

Chemical and Physicochemical Composition of Watermelon Seed Oil (*Citrullus lanatus* L.) and Investigation of the Antioxidant Activity

Chemical constituents and Antioxidant Activity of *Citrullus lanatus* L. Seed Oil

[Oloyede, G. K.^{1*} and Aderibigbe S. A.²]

Abstract— *Citrullus lanatus* L. (Cucurbitaceae) known as Watermelon is an edible fruit in many countries especially Nigeria. Chemical composition, phytochemical, physicochemical and antioxidant properties of the dried seeds of *C. lanatus* were determined to understand its potential health benefits since oil extract from a number of fruits, nuts and seeds are used in cooking, soap making and as ingredient in confectioneries of baked or fried foods. Dried and pulverized Watermelon seeds were extracted using hexane at 60-70°C. Standard methods were used to determine phytochemical and physicochemical parameters. The chemical composition of the oil was determined using Gas-Chromatography – Mass Spectrometry (GC-MS) while antioxidant activity was determined using 2, 2-diphenylpicrylhydrazyl radical (DPPH) and reducing power methods. Results showed that the light yellow oil with an average yield of 41.32% showed the presence of terpenoids, phenolics, glycosides, and carbohydrate. Physicochemical parameters such as acid value 2.03, saponification value 183.13, iodine value 121.51, specific gravity 0.85, viscosity 2.48, refractive index 1.47 and power of hydrogen 6.20 were reported. Oleic acid methyl ester (62.38%) was the most abundant compound detected in the seed oil. The seed oil showed moderate activity when compared with ascorbic acid in the antioxidant screening when DPPH and reducing power methods were used. This present study has greatly justified the use of watermelon as an edible medicinal fruit.

Keywords— Methyl 9-cis, 11 –trans-octadecadienoate, methyl palmitate, seed oil, 2,2-diphenyl-1-picrylhydrazyl, *Citrullus lanatus*

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I. Introduction

Fruits are the fleshy seed-associated structures of a plant that is sweet or sour, and edible in the raw state, for example oranges, apples and banana, but in botanical usage, "fruit" includes many structures that are not commonly called "fruits", such as tomatoes, bean pods, wheat grains and corn kernels (Desai and Salunkhe, 1991; Munisse, *et al.*, 2011). Fruits and vegetables are of particular interest for their content in phytochemicals, antioxidants, vitamins, minerals and dietary fiber. All these substances are reported to lower the risk of development of health problems such as certain types of cancer, cardio vascular diseases, type 2 diabetes, obesity and constipation. Low intake of fruits can lead to increased risk of lung cancer and nutritional deficiencies (El-Adawy and Taha 2001; Sun, 2002).

Watermelon (*Citrullus lanatus* L.) of the family Cucurbitaceae is an important horticultural crop, mostly grown for its sweet and juicy fruit in warm climates all over the world. It is a vine-like flowering plant. It is referred as a pepo by botanists, which is a berry having a thick rind (exocarp) and fleshy center (mesocarp and endocarp). When used fresh or processed into juice, it generates much waste in the form of rind and seeds (Aranceta, 2004; Parry, *et al.*, 2005). The crops are primarily harvested for juice and juice concentrate. Although the seeds are considered waste, they have been shown to be highly nutritive and contain large amounts of proteins, vitamin A, C, E and many beneficial minerals (Rossell, 1991; Taiwo, *et al.*, 2008). Melons have their origin in Africa and Southwest Asia, but later started appearing in Europe at the end of the Roman Empire. Melons are nourishing food as its seeds are used to treat tuberculosis because of the high levels of potassium and are considered diuretics due to their high water content which is as high as 92% of the total weight. The presence of lycopene, an antioxidant found in some fruits and vegetables gives it the ability to lower the risk of cancer (Ensminger and Ensminger, 1986; Tarazona-Díaz *et al.*, 2013; Gul *et al.*, 2014).

