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Antibacterial studies, physicochemical properties and fatty acid composition of fixed oil from *Citrullus lanatus* seed cultivated in South-South Nigeria

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Abstract
Citrullus lanatus seed oil was extracted from its powered seeds in a Soxhlet apparatus using diethyl ether. Physicochemical, fatty acid and antibacterial analyses of the oil was carried out using standard methods. Extraction yielded 50 % oil characterised with a light yellow colour, an agreeable odour, specific gravity of 0.89 and refractive index of 1.48±0.02. The iodine, peroxide, saponification and acid values were found to be 59.01±0.05, 5.17±0.05, 352.54±0.05, and 6.45±0.05 respectively. The results of the fatty acid analysis showed that the oil contains linoleic acid (56.85%), oleic acid (14.79%), palmitic acid (13.95%) and stearic acid (9.17%) among other acids. Antibacterial evaluation revealed that the oil was active against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* with the diameters of zone of inhibition ranging from 12-20 mm wide, the oil was however not active against *Enterorbacter aerogenes*, *Klebsiella pneumonia* and *Proteus mirabilis*. Moreover, these experimental findings support the ethnomedical usage of the oil in treatment of certain skin infections and rich source of polyunsaturated fatty acids (parent fatty acids of omega 6- series), which has ample health benefits.

Keywords: *Citrullus lanatus*, antibacterial analysis, physicochemical, fatty acid analysis

Introduction
Citrullus lanatus (water melon) is one of the most popular species of the family Cucurbitaceae with high water content as high as 92% of the total weight and it plays a very important role in Africa as it is used to quench thirst when there is shortage of water (Mirjana and Ksenija, 2005) [15]. It is an oval-shaped fruit with a hard and thick outer skin. Externally the fruit features smooth deep green colour, internally the flesh is juicy, pink, red or yellow with numerous small black seeds embedded inside. The crops are primarily harvested for juice and juice concentrate as an excellent source of Vitamin C and Vitamin A (Baboli and Kordi, 2010) [8]. The young fruit and leaves can be cooked and eaten as vegetable. Flour of watermelon contains several antioxidants compounds e.g. stachyose, raffinose and verbascose. The dry seed of watermelon has been reported to contain proteins and fats. However, seedless watermelons are produced in many areas of the world e.g. in Florida, USA (Pearsons, 2002 and, Mossler, 2007) [20].

The dry seed can be bruised and rubbed up with water to form an emulsion, which is used in the treatment of catarrhal infections, disorders of the bowels, urinary passage and fever. It is also being used as worm expeller; in recent years it has been used to expel tape worms and as a natural Viagra (Mossler, 2007) [16].

According to Erhirhie and Ekene, (2013) [10] *C. lanatus* seed oil has some therapeutic properties which include emollient, antioxidant, anti-inflammatory, anthelminthic, diuretic. Fatty oil in the seed, as well as aqueous or alcoholic extracts, has been reported to paralyze tapeworms and roundworms (Chopra, 1958) [9]. The rind of the fruit is prescribed in poisoning and diabetes. *Citrullus lanatus* is used in Northern Sudan for burns, swellings, rheumatism and gout (Schippers and Budd, 1997) [21]. The fruits are used as a drastic purgative in Senegal; they are also used to treat diarrhoea and gonorrhoea in Nigeria. Tar is extracted from the seeds and used for the treatment of scabies and for skin tanning (Schippers and Budd, 1997) [21].

The present investigation was carried out to determine the available oil quantities in the seeds, some of its physicochemical characteristics, antibacterial activity as well as fatty acid profile for possible future commercial uses and/or inclusion into human diets.

Materials and Methods

Sample collection and preparation

The matured fruits of the study plant (*C. lanatus*) were purchased from Jattu market in Edo state, were cut open into pieces and the seeds extracted. The extracted seeds were sun-dried for several days and later in a hot-air oven at 105 °C for 1 hour. The dried seeds were de-shelled, blended into flour which was put into a plastic container with cover and stored in a refrigerator for further use.

Extraction of oil

174.60 g of the seed flour was placed in the thimble of a Soxhlet apparatus and extracted with diethyl ether (Analytical grade). The oil extracted was separated from the solvent by evaporation and quantified gravimetrically.

Determination of physicochemical properties of the oil

Odour, colour, and physical state of the oil at room temperature were determined through visual inspection. The refractive index, peroxide value, saponification value, acid value and iodine value were determined according to the methods of AOAC (1990).

Antibacterial screening of oil

Test organisms

Clinical isolates of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Kiebsiella pneumoniae, Enterobacter aerogenes, Proteus mirabilis* were obtained from the Department of Medical Microbiology, University of Benin Teaching Hospital (UBTH) Benin City, Nigeria. All the organisms were checked for purity at PAX Herbal Clinic and Research Laboratories, Ewu, Edo state and maintained at 4^{o}C slants of nutrient agar.

