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Physicochemical and *in vitro* antimicrobial activity of the oils and soap of the seed and peel of *Citrus sinensis*

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Citrus sinensis **seed and peel oils were extracted by solvent extraction using n-hexane, after air drying and grinding. Soaps were formed by saponification methods. Fatty acid composition of the oil samples were analyzed using Gas Chromatograph-Flame Ionization Detector (GC-FID). Physicochemical properties of the oils and soaps were determined following standard methods. Antimicrobial activities were assessed by the agar disc and hole-in plate methods. The seed and peel oil yield were 38 and 30%, respectively and the colors were golden yellow and brownish-yellow, respectively. Physicochemical properties of the oil samples determined were: refractive index (RI): 1.46 and 1.47, smoke point: 140 and 149, flash point: 150 and 160, pH: 5.2 and 4.2, acid value (AV): 23.6 and 25.1 mgKOH/g, free fatty acid (FFA): 11.86% as oleic acid and 12.61% as oleic acid, iodine value (IV): 78.83I_2 g/100 g and 120.10I_2 g/100 g, peroxide value (PV): 18.00 mgKOH/g and 5.40 mgKOH/g, saponification value (SV): 222.58 and 41.25 mgKOH/g, ester value (EV): 178.24 and 28.96 mgKOH/g for the seed and the peel oil respectively. Inhibitory antimicrobial activities were assessed for the two oils and the soap produced at concentrations of 40 mg/ml and below, against most of the gram positive and gram negative bacteria as well as the two candida strains, screened as compared with streptomycin (1 mg/L) and acriflavin (6.3 mg/ml) standard controls. Seed oil demonstrated better activities than the peel oil with growth inhibitions obtained against *Staphylococcus aureus* and *Candida albicans* at a concentration as low as 2.5 mg/ml. This study has shown that the results obtained for the physicochemical and antimicrobial properties of the oils provide a synergy for the oil samples as suitable raw materials for the cosmetic and pharmaceutical industries**.

Key words: Physicochemical properties, antimicrobial activity, soap, seed oil, peel oil.

INTRODUCTION

Citrus sinensis (sweet orange) is one of the natural staple food of man, containing essential nutrients in adequate proportion. The nutritional and medicinal values of the fruit juice has made it essential and important part of

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human diet for ages (Okwu and Emenike, 2006; Ezejiofor et al., 2011). Generally, citrus are excellent sources of minerals, vitamins and enzymes. They have been reported to be free from fat and cholesterol, but contain important mineral elements such as potassium, calcium, phosphorus, magnesium and silicon (Assa et al., 2013). They are easily digested and bring about a cleansing effect on the blood and the digestive tract. Orange fruits have been discovered to have anti-scurvy property (Rapisararda et al., 1999). Furthermore, they are rich in vitamin C, folic acid and fiber; these contribute to the prevention of degenerative processes, particularly reducing the incidence and mortality rate of cancer as well as cardio- and cerebro-vascular diseases (Rapisararda et al., 1999; Cushnie et al., 2005; Pultrini et al., 2006).

C. sinensis belongs to the race *var. sinensis*, of the family Rutaceae. It is an hybrid between Pomelo (*C. maxima*) and Mandarin (*C. reticulata*) originating from Southeast Asia. The fruit size varies with cultivar and crop load, but most often measures between 2.5 to 4 .0 inches in diameter (Manthey, 2004). The shape of the fruit is spherical to oblong, with a peel thickness between that of grape fruit and tangerine, and is either smooth or roughly pebbly (Hilditch et al., 1950). It is usually very closely adhered to the flesh of the fruit. Its colour tints from green to light orange, depending on the cultivar. The presence and amount of seed depends also on cultivar, starting from 15 to 25 seeds per fruit (Nwobi et al., 2006). Of all the citrus fruits, *C. sinensis* is the commonest in the forest zone of Western Nigeria, Middle Belt, Eastern and some part of South-south Nigeria (Odbanjo and Sangodoyin, 2002).

The yield of orange juice is about half of the fruit weight thereby generating a very high amount of waste annually (Bovili, 1996). Citrus waste as huge as 36 metric tons are produced annually with Florida citrus industry generating 3.5 to 5 tons, used and sold as feed stock for cattle, and Nigeria generating about 0.3 million tons with potential to generate more annually (Ezejiofor et al., 2011). These agro wastes are common in Nigeria along major roads where retailers peel and sell to motorists and others. The wastes in market places constitute menace, causing environmental pollution.

