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Antioxidant and cytotoxicity effects of seed oils from edible fruits

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ABSTRACT

Objective: To propose a natural remedy for the some acute diseases the fatty acids profile, antioxidant and cytotoxicity potentials of seed oils from natural sources have been examined. **Methods:** The fatty acids profile of seed oils from sweet orange, grape, lime and watermelon obtained by soxhlet extraction were trans-esterified and examined by gas chromatography-mass spectrometry (GC-MS). The antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl assay were examined and compared with gallic acid and α-tocopherol while the cytotoxicity were examined via the brine shrimp cytotoxicity assay using cyclophosphamide as a reference standard. **Results:** Sweet orange seed contained 9,12-octadecadienoic acid (62.18%), grape seed, erucic acid (43.17), lime seed, oleic acid (52.42%) and watermelon seed linoleic acid (61.11%) as the major fatty acid present. Among the four oils tested, grape seed oil had the highest acute toxicity with LC_{50} value of (156.2 ± 0.37) μg/mL while orange seed oil had the highest lethal toxicity with LC_{50} (7.59 ± 0.35) μg/mL value. Lime seed oil IC_{50} (14.49 ± 3.54) μg/mL showed the highest antioxidant potential of about 70% at 1 mg/mL concentration which was more significant than the reference compounds gallic acid and α-tocopherol with IC_{50} value of (201.10 ± 1.65) and (54.86 ± 2.38) μg/mL respectively. The yield of oil from these seeds varied from 9.583% to 24.790% with the oils being rich in essential fatty acids. **Conclusion:** Utilization of the seeds will reduce wastes, improve commercialization and procures hitherto neglected substances for technological and nutritional applications.

1. Introduction

Experiments involving human have shown significant connections between antioxidants such as vitamins A and E, and carotenoids (alpha-carotene, beta-carotene, xanthine, beta-cryptoxanthine, lycopene) and acute diseases[1]. More recently, Curcumin a natural food addictive was reported to be a shield against acute and chronic diseases[2]. Recently, warfain, a synthetic derivative of naturally occurring dicoumarol was observed to induce sublingual hematoma, an acute situation[3]. The advocacy for the use of natural product for the treatment of acute, subacute and chronic diseases is gaining reputation.

Fruits seeds are waste products of the wine and juice industries but a proper utilization of these waste products could lead to an important new source of oil, nutraceuticals and meal thereby limiting the problem of disposal. Seed oils from the seed of edible fruits are important common food ingredients and growing evidence has suggested that individual fatty acids from seeds may play different roles in human health especially in acute and chronic diseases management. Diets rich in specific fatty acid may provide potential prevention of a number of health problems or diseases. For instance, omega-3 (n-3) unsaturated fatty acids have been suggested to possess health benefits which include the prevention of cancer, heart diseases, hypertension, and autoimmune disorders[4]. Currently, consumer's growing interest in improving their dietary nutrition is driving the development of novel seed oils having unique fatty acid profiles and other beneficial components, including phytosterols and natural antioxidants[5].

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Watermelon (*Citrullus vulgaris*) is taxonomically classified as a member of the Curcubitaceae family, which is also known as the gourd family. Other gourds include pumpkins, cucumbers, squash, and other melons. It prefers warm climate growing conditions and is produced worldwide where conditions permit. The fruit is widely consumed in regions where it grows and the seed are sometimes extracted traditionally for its highly nutritive oil[6].

Orange fruits are the most popular ones for consumers throughout the world due to their pleasant flavors and nutritional value[7]. The fruits are both consumed fresh and industrially processed. The pulps, which are rich in soluble sugars and significant amounts of vitamin C[8] are mainly process into juice while the seed are mainly discarded.

Acid lime (Citrus aurantifolia Swingle) is an important citrus fruit crop in many countries. It is generally grown under both tropical and subtropical climatic conditions in the plains and up to 1 200 m. It is a good source of vitamin C and has good antioxidant properties; it is an appetizer, stomachic, antiscorbutic and antihelmintic and it checks biliousness[9].

Grapefruit (Citrus paradisi Macf.) contain various functional components including flavonones (naringin, and narirutin), carotenoids, limonoids (limonin, nomilin, and limonoid glucosides), folic acid, aurapatene, and vitamin C. which have potential benefits some of which includes cancer and cardiovascular disease prevention in human[10].

