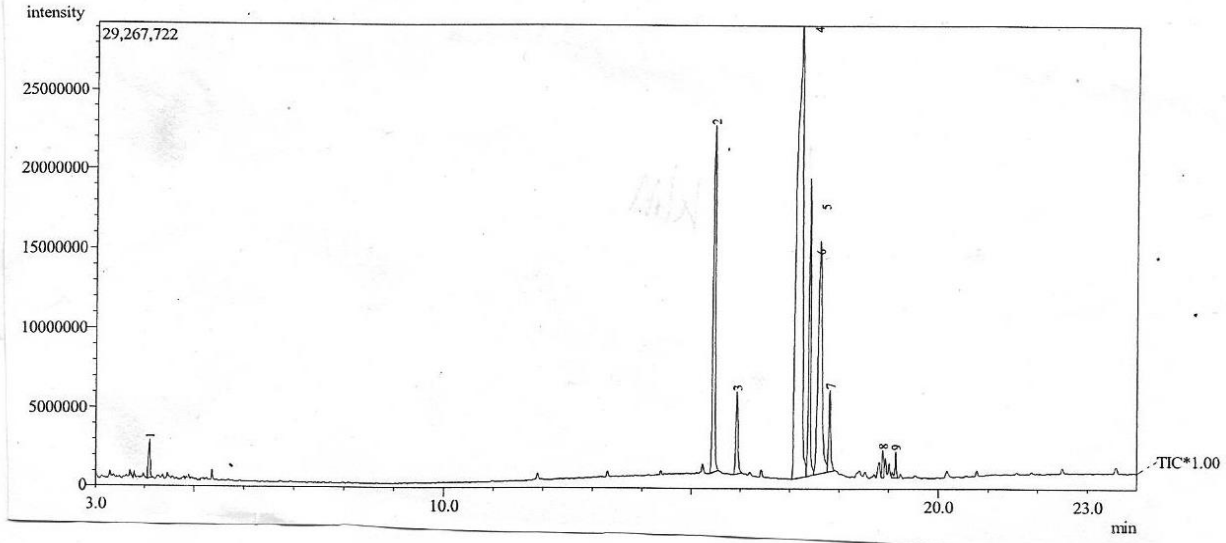
Peak #	Retention Time	Component	% Composition
1	4.094	2-Ethyl-2-hexenal ($C_8H_{14}O$)	0.94
2	15.226	Palmitoleic acid methyl ester ($C_{17}H_{32}O_2$)	0.45
3	15.454	Palmitic acid methyl ester ($C_{17}H_{34}O_2$)	13.80
4	15.925	Palmitic acid ($C_{16}H_{32}O_2$)	6.00
5	17.222	11-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)	39.07
6	17.379	Stearic acid methyl ester ($C_{19}H_{38}O_2$)	8.79
7	17.663	Oleic acid ($C_{18}H_{34}O_2$)	25.09
8	17.808	Stearic acid ($C_{18}H_{36}O_2$)	3.90
9	18.931	Cyclopropaneoctanoic acid-2-hexyl-methyl ester ($C_{18}H_{34}O_2$)	0.99
10	19.136	Arachidic acid methyl ester ($C_{21}H_{42}O_2$)	0.96
11	20.769	Unknown ($C_{18}H_{31}O_2$)	0.46