Recently, more attention has been paid to the utilization of by-products and wastes, as well as underutilized agricultural products. Such utilization will contribute to maximizing available resources and can also result in the production of new foods (Oyediji and Oderinde, 2006). Watermelons are one of the major underutilized fruits grown in warmer parts of the world. Farmers in Namibia grow three types of watermelons: dessert, seed and the cooking types. Watermelon is basically classified into the seeded (diploid) and the seedless (triploid) watermelon (Lawrence, 1985). The crop is a natural and rich source of phytochemical compounds which are believed to be beneficial for human health and well-being (Abu-Reidah *et al.*, 2013). The seeds have a high nutritive value and are a potential source of unsaturated fat, vitamins, antioxidants, minerals

and proteins. Other benefits of watermelon include promoting a healthy complexion and hair, increase energy, and lower weight. Choline, found in watermelon is a very important and versatile nutrient; it aids sleep, muscle movement, learning, and memory. It maintains the structure of cellular membranes, aids in the transmission of nerve impulses, assists in the absorption of fat, and reduces chronic inflammation. L-citrulline present in watermelon has been reported to be responsible for muscle soreness reduction and improve recovery time following exercise in athletes (Pari and Umamaheswari, 2000; Rahman, *et al.*, 2013; Tarazona-Díaz, *et al.* 2013). The seed oil is light, pale yellow in colour, very stable with nutty aroma and is used as carrier oil for essential oil during aromatherapy massage. It is also used as emollient, antioxidant, anti-aging, detoxifying agent, anti-inflammatory, antihelminthic, and diuretic (El-Adawy and Taha, 2001; Erhirhie and Ekene, 2013). The oil has been used for cooking by native people of Kalahari Desert, although it is not used for cooking in most places in recent times (El-Adawy and Taha, 2001; Leland, *et al.*, 2006; Munisse, *et al.*, 2011). Little is documented about watermelon and its seeds in Africa, but the indications are that it has versatile uses. Therefore, this study seeks to investigate the phytochemical, physicochemical, chemical composition and antioxidant properties of the extracted watermelon (*C. lanatus*) seed oil from Nigerian soil. Investigation of the free radical scavenging activity is relevant since free radical and UV radiation induced damage are major causes of accelerated aging and many other diseases. Free radicals are neutral, short lived, unstable and highly reactive chemical species associated with odd or unpaired electron which is capable of attacking the healthy cells of the body, causing them to lose their structure and function. Antioxidants however are capable of stabilizing or deactivating free radicals before they attack cells. Antioxidant is a substance that can efficiently reduce a pro-oxidant with concomitant formation of products having no or low toxicity (Halliwell *et al.*, 1995; Boligon *et al.*, 2014)

II. Materials and Methods

Materials

Collection and Preparation of Sample

Samples of watermelon seed (*Citrullus lanatus* L.) were purchased from Bodija market in Akinyele Local Government Area of Oyo State, Ibadan, Nigeria in June 2016 and identified by a Taxonomist. The melon seeds were air-dried for two weeks, ground and kept in a desiccator till when needed.

Reagents: Hexane, methanol, hydrogen peroxide (BDH chemical), 2,2-diphenyl-1-picrylhydrazyl, potassium ferricyanide, ferric chloride and trichloroacetic acid (Sigma-Aldrich). Ascorbic was used as antioxidant reference standard.

Equipment

The following apparatus and equipment were used: Buchi Rotary Evaporator fitted with Vacuum pump V-700 and B-490 heating bath was used to concentrate samples. Oven (Carbolite), Ultraviolet-visible (UV-visible) spectrophotometer (Unico1200 & Perkin Elmer lambda 25 model, UK), and Gas Chromatography-mass spectrophotometer (GC-MS) (Gas chromatograph/GC-MS (HP 6890, UK), heating mantle, electronic weighing balance (OHAUS), desiccators, syringes, sample bottles, round bottom flask.

Methods

Extraction of Seed Oil

The ground melon seeds (*Citrullus lanatus* L.) sample (230 g) was transferred into a 10 Litre capacity round bottom flask and 680 ml of pure n-hexane was added, stirred every two hours with a glass rod and allowed to stay for 72 hours. The mixture was collected using muslin bag. This process was repeated by adding another 680 ml of pure n-hexane to the shaft. The combined filtrate was filtered using Whatman filter paper (1mm). The filtrate was concentrated with the aid of rotary evaporator set at 35^oC and the concentrate was transferred into a vacuum oven set at 35^oC and 700 mmHg pressure. Phytochemical screening was carried out on watermelon seed oil according to the methods of Kokate (1993) and Chitravadivu *et al.*, (2009). Proximate analysis of refractive index, free fatty acid, iodine, saponification, peroxide and acid values were determined for the sample in triplicate in accordance with the Association of Official Analytical Chemist (AOAC) procedures (Pritchard and Rossell, 1991).