With the growing popularity of seed and vegetable oils as great sources of sustaining oil (for cooking) in various homes, owing largely to consumers keen concern about the ratio of saturated and unsaturated fatty acids in the diet, the lipid composition of fruit and vegetable has recently received special attention. Consumers are especially interested in essential fatty acids, with emphasis on the health implication of polyunsaturated fatty acid. Oils with unique fatty acid profile from seeds of edible fruits hold promising answers for home use oil, tailor-made nutraceuticals and functional foods. In the light of the economic and pharmacological importance of seed oils from edible fruits, the fatty acid profiles, antioxidant potential and cytotoxicity of three rutaceae family: lime, orange, grape and one curcubitaceae family: water-melon seed oil have been evaluated.

2. Materials and methods

2.1.Chemicals

α - Tocopherol, Gallic acid, DPPH and solvent (analytical grade) were obtained from Sigma-Aldrich (Germany).

2.2. Instruments

Absorbance measurements for the DPPH assays were recorded on a UV/Vis Spectrumlab 23A Spectrophotometer (England). The infrared spectrum of the oil was recorded using a Shimadzu (8400S) Fourier Transform-Infrared using KBr pellet. A Gas Chromatography- Mass Spectroscopy (GCMS-QP 2010) PLUS (Shimadzu Japan) system coupled with a finigan MAT ion trap detector was used with the column being an RTX5MS column packed with 100% grade dimethylpolysiloxane. The GC-MS was operated under the following conditions; column temperature was initially held at 60 ℃ for 5 min with injection volume of 1 μL and then programmed to rise at the rate of 5 ℃ per min to 250 ℃. The injector temperature was set at 200 ℃ while the detector (mass spectrophotometer) temperature was maintained at 250 ℃. Helium was used as the carrier gas at a linear velocity of 46.3 cm/s and pressure of 100.2 Kpa. Ionization mode was electron impact (EI) at a voltage of 70 eV. Identification of the volatile component was carried out using the peak enrichment technique of reference compounds and as final confirmation of the peak identification by GC-MS, their spectral were compared with those of NIST library mass spectra.

2.3. Materials

The fruits, bearing the seeds were purchased from a local market in Lagos, Nigeria. All the solvents used were of analytical grade. They were obtained along with other chemicals as previously stated.

2.4. Extraction and methanolysis of fixed oil

The seeds were carefully obtained from the fruits and air-dried. 30 g each of the air-dried seeds were pulverized and extracted with hexane for about 5 h in a soxhlet extractor. The oil extracts were concentrated under reduced pressure. Oil (1 mL) was dissolved in 20 mL petroleum ether and 2 mL methanolic KOH (2 M). The mixture was shaken for 2 min and allowed to stand for about 30 min. The upper layer was removed and washed with water[11]. The resulting Fatty acid methyl esters (FAME) were subjected to GC-MS analysis.

2.5. Antioxidant assay

The free radical scavenging activity of the extract was examined in vitro using the DPPH assay. This spectrophotometric assay was carried out according to the modified method previously described[12,13]. The DPPH free radical was prepared at a 0.1 mM concentration in methanol

and protected from light after preparation. Stock solutions of the seed oils (1 mg/mL) were prepared and diluted to final concentrations of 500, 250, 200, 100 and 50 μg/mL in methanol. 1 mL of 0.1 mM DPPH methanol solution was added to solutions of the sample or standards (α-tocopherol and gallic acid separately) and incubated for 30 min in the dark. The absorbance was determined at 518 nm. Blank experiment was also carried out to determine the absorbance of DPPH before interacting with the sample. The antioxidant activity, AA was calculated using the equation given below. The IC_{50} was determined on graphpad prism 3 software through a non-regression analysis. The IC_{50} was taken as the concentration that scavenged 50% of the radicals.

$AA = 100 \times [(Abs_{control} - Abs_{sample})]/(Abs_{control})$

Where $Abs_{control}$ is the absorbance of the control sample and Abs_{sample} is the absorbance of the various samples.

2.6. Artemia salina lethality test

The brine shrimp larva Artemia salina is highly sensitive to a variety of chemical substances and therefore used for the determination of cytotoxicity. The method described by Hossain et al[14] was adopted with slight modifications. The non-esterified seed oils were dissolved in DMSO and made up with natural sea water to 5 mL in four different concentrations (1 000, 200, 20 and 2 μg/mL). The test was carried out in replicate and a control was included with only DMSO in seawater. Eggs were sown in natural sea water and nauplii were obtained after 48 h and inoculated with the test samples. After one hour and 20 h of incubation at room temperature in the light, the number of survivors in each test tube was calculated for acute and lethal toxicity respectively. Cyclophosphamide was used as a standard cytotoxic test drug. Using the dose response curve, the LC_{50}

Table 1
Physico-chemical properties of the seed oils.