Table 3: Fatty Acid Chemical composition of Ripe Pawpaw Seed Oil

Peak #	Retention Time	Component	% Composition
1	4.096	2-Ethyl-2-hexenal ($C_8H_{14}O$)	0.60
2	12.544	Geijerone(3-Isopropenyl-4-methyl-4-vinylcyclohexanone($C_{12}H_{21}O$)	0.61
3	13.306	Myristic acid methyl ester- ($C_{15}H_{30}O_2$)	0.33
4	15.227	Palmitoleic acid methyl ester ($C_{17}H_{32}O_2$)	0.48
5	15.458	Palmitic acid, methyl ester ($C_{17}H_{34}O_2$)	13.71
6	15.927	Palmitic acid ($C_{16}H_{32}O_2$)	5.03
7	17.233	14-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)	40.25
8	17.384	Stearic acid, methyl ester ($C_{19}H_{38}O_2$)	9.20
9	17.660	Oleic acid ($C_{18}H_{34}O_2$)	23.37
10	17.806	Stearic acid ($C_{18}H_{36}O_2$)	3.15
11	18.931	Cyclopropaneoctanoic acid, 2-hexyl- methyl ester ($C_{18}H_{34}O_2$)	1.09
12	19.135	Arachidic acid, methyl ester ($C_{21}H_{42}O_2$)	1.23
13	19.239	11-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)	0.45
14	20.767	Hexacosanoic acid methyl ester ($C_{27}H_{54}0_2$)	0.50

Phenolic content of Watermelon, ripe Pawpaw and Unripe Pawpaw Seed Oils

The results of the phenolic content of the oil samples are represented in Table 4. The phenolic content of the oils ranged from 1.41± 0.01 to 1.55± 0.01mgGAE/g and is shown to be in the order; unripe pawpaw seed oil > watermelon seed oil > ripe pawpaw seed oil. The phenolic contents of the oils are