Analysis of the Seed Oil

Gas Chromatography: The seed oil was analysed using an HP 6890 Gas Chromatograph powered with ChemStation Rev. A09.01 [1206] Software at the following specifications: split injection temperature, split ratio: (20:1), with hydrogen as carrier gas. Flow rate: 1.0 mL/min, inlet temperature: 150^oC, column type: HP 5MS, column dimensions: 30 m x 0.25 mm x 0.25 µm, oven program: initial at 40^oC, ramped at 5^oC/min to 200^oC, and run at 220^oC for 5 minutes (David *et al.*, 2011; Oloyede and Egbewole, 2014).

Gas Chromatography–Mass Spectrometry: GC oven temperature and conditions were as described above using HP 6890 powered with ChemStation Rev. A09.01 [1206] Software. Mass spectra were recorded at 70 eV (Marriot *et al.*, 2001; Oloyede *et al.*, 2012).

Identification of Components: Relative percentage compositions of constituents were obtained from electronic integration measurement using a Flame Ionization Detector (FID) set at a temperature of 300^oC while individual components of the oil were identified on the basis of their retention indices determined with reference to a homologous series of n-alkanes and by comparison of their mass spectra fragmentation pattern (NIST0.8 L database/chem. Station system) with data previously reported in literature (McLafferty and Stauffer, 1989; Gohlke *et al.*, 1993; Adams, 2007; Diomande, 2012). The peak numbers and relative percentages of the characterized components are given in Table 1.

Determination of Antioxidant Activity

DPPH Method

The ability to scavenge radical was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method. A 3.94 mg of DPPH radical was dissolved in 100 mL of methanol to give a 100 µM solution. The sample extract (0.2 mL) from stock solution of 1g/L was diluted with methanol and 2 mL of DPPH solution (0.5 mM) was added. The decrease in absorption of DPPH at 517 nm was measured in UV spectrophotometer after 30 minutes of incubation for the various concentrations (100 - 1000 µg/mL). Analysis was carried out in triplicates and the average results were recorded. The same experiment was carried out on ascorbic acid used as standard. Percentage inhibition was also calculated (Onocha *et al.*, 2011; Oloyede *et al.*, 2012; NurAlam, *et al.*, 2013).

Reducing Power Method (RP)

This method is based on the principle that increase in the absorbance of the reaction mixtures indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a colour complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured at 700 nm in a UV spectrophotometer. Increase in absorbance of the reaction mixture indicates the reducing power of the samples (Jayaprakash *et al.*, 2001). In the method described by Oyaizu (1986), 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) are added to 1.0 mL of sample dissolved in distilled water. The resulting mixture is incubated at 50°C for 20 min, followed by the addition of 2.5 mL of trichloro acetic acid (10% w/v). The mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%, w/v). The absorbance was measured at 700 nm against blank sample and recorded.

III. Results and Discussion

The extracted oil with an average yield of 41.32±0.5 per 100 gm (41.32% w/w) was light yellow in colour. This quantity of extract is considerable and the value is commensurate with reported values for some other similar oil seeds contents such as Cucumismelo (44.85%) and pumpkin seed oil (41.59%) (Tilak, *et al.*, 2006; Ziyada and Elhussien, 2008). Phytochemical screening revealed the presence of phenolics, terpenoids, carbohydrate and glycosides while flavonoids, saponin, tannins, reducing sugar and alkaloid were absent. Results of the physicochemical parameters determined are shown in Table 1.








Table 1: Average values of physicochemical analysis of Watermelon seed oil

S/N	Property	Value
1	Acid Value	2.03
2	Saponification value	183.13
3	Iodine value	121.51
4	Specific gravity	0.85
5	Viscosity	2.48
6	Refractive index	1.47
7	Power of Hydrogen Free fatty acid content (% as oleic acid)	6.20%

The values obtained for measure of un-saturation of oil (iodine value), percentage of volatile oils (free fatty acids content), measure of the average carbon chain length (saponification value) and specific gravity (Table 1) obtained in this analysis were in conformity with was previously reported by Duduyemi *et al.*(2013) which were 121.51, 6.40 %, 183.13 and 0.85 respectively. Baboli and Kordi

(2010) also reported that watermelon seeds oil from Iran produced yields of 50% (w/w) oil and the refractive index, saponification and iodine value were 1.4712 (at 25°C), 200 mg KOH/g and 156 g I/100 g, respectively. The acid and peroxide values were 2.4 mg KOH/g and 3.24 mequiv/kg, respectively. The values obtained were at par with the result obtained from this present study. The specific gravity of 0.85 for watermelon seed oil was below the range of 0.87–0.90 recommended for oil requirement for biodiesel production and so may not be fit as ingredient in biofuel production. Saponification value was used in checking the quality of the oil and found to be far greater than 100. It indicated the presence of unsaturated fatty acid characteristics of foaming ability. Foaming is a desired characteristic of good surfactants with applications in preparation of emulsions, soaps, foam and detergents formulation.