Seed oil	%Yield	Physical colour	State at room temp.
Lime seed oil	24.790	Golden yellow	Liquid
Grape seed oil	9.583	Yellow	Solid
Orange seed oil	12.279	Yellow	Liquid
Water melon seed oil	22.795	Orange	Liquid

Table 2
% Fatty acids profile of the seed oils.

Fatty acids	Short name	Sweet orange seed oil (%)	Grape seed oil (%)	Lime seed oil (%)	Water melon seed oil (%)
Hexadecenoic acid	C16:1, n-9	–	–	1.16	–
Palmitic acid	C16:0	27.53	33.90	26.17	12.49
Margaric acid	C17:0	–	–	0.74	–
Oleic acid	C18:1 n-9	–	–	52.42	–
Linoleic acid	C18:2 n-9,12	1.67	10.23	–	61.75
Linolenic acid	C18:3 n-9,12,15	–	–	1.30	–
9,12-Octadecadienoic acid	C18:2 n-9,12	62.18	–	–	–
16-Octadecenoic acid	C18:1 n-16	–	–	–	15.21
Stearic acid	C18:0	8.61	12.69	16.46	9.94
Arachidic acid	C20:0	–	–	1.74	0.61
Erucic acid	C22:1 n-13	–	43.17	–	–
Saturated		36.14	46.59	45.11	23.04
Monounsaturated		–	43.17	53.58	15.05
Polyunsaturated		63.85	10.23	1.30	62.14

Table 3
Brine Shrimp lethality test and DPPH antioxidant results of the seed oils.

Samples tested	Cytotoxicity test		Antioxidant assay	
	LD_{50} (μg/mL) (1 h)	LD_{50} (μg/mL) (1 d)	%DPPH (1 mg/mL)	IC_{50} (μg/mL)
Lime seed oil	955.90 ±1.12	32.28 ±0.04	70.90	14.49 ±3.54
Grape seed oil	156.20 ±0.37	12.76 ±0.41	61.81	209.00 ±2.27
Orange seed oil	920.40 ±2.50	7.59 ±0.35	49.09	564.10 ±1.24
Water melon seed oil	1084.00 ±2.50	0.14 ±0.38	56.36	313.80 ±3.80
Gallic acid	ND	ND	61.86	201.10 ±1.65
α-Tocopherol	ND	ND	45.45	54.86 ±2.38
Cyclophosphamide	2506.00 ±0.47	0.0	ND	ND

The results are presented as LC_{50} and IC_{50} values (μg/ml) at 95% Confidence Intervals (*CI*). ND means not determined.

was determined on graphpad prism-3 software by applying a non-linear regression analysis.

3. Results

The physico-chemical properties of the oils are shown in Table 1. Grape seed oil, with the least percentage oil yield of 9.583 was the only one that was solid at room temperature among the studied oils. The FTIR analysis of the four oils shows much similarity in the absorption bands with slight differences in the intensity of their peaks (Figure 1). The C-H stretching of aliphatic carboxylate ester was seen between 2 960-2 850 cm^{-1}, the vibrational frequencies found around 1 780-1 655 cm^{-1} were attributed to the presence of C=O functional groups while the band at 3 300 cm^{-1} in grape seed oil could be due to the presence of O-H group in this oil.

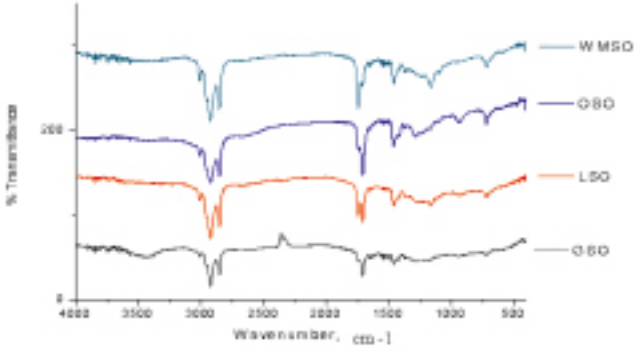

Figure 1. FTIR Spectrum of the seed oils.
GSO = Grape seed oil, LSO = Lime seed oil, OSO = Orange seed oil, WMSO = Water melon seed oil.