however not too far from each other. Phenolics are a group of polyphenols that have been known for their prominence in functioning as antioxidants. They are able to perform this function because of their possession of electrons or hydrogen atoms which they donate and with which they are able to arrest the free radicals or terminate the continuous chain of free radical formation. Once the free radical formation chain is terminated by these antioxidants, then the various oxidation reactions that occur due to the presence of these free radicals are also invariably terminated thereby protecting the body from diseases such as cancer, cardiovascular diseases, cataract formation that result from the presence or propagation of the free radicals also referred to as oxidants. The presence of phenolics in these oils therefore makes them good prospects to be exploited for antioxidant uses in the pharmaceutics. Phenolics are however not the only group of phytochemicals that confer antioxidant properties on plants.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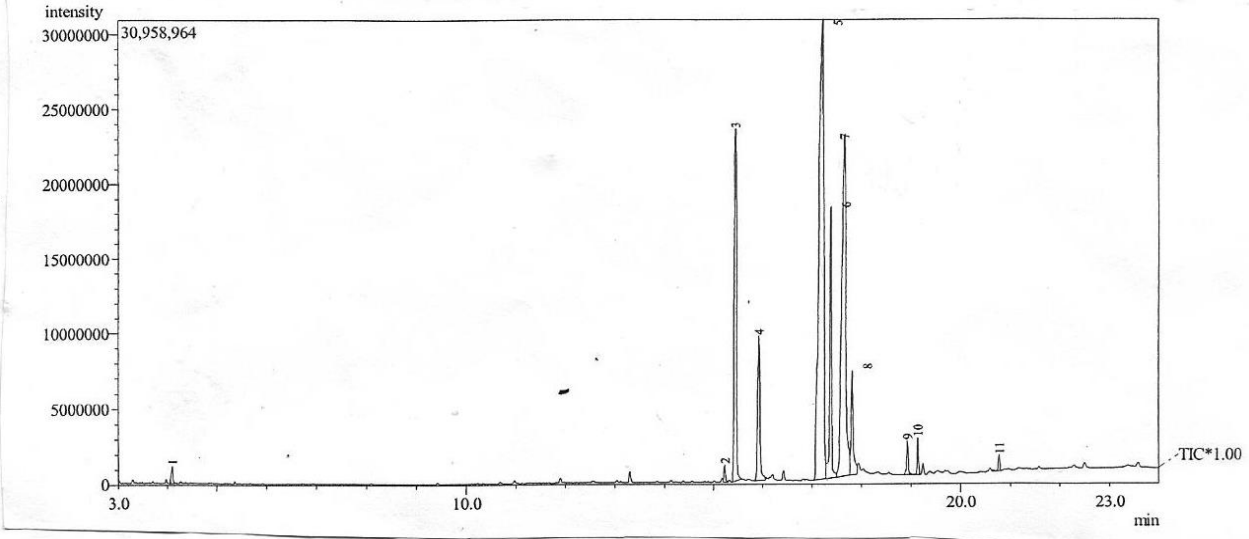
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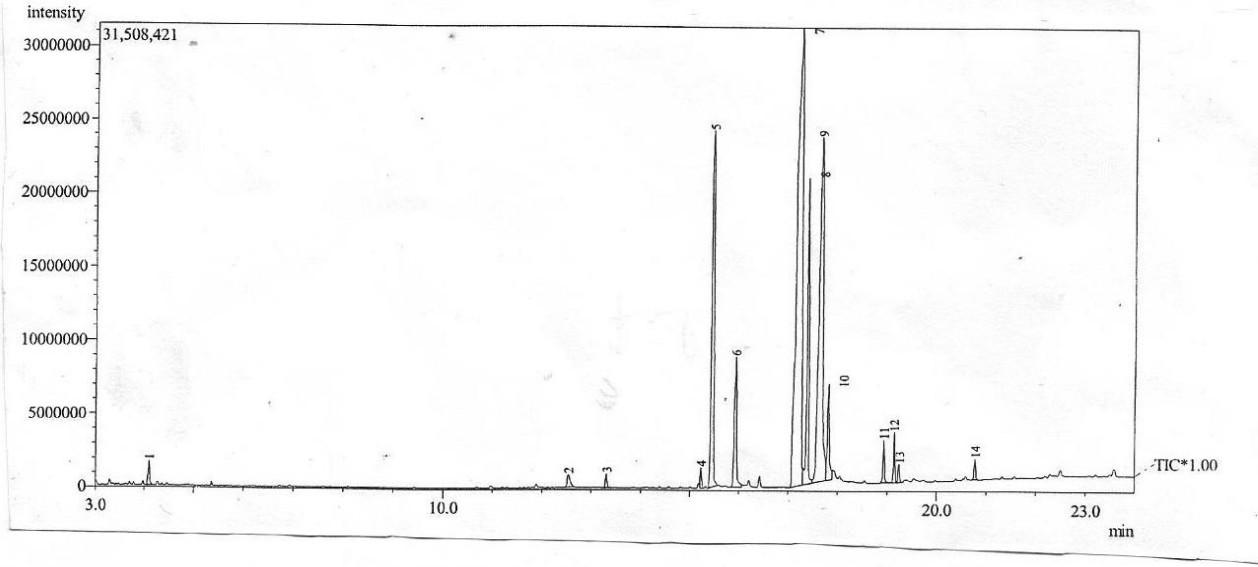
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Figure 1: GCMS Chromatograms of (a) Watermelon seed oil (b) Unripe pawpaw seed oil and (c) Ripe pawpaw seed oil