Screening of the oil

The Agar-well diffusion method of Karou *et al*., (2006) [13] was used to determine the antibacterial activity of the oil. Pure culture of the organisms were innoculated on Mueller-Hinton Agar and incubated for 24 h at 37 °C about 5 discrete colonies were asceptically transferred using sterile wire loop into tubes containing sterile normal saline and were adjusted to a turbidity of 0.5 MacFarland standard. The suspensions were then innoculated on the surface of sterile Mueller-Hinton agar plates using sterile cotton swabs. A sterile 6 mm diameter cork borer was used to make holes (wells) into the set of innoculated Mueller-Hinton agar plates. The wells were filled 100 microlitre of the stock concentration of the oil. The plates were incubated at 35 °C for 24 hours. The plates were observed for clear zones of growth inhibition, the diameters of which were measured in millimeters using a transparent ruler and recorded. Two wells, one filled with sterilised Dimethylsulphoxide (DMSO) and the other with 5 mg/ml Ampiclox served as negative and positive controls respectively. All tests were performed in triplicates.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of the oil was determined using micro broth dilution technique in accordance with NCCLS, 2002. Different concentrations of the oil were prepared by serial dilution of the stock using test tubes containing 9 ml of double strength nutrient broth (OXOID). The test tubes were innoculated with the suspension of the standardised innocula and incubated at 37 °C for 18 h. Minimum inhibition concentration were recorded as the lowest concentration of the oil that showed no visible growth (turbidity) in the broth.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentrations were determined by aseptically subculturing aliquots of the contents of the MIC tubes (i.e the tubes that showed no growth) on sterile agar plates and incubating at 37 °C for 24 h. The MBCs were recorded as the lowest concentration of oil showing no bacterial growth at all.

Fatty acid analysis of oil

Fatty Acid Methyl Esters (FAMEs) were prepared by direct *transesterification* of the oil according to the method described by Akintayo *et al.,* (2004) [3]. 0.2 ml of the clear supernatant of FAMEs was injected into Hewlett-Packard 5890 gas liquid chromatograph equipped with flame ionisation detector (FID) and HP chemist action Rev H09.01 (1206) software. The column was packed with J and W scientific fused silica column DB5 coated with cross linked 5 % Phenol and 95 % polysiloxane, 25 x 0.25 mm, 0.2 coating thickness. The column initial temperature was 120 °C for 2 min, temperature increased at the rate of 4^{o}C/ min up to 260 °C and maintained at this temperature for 5 mm, injector and detector temperatures were 230 °C and 300 °C respectively. The carrier gas (nitrogen) flow rate was maintained at 50cm^3/min FAMEs peaks were identified by comparison of their retention times with those of a standard mixture chromatographed under the same conditions as the sample oil.

Results

Results obtained for all the analyses carried out are shown in Tables 1-3.

Table 1: Physciochemical properties of oil

Physicochemical Parameters	Results
Percentage Oil yield	50%
Specific Gravity	0.89
Refractive Index	1.48±0.02
State at 30 °C	Liquid
Colour	Light yellow
Odour	Agreeable
Peroxide value (meq/kg)	5.17±0.05
Saponification (mgKOH/g)	352.54±0.05
Acid Value (mgKOH/g)	6.45±0.05
Iodine Value (g12/l00g sample)	59.01±0.05

The value above is a mean of three replicates: x ± SEM

Table 2: Antibacterial activity, minimum inihibitory concentration (MIC), minimum bactericidal concentration (MBC) of oil on test organisms

Isolates	Mean Diameter Zone Of Inhibition (mm)			MIC	MBC
	CLO	Positive Control (Ampiclox)	Negative Control (DMSO)		
Bacillus sublitis	17	24	0	10	20
Enterobacter aerogens	0	18	0	-	-
Escherichia coli	12	30	0	40	80
Klebsiella pneumonia	0	31	0	-	-
Pseudomonas aeruginosa	15	33	0	20	40
Staphylococcus aereus	20	19	0	5	10
Proteus mirabilis	0	31	0	0	0

Keyword: CLO (*C. lanatus* Oil), DMSO (Dimethylsulphoxide)