Table 2: Chemical constituents of Watermelon Seeds Oil *

S/N	RT	AI	Compound	Mol. formula	Structure	%Area
1	14.359	1926	Methyl palmitate	$C_{17}H_{34}O_2$		9.32
2	16.104	2089	Oleic acid, methyl ester	$C_{19}H_{34}O_2$		62.38
3	16.133	2084	Methyl eladate	$C_{19}H_{36}O_2$		8.41
4	16.339	2109	Methyl stearate	$C_{19}H_{38}O_2$		6.24
5	16.671	2155	Ethyl linoleate	$C_{20}H_{36}O_2$		8.83
6	18.697	2012	Methyl 8,11,14-heptadecatrienoate	$C_{18}H_{30}O_2$		2.17
7	25.529	1745	I. 5-Methyl-2-Phenyl-Indolizine	$C_{15}H_{13}N$		2.66
Total =						100%

*Percentages calculated from the flame ionization detection data. RT = Retention Time; AI = Arithmetic Retention Index on HP-5MS column.

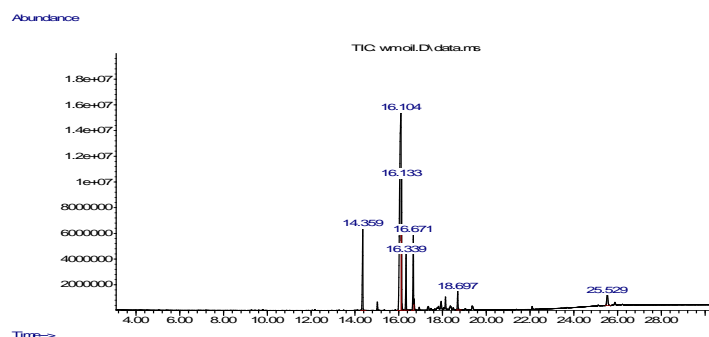


Figure 1: GC Spectrum of Water melon seed oil

A total of 7 components were detected in the watermelon seed oil, totalling 100% and dominated by oxygenated terpenes and an indolizine alkaloid (Table 2). The GC spectrum is shown in Figure 1. The watermelon seed oil had a high concentration of unsaturated fatty acids when compared with other similar oil seeds in literature. The main component was oleic acid, methyl ester (62.38%), methyl palmitate (9.32%) and methyl stearate (6.24%). Linoleic acid was however the dominant fatty acid (68.3%) in the watermelon seed oil from Iran (Baboli and Kordi, 2010). Figure 2 shows the mass spectrum of the most abundant compound, Methyl 9 cis, 11- methyl 9-cis, 11-trans-octadecadienoate (oleic acid, methyl ester) obtained from water melon seed oil from Nigeria. Oleic acid and its derivatives are used as an emollient. Methyl palmitate and methyl stearate are used as food additives and flavouring agents while ethyl linoleate (8.83%) detected in the oil is one of the most abundant essential polyunsaturated fatty acids which can be used as a reference material in assays that quantify fatty acid ethyl esters (FAEEs) for detection of alcohol abuse and studied as an acetylcholinesterase (AChE) inhibitor (Vick, 1993). Other compounds are Methyl eladate (8.41%), Methyl 8,11,14-heptadecatrienoate (2.17%) and 5-Methyl-2-Phenyl-Indolizine (2.66%).

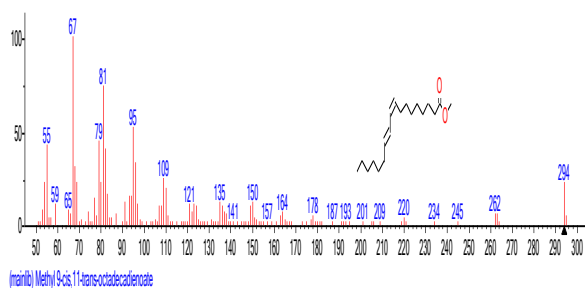


Figure 2: Mass Spectrum of Methyl 9 cis, 11- methyl 9-cis, 11-trans-octadecadienoate (Oleic acid, methyl ester) obtained from Water melon seed oil.