Table 4 TOTAL PHENOLIC CONTENT OF OIL SAMPLES

Oil Sample	Phenoilc Content mgGAE/g
WMS oil	1.50 ± 0.01
URPS oil	1.55 ± 0.01
RPS oil	1.41 ± 0.01

Data are expressed as mean ± standard error of three replicates. RPS=Ripe Pawpaw Seed URPS= Unripe Pawpaw Seed WMS= Watermelon Seed

Antioxidant potential of Watermelon, ripe Pawpaw and Unripe Pawpaw Seed Oils

Table 5 shows the results of the antioxidant activity of the oils of watermelon, ripe and unripe pawpaw seeds obtained by evaluating the free radical scavenging effect of the seed oils on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical. Gallic acid was used as the reference substance and its DDPH radical scavenging ability is represented in Table 6. The three oils showed significant DDPH radical scavenging potential but at about three orders of magnitude lesser than Gallic acid; the reference substance. The mean percentage inhibition of DDPH radical by the oils ranged from 45.96±0.14 to 65.28±0.11% with the watermelon seed oil showing the highest inhibition followed by ripe pawpaw seed oil and the least inhibition shown by the unripe pawpaw seed oil. This order of the antioxidant capacity of the seed oils was also reflected in their IC50 values; which represent the concentration of the sample that scavenges 50% of the free radicals. The higher the antioxidant strength of a sample, the lower its amount needed to cause significant scavenging of free radicals. The results showed that watermelon seed oil with the highest percentage free radical inhibition was required at the least concentration; ~36 mg/mL to cause 50% free radical inhibition (i.e. lowest IC50 value). Ripe pawpaw seed oil required ~44mg/mL while the unripe pawpaw seed oil with the lowest percentage inhibition showed the highest IC50 value of ~56 mg/ml required to inhibit 50% of the free radicals. This suggests that the best antioxidant activity of pawpaw seed oil is obtained from the seed of ripe pawpaw fruit. The higher the percentage inhibition of free radicals by plant extracts, the lower the IC50 value and vice-versa. Gallic acid scavenged the DPPH free radical sufficiently well at microgram concentration such that at 10µg/ml, its percentage inhibition of DPPH free radical was ~95 %; on the average it inhibited ~74 % of the free radical and it showed an IC50 of 3.82µg/ml. However, despite the fact that the antioxidant capacity of the three seed oils was shown to be lower than that of Gallic acid in three orders of magnitude, the oils however still showed significant antioxidant potential. This suggests that the three oils apart from been nutritious with a lot of minerals [8] may also be good sources of antioxidants; therefore making them suitable prospects for pharmaceutical purposes. The consumption should however be monitored to avoid excessive in-take due to the presence of more saturated fatty acids in the oils as determined in this work making them unsuitable for long term consumption. Many authors have reported positive correlation

between phenolic content and antioxidant activities of medicinal plants or their extracts [19, 20, 21, 22, 23]. This present study however does not exactly show a correlation between them. This is because, while the phenolic contents of the oils occurred in the order URCPS oil > WMS oil > RCPS oil, the antioxidant activity of the oils occurred in the order WMS oil > RCPS oil > URCPS oil. This may imply that the antioxidant activity of these oils is not due to the presence of the phenolic substances alone, rather may be due to the presence of other substances such as the fatty acids or synergistic effect of other constituents with the phenolic substances. This is because antioxidant strength of plant extracts does not depend on the presence of phenolic compounds alone. Other phytochemicals that may be responsible for or contribute to the antioxidant activity of plants extracts include but not limited to ascorbic acid, carotenoids and fat-soluble vitamin [24]. However, the seed oil of ripe pawpaw was higher in terms of phenolic content and DPPH scavenging capacity than the seed oil of unripe pawpaw. Worthy of note is the fact that, watermelon seed oil which was the only oil with Linoleic acid, an essential fatty acid, also showed the best antioxidant activity. Meanwhile, the possession of two additional fatty acids; Myristic acid and Hexacosanoic acid by the ripe pawpaw seed oil may be responsible for its better antioxidant activity over the unripe pawpaw seed oil.

Table 5 DPPH SCAVENGING ACTIVITY OF OIL SAMPLES

Sample	DPPH Scavenging Activity (%)					Mean%DPPH Inhibition	IC50(mg/mL)
Conc. (mg/ml)	10	20	40	80	100		
WMS oil	26.70±0.28	56.70±0.28	69.00±0.42	84.00±0.14	90.00±0.28	65.28±0.11	36.04±0.11
URPS oil	4.50±0.14	26.60±0.28	42.80±0.21	73.70±0.21	82.20±0.28	45.96±0.14	55.51±0.41
RPS oil	18.30±0.35	35.10±0.14	66.70±0.50	84.10±0.14	85.30±0.07	57.90±0.04	43.80±0.04

Data are expressed as mean ± standard error of three replicates. RPS=Ripe Pawpaw Seed URPS= Unripe Pawpaw Seed WMS= Watermelon Seed

Table 6 DPPH SCAVENGING ACTIVITY (%) FOR GALLIC ACID STANDARD

Conc.(µg/ml)	%DPPH Inhibition	Mean % DPPH Inhibition	IC50 (µg/ml)
2	34.87±0.03	73.39±0.22	3.82 ± 0.01
4	61.55±0.32		
6	85.50±0.51		
8	90.10±0.21		
10	94.91±0.02		