Table 3: Fatty acid composition of CLO

Fatty Acid (Methyl Ester)	Percentage Composition
Caprylic Acid (C8:0)	0.00
Capric Acid (C10:0)	0.00
Lauric Acid (C.12:0)	0.27
Myristic Acid (C14:0)	0.84
Palmitic Acid (C16:0)	13.95
Palmitoleic Acid (C16:1)	2.81
Margaric Acid (C17:0)	0.00
Stearic Acid (C18:0)	9.17
Oleic Acid (C18:1)	14.79
Linoleic Acid (C18:2)	56.85
Linolenic Acid (C18:3)	0.93
Arachidonic Acid (C20:4)	0.01
Behenic Acid (C22:0)	0.38
Erucic Acid (C22:1)	0.00
Lignoceric Acid (C24 0)	0.00

Discussion

Table 1 shows the results of the physicochemical properties of water melon seed oil. The oil is light yellow in colour, its specific gravity is 0.89, which shows that it is less dense than water and compares favourably with the 0.87 reported for melon seed oil (Eze, 2012) [11]. Iodine value is a measure of the degree of unsaturation in oil and is a reflection of the susceptibility of oil to oxidative and hydrolytic rancidity (Amoo, *et al.*, 2004) [4]. It could also be used to classify oils as dry and non- drying. A good drying oil should have iodine value of 180 (Abayeh *et al.*, 1998) [1]. The iodine value obtained in this study indicates that *C. lanatus seed oil* contains appreciable level of unsaturation and places it as a non-drying oil. The oil may be used as a raw material in industries for the manufacture of vegetable oil-based ice cream (Oderinde *et al.*, 2009a and 2009b) [18, 19]. The peroxide value of the oil, 5.17 meq/kg, is higher than the 1.03 meq/kg reported for groundnut seed oil (Eze, 2012) [11] but lower than 6.65 meq/kg for *Hura crepitans* seed oil (Amoo *et al.*, 2002) [5]. The peroxide value is used as an indication of oil deterioration. The peroxide value shows that the oil has low level of peroxide and also suggests presence of antioxidant. The saponification value (SV) is very, high, higher than the 193.20 mgKOH/g for groundnut oil (Atasie, *et al.*, 2009) [7] and 300 mg KOH/g for *Blighia unijugata* seed oil (Oderinde *et al.*, 2009) [19]. This high SV means that *C. lanatus seed oil* will be useful in soap making. The acid value, which is an indication of the level of oxidative deterioration of an oil by enzymatic or chemical oxidation, is 6.45 mgKOH/g oil for *C. lanatus seed oil*. This low value compared to 11 5mgKOH/g for *Plukeneta conophora* (Akintayo and Bayer, 2002) [2] suggest that the oil has low levels of free fatty acids and will therefore be suitable for consumption.

Table 2 presents the fatty acids profile of *C. lanatus seed oil*. The results show that the oil contains both saturated and unsaturated fatty acids. The saturated fatty acids identified are the non-essential fatty acids lauric acid (0.27%), Myristic acid (0.84%), Palmitic acid (13.95%), Stearic acid (9.17%) and Behenic acid (0.38%). In all, the saturated fatty acids account for 24.61% of the fatty acids in the oil while the unsaturated ones make up the ramming 75.39% comprising 17.60% monosaturated acids (Oleic, 14.79% and Palmitoleic, 14.79%) and 57.73% polyunsaturated acids (56.85% linoleic, 0.93% linolenic and 0.01% Arachidonic). It has been established that relative to carbohydrates, the saturated fatty acids elevate serum cholesterol while the polyunsaturated fatty acids (PUFA) lower it (Kanderson and Grande, 1957; Hegsted, *et al.*, 1993) [14, 12]. Thus *C. lanatus seed oil* which contains predominantly polyunsaturated fatty acids will play an important role in stemming/reducing the risk of developing cardiovascular diseases.

Results of the antibacterial activity assay of the oil against the test organisms are presented in Table 3. On the basis of the measured diameters of zones of inhibition, it can be seen that the oil was active against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginos* and *Eschericia coli*. The oil, however, exhibited no activity against *Enerobacter aerogenes, Klebsielia pneumonia* and *Proteus mirabilis*. The oil was most active against *Staphylococcus aureus*, followed by *Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* in that order. Although the Ampliclox used as a positive control had higher antibacterial activities than the oil against all the organisms, the oil could be used as alternative natural antibiotics against the test organisms that showed susceptibility to it.

Conclusion

The physicochemical properties and fatty acids composition of the oil have been determined. The antibacterial activities of the oil against selected organisms have also been evaluated. The oil has remarkable physicochemical properties that enhance its suitability for both domestic and industrial applications. The preponderance of polyunsaturated fatty acids (PUFA) in the oil makes it a health friendly source of these essential fatty acids. The antibacterial activity of the oil makes it a choice natural antibiotic, devoid of any side effects, against susceptible organisms. Further studies are on-going to identify and isolate the active principles in the oil responsible for its antibacterial potency.

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