Table 2 shows the fatty acids profile of the trans-esterified seed oils as indicated by the GC-MS. In the four trans-esterified oil samples, a total of twelve fatty acids were identified. Erucic acid was dominant in the grape seed oil (43.17%). This is in contrast to what was obtained in an Egyptian grown grape seed oil[15] in which linoleic acid constituted more than 50% of the total fatty acid while oleic acid was the second major fatty acid reported. Meanwhile, oleic acid was not detected in the grape seed oil in this present study. Water melon seed oil contains 61.11% linoleic acid and 77.19% total unsaturated fatty acid which is in agreement with previously reported data[16]. Linoleic acid is an essential fatty acid that must be obtained through diet. Preliminary research has found evidence that α-linolenic acid is related to a lower risk of cardiovascular disease[17]. Grape seed oil is the only oil solid at room temperature which suggested its possible usefulness in production of specialized liquid soap and shampoo. The lime seed oil is golden yellow oil with the highest yield (24.79%). The relatively high oil content of the lime seed indicated that its oil could be economically viable. Its major fatty acid was oleic acid (52.42%) followed by palmitic acid (26.17%) and stearic acid (16.46%). It has a composition of 54.88% total unsaturated fatty acids. The sweet orange oil was found yellow with 9,12-octadecadienoic acid (62.18%) as the major fatty acid present. All the oils have a high unsaturation to saturation fatty acid ratio which is an indication of a good health value.

Brine Shrimp Toxicity: In the cytotoxicity assay, the degree of acute and lethal toxicity obtained was directly related to the concentration of the oil samples examined. This lethality assay is a primarily aimed at screening the seed oils for its cytotoxicity potential based on reported method[18]. Grape seed oil elicited the highest acute toxicity with LD_{50} value of (156.20 ± 0.37) μg/mL while water melon seed oil elicited the highest lethal toxicity with LD_{50} value of (0.14 ± 0.38) μg/mL (Table 3) thus showing a relatively moderate cytotoxicity.

In vitro antioxidant activity: DPPH is a stable free radical that can easily accept an electron or hydrogen radical to become a stable diamagnetic molecule. It is typically used as a substrate to evaluate the antioxidant potential of various antioxidants substance. The seeds oils were subjected to screening for their possible antioxidant activity by adopting the DPPH free radical scavenging assays. Gallic acid and α-tocopherol were used as positive control in the test. From the DPPH result obtained (Table 3 and Figure 2), the lime seed oil showed the highest antioxidant potential of about 70% at 1 mg/mL concentration which was more significant than the reference compounds gallic acid and α-tocopherol with IC_{50} value of (201.10 ± 1.65) μg/mL and (54.86 ± 2.38) μg/mL respectively. There is no much significant difference between the antioxidant values of both gallic acid and grape seed oil. However, from the present study, orange seed oil had the lowest antioxidant potential among all the seed oil examined. From the above results, it may be concluded that the seed oil possesses functional biological values which may be able to alleviate the effect of some acute and subacute disorders. The seed oils do not only possess the potential to act as natural antioxidant, but also possess the ability to act as alternative and cheap source of oil as well as functional food for household purpose.

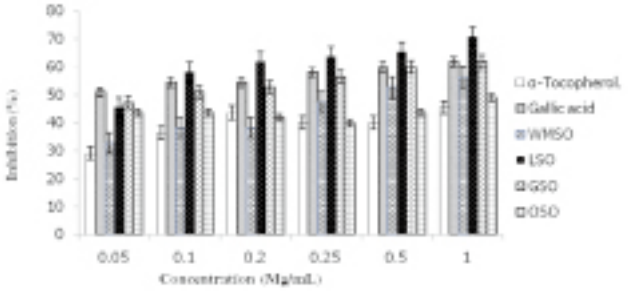

Figure 2. DPPH antioxidant capacity of sweet orange, grape, lime and watermelon seed oils. Results represent the mean ± standard error of mean (displayed at 5%) of replicate values.

4. Discussion

The results herein presented provides empirical data on the fatty acids profile of seed oils from sweet orange, grape, lime and watermelon as well as their antioxidant and cytotoxicity potentials. Fatty acid composition is essential in determining the bioactivities of oils obtained from natural resources.

It is obvious that the demand towards natural ingredients and products promoting health is likely to increase. The data obtained in this study from the seed oils from edible fruits will be important as indication of the potential dietary, nutraceutical and economic utility in the future. Moreover, these results provided useful information for the industrial application of lime, grape, sweet orange and water melon seed oils. Evidently, the seeds provided relatively fair yield of oils which are rich in essential fatty acids. Utilisation of these seeds will reduce environmental wastes; enhance their economic value as well as providing veritable outlets for the technological and nutritional channeling of these neglected bio-resources.

Conflict of interest statement

The authors affirm that there is no conflict of interest.

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