Data are expressed as mean ± standard error of three replicates

CONCLUSION

This present study examined comparatively the fatty acid compositions as well as the antioxidant potential of oils obtained from seeds of watermelon, unripe and ripe pawpaw. The results of the analysis of these oils showed the presence of various fatty acids ranging from C15-C27. Watermelon seed oil contained eight fatty acids; unripe pawpaw seed oil contained eleven while ripe pawpaw seed oil had twelve fatty acids. The unripe and ripe pawpaw seed oil had no essential fatty acids while watermelon seed oil had one; Linoleic acid at a concentration of 17.23 %. Also, Myristic and Hexacosanoic acids were found present in the ripe pawpaw seed oil but absent in the unripe pawpaw seed oil. Owning to the fact that more fatty acids were discovered in the oil of the ripe pawpaw seed, we conclude that ripening of pawpaw fruit may enhance the number and concentration of fatty acids present in its seed oil. The oils contain mixtures of saturated and unsaturated fatty acids with more saturated fatty acids. This makes the oils not recommended for long term consumption. However, because there are useful minerals present in the oils, they may be useful for treating specific ailments but not for long term consumption. In the same vein, the antioxidant potential of these oils was also examined by determining their phenolic content and ability to scavenge standard DPPH free radical in comparison with Gallic acid; a standard antioxidant. The highest phenolic content was obtained for watermelon seed oil while the least was obtained for unripe pawpaw seed oil. These seed oils showed significant free radical scavenging activity; although at three order of magnitude less than Gallic acid. The order of free radical inhibition by the three oils was watermelon seed oil> ripe pawpaw seed oil> unripe pawpaw seed oil. This was also reflected in their IC50 values with watermelon and unripe pawpaw seed oils showing the least and highest IC50 values respectively. The possession of significant antioxidant activity makes the oils suitable prospects for pharmaceutical purposes. Therefore based on our findings in this present study as well as in our previous work on these seed oils, further works are recommended to look into the potential usability of each of these oils for treating specific diseases.

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COMPETING INTERESTS

This is to declare that no competing interests exit as far as this research work is concerned.

AUTHORS' CONTRIBUTIONS

Author 1 designed the study. Each author contributed equally to the analysis of the samples and generation of data. Authors also contributed equally to the development of the manuscript and also the proofreading of the final manuscript. Both authors read and approved the final manuscript.

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Table 1: Fatty Acid Chemical composition of Watermelon Seed Oil

Peak #	Retention Time	Component	% Composition
1	4.092	2-Ethyl-2-hexenal ($C_8H_{14}O$)	1.20
2	15.457	Pentadecanoic acid, 14-methyl, methyl ester ($C_{17}H_{34}O_2$)	14.07
3	15.925	Palmitic acid ($C_{16}H_{32}O_2$)	3.14
4	17.213	11-Octadecenoic acid, methyl ester($C_{19}H_{36}O_2$)	49.41
5	17.392	Stearic acid methyl ester ($C_{19}H_{38}O_2$)	10.30
6	17.602	Linoleic acid, methyl ester ($C_{19}H_{34}O_2$)	17.23
7	17.799	Stearic acid ($C_{18}H_{36}O_2$)	2.92
8	18.878	Ricinoleic acid, methyl ester. ($C_{19}H_{36}O_3$)	0.88
9	19.134	Arachidic acid methyl ester ($C_{21}H_{42}O_2$)	0.85