Watermelon seeds oil is one of the most undermined seed oil, with the most utilised being the soybean, rapeseed (*Brassica napus*), cotton (*Gossypium hirsutum*), peanut (*Arachis hypogaea*) and sunflower oils in decreasing order. Oil seed plants are plants that have seeds with a high level of oils used as energy reserves and can be used for human food and/or biodiesel production. They also possess reasonably balanced amounts of carbohydrates, fats and proteins (El-Adawy and Taha, 2001; Rodrigues *et al.*, 2012). The seeds of watermelon are increasingly being used in the oil industry in semi-arid regions by cosmetics and pharmaceutical industries and also the prospect of use of the seeds in the improvement of infant formulation due to their high protein and fat content is on the increase (Poysa and Woodrow, 2002; Nwanko *et al.*, 2014). The most abundant compound in this oil, oleic acid methyl ester is a monounsaturated fat in human diet which decreases low density lipoprotein (LDL) cholesterol, decreased risk of breast cancer and hypotensive (Martin-Moreno *et al.*, 1994; Pala *et al.*, 2001; Teres *et al.*, 2008). Thus an important contribution to food resources or industrial products can be made.

Antioxidant Activity of the Essential Oils

The increase in awareness of the use of watermelon seed oil especially as anticancer agent has necessitated investigation of its biological relevance. Therefore screening of watermelon seed oil for antioxidant activity will justify its use as free radicals are reported to

be the major causative agents of many degenerative diseases including cancer. DPPH assay and Reducing Power Methods were used. Results obtained (Table 3) showed that there was reduction in absorbance values in the oil sample after incubation in DPPH as concentration is increased unlike in the reducing power method where there was increase in the absorbance of the reaction as concentration is increased, indicating an increase in the antioxidant activity. At the highest concentration (1000 µg/mL), absorbance values were 0.788±0.013 and 0.868±0.002 whereas at the lowest concentration (100 µg/mL), absorbance values were 0.892±0.001 and 0.487±0.001 for the two assays respectively. Ascorbic acid however showed better activity in the DPPH assay with absorbance value of 0.090±0.001 at 1000 µg/mL (Table 3). The percentage inhibition as shown in Figure 1 indicated that water melon seed oil displayed moderate activity in the two assays.

Table 3: Absorbance values obtained in the DPPH assay and Reducing Power Method (RPM) of Water melon seed oil*

Conc (µg/mL)	DPPH ASSAY	ASCORBIC ACID	RPM
1000	0.788±0.013	0.090±0.001	0.868±0.002
800	0.799±0.020	0.092±0.004	0.629±0.011
600	0.825±0.011	0.093±0.000	0.620±0.001
400	0.835±0.015	0.093±0.005	0.577±0.004
200	0.836±0.014	0.094±0.001	0.562±0.007
100	0.892±0.001	0.095±0.005	0.487±0.001

*Absorbance measurement of Water melon seed oil and standard: ascorbic.

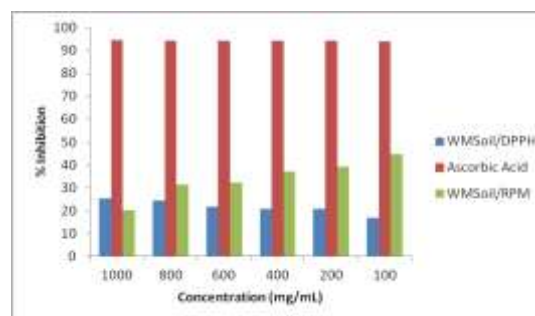


Figure 1: Percentage Inhibition of DPPH free radical scavenging activities and Reducing Power of Water melon seed (WMS) oil and Ascorbic.

IV. CONCLUSION

Watermelon seed oil contains mainly esters of fatty acids dominated by methyl 9-cis, 11-trans-octadecadienoate (Oleic acid methyl ester). The free radical scavenging activity in terms of hydrogen donating ability, using the stable radical 2,2-diphenyl picrylhydrazyl radical (DPPH) and reducing power methods indicated that Watermelon seed oil showed moderate activity. The high oil content of watermelon seed coupled with fairly high concentration of fatty acid make the seed suitable as food supplement. It may also enjoy applications as industrial ingredients in soap production, cosmetics, and foam ingredient. The by-products emanating from the processing could be useful in firing boilers for plants or as animal feed if properly processed.

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Phytochemical evaluation of Medicinal Plants with emphases on antioxidant, anticancer and hepatotoxic activities.

Isolation and Characterisation of bioactive chemical compounds from plants

Synthesis of Schiff and Mannich Bases and investigation of their biology activities.



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