Table 2: Fatty Acid Chemical composition of Unripe Pawpaw Seed Oil

Peak #	Retention Time	Component	% Composition
1	4.094	2-Ethyl-2-hexenal ($C_8H_{14}O$)	0.94
2	15.226	Palmitoleic acid methyl ester ($C_{17}H_{32}O_2$)	0.45
3	15.454	Palmitic acid methyl ester ($C_{17}H_{34}O_2$)	13.80
4	15.925	Palmitic acid ($C_{16}H_{32}O_2$)	6.00
5	17.222	11-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)	39.07
6	17.379	Stearic acid methyl ester ($C_{19}H_{38}O_2$)	8.79
7	17.663	Oleic acid ($C_{18}H_{34}O_2$)	25.09
8	17.808	Stearic acid ($C_{18}H_{36}O_2$)	3.90
9	18.931	Cyclopropaneoctanoic acid-2-hexyl-methyl ester ($C_{18}H_{34}O_2$)	0.99
10	19.136	Arachidic acid methyl ester ($C_{21}H_{42}O_2$)	0.96
11	20.769	Unknown ($C_{18}H_{31}O_2$)	0.46

Table 3: Fatty Acid Chemical composition of Ripe Pawpaw Seed Oil

Peak #	Retention Time	Component	% Composition
1	4.096	2-Ethyl-2-hexenal ($C_8H_{14}O$)	0.60
2	12.544	Geijerone(3-Isopropenyl-4-methyl-4-vinylcyclohexanone($C_{12}H_{21}O$)	0.61
3	13.306	Myristic acid methyl ester-($C_{15}H_{30}O_2$)	0.33
4	15.227	Palmitoleic acid methyl ester ($C_{17}H_{32}O_2$)	0.48
5	15.458	Palmitic acid, methyl ester ($C_{17}H_{34}O_2$)	13.71
6	15.927	Palmitic acid ($C_{16}H_{32}O_2$)	5.03
7	17.233	14-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)	40.25
8	17.384	Stearic acid, methyl ester ($C_{19}H_{38}O_2$)	9.20
9	17.660	Oleic acid ($C_{18}H_{34}O_2$)	23.37
10	17.806	Stearic acid ($C_{18}H_{36}O_2$)	3.15
11	18.931	Cyclopropaneoctanoic acid, 2-hexyl- methyl ester ($C_{18}H_{34}O_2$)	1.09
12	19.135	Arachidic acid, methyl ester ($C_{21}H_{42}O_2$)	1.23
13	19.239	11-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)	0.45
14	20.767	Hexacosanoic acid methyl ester ($C_{27}H_{54}0_2$)	0.50

Table 4 TOTAL PHENOLIC CONTENT OF OIL SAMPLES

Oil Sample	Phenoilc Content mgGAE/g
WMS oil	1.50 ± 0.01
URPS oil	1.55 ± 0.01
RPS oil	1.41 ± 0.01

Data are expressed as mean ± standard error of three replicates. RPS=Ripe Pawpaw Seed URPS= Unripe Pawpaw Seed WMS= Watermelon Seed

Table 5 DPPH SCAVENGING ACTIVITY OF OIL SAMPLES

Sample	DPPH Scavenging Activity (%)		Mean% DPPH Inhibition			IC50(mg/mL)	
Conc. (mg/ml)	10	20	40	80	100		
WMS oil	26.70±0.28	56.70±0.28	69.00±0.42	84.00±0.14	90.00±0.28	65.28±0.11	36.04±0.11
URPS oil	4.50±0.14	26.60±0.28	42.80±0.21	73.70±0.21	82.20±0.28	45.96±0.14	55.51±0.41
RPS oil	18.30±0.35	35.10±0.14	66.70±0.50	84.10±0.14	85.30±0.07	57.90±0.04	43.80±0.04

Data are expressed as mean ± standard error of three replicates. RPS=Ripe Pawpaw Seed URPS= Unripe Pawpaw Seed WMS= Watermelon Seed

Table 6 DPPH SCAVENGING ACTIVITY (%) FOR GALLIC ACID STANDARD

Conc.(µg/ml)	%DPPH Inhibition	Mean % DPPH Inhibition	IC50 (µg/ml)
2	34.87±0.03	73.39±0.22	3.82 ± 0.01
4	61.55±0.32		
6	85.50±0.51		
8	90.10±0.21		
10	94.91±0.02		

Data are expressed as mean ± standard error of three replicates