Citrus fruit peels are also known to have flavonoids, an anti-oxidant (Bocco et al., 1998; Cushnie et al., 2005; Ghasemi et al., 2009). Essential oil had been generated in sweet orange and grape fruit (*C. paradisi*) peels (Ezejiofor et al., 2011; Okunowo et al., 2013) and the antimicrobial activities of grape peel oil had been documented (Okunowo et al., 2013). Essential oils in plant products have tremendous applications in food, cosmetic and aromatherapy (Ramadan et al., 1996; Haddouchi et al., 2013; Narmadha et al., 2013). Research in medicinal chemistry have also shown that screening plant products for antimicrobial activities have led to detection and development of new potential anti-infective agents (Ordonez et al., 2003; Arias et al., 2004; Rasool et al., 2008). The peel of citrus fruits is a rich source of flavones and many polymethoxylated flavones which are very rare in other plants (Ahmed et al., 2006). The antimicrobial abilities of essential oils from citrus plants have shown to be of particular interest for applications within the food industries (Caccioni et al., 1998).

In this study, the physicochemical properties and fatty acid compositions of the fixed oil from the seeds and peels of sweet orange were determined. Alkali generated from the peel and seed oil were used to prepare soaps. The antimicrobial properties of these oils and the soap were also determined with a view to investigate their suitability as possible alternative to the orthodox antibacterial soaps. The results of this investigation is expected to contribute to information on the usefulness of *C. sinensis* in the cosmetic industries for the health benefit of man and to reduce the menace of pollution caused by the peel wastes in the environment.

MATERIALS AND METHODS

Collection and preparation of sample

C. sinensis were collected mainly from Oje market in Ibadan, Oyo State, Nigeria (Specimen ID: 006653. Herbarium: PTBG). Its seeds and peel were manually removed and were then air dried to remove the moisture content. The dried seeds and peel were then grinded to particles with the aid of an electric grinding machine.

About 1430 and 2350 g of the ground *C. sinensis* seed and peel were weighed separately and were transferred into a porous thimble and kept in the Soxhlet apparatus for extraction. Anti-bumping granule was dropped into the flask to prevent the build of pressure in the flask and n-hexane was added as the extracting solvent. The oil was recovered from the mixture by evaporating the residual extracting solvent using a rotary evaporator. The weight of oil was noted (Soxhlet, 1879, Laurence et al., 2012).

After the extraction, the oil was transferred into a weighed round bottom flask. The weight of the oil was determined by weighing the oil and the flask and subtracting the weight of empty flask. The percentage yield was determined.

Physical properties of the oils

The specific gravity of the seed and peel oil were determined by measuring 10 mL of the oil samples into a pre-weighed measuring cylinder. The values obtained were used to determine the specific density of the oil. The pH of the oils were determined using Hannah instruments, pH 210 Microprocessor pH meter while the refractive index was determined at room temperature using the Abbey refractometer at the Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

Other physical parameters such as flash and smoke points, cloud and pour points and viscosity test were carried out using ASTM D56 (2001), TCWI (2009) and ASTM D445 (1965).

Chemical properties of the oils

The chemical properties such as the acid value (AV), free fatty acid (FFA), Iodine value (IV), saponification value (SV) and peroxide value

(PV) of the seed and peel oil were determined by standard method of AOAC (1990).

Determination of fatty acid composition

Fatty Acid composition of the oil samples were analyzed using PERKIN Elmer Clarus 500 Gas Chromatograph employing the following conditions: capillary column (RT-2560, 50 m x 0.25 mm ID, 0.25 micron dry film); Nitrogen was used as a carrier gas, a flame ionization detector and a sample volume of 1.0 L was employed. The temperature programming of the instrument: Initial temperature was 50°C held for 5 min, with an increase of 4/min to 190, then 0.8/min to 212, then 0.4°C /min to 220. The total GC-FID running time was 85.49 and 78.90 min for the orange peel oil and orange seed oil respectively.

Preparation of orange peel ash

The oranges were peeled and the peels were washed with double distilled water and dried in an oven at (105°C ± 2) for two days to constant weight. The dried peels were ashed in a porcelain crucible placed in a Gallenkamp muffle furnace for 6 h by stepwise increase of the temperature up to 500°C. The ashed samples were homogenized in porcelain mortar and pestle and sieved. Sixty (60) g of the sample were weighed into poly ethylene buckets of 2 L capacities and one liter of water was added (Onyegbado et al., 2002; Olabanji et al., 2012). The buckets were covered to prevent contamination and extractions were done for 24 h. The extracts were carefully decanted and double distilled water were added in ratios of 1:4 of sample to double distilled water and were analyzed by atomic absorption spectrophotometer (AAS) Buck Model 205 at the Center for Energy Research Development, Obafemi Awolowo University, Ile- Ife. These extracts were alkaline to litmus paper and methyl orange.

Determination of molarity of orange peel ash alkali

Primary standard (Na_2CO_3) of known molarity was prepared and used to standardize the acid (HCl) which was titrated against the derived alkali using methyl orange indicator to determine its molarity.

Saponification reaction using the ash-extracts

Two hundred milliliter of the ashed peel extract was concentrated to 50% by heating in a beaker (Babayemi et al., 2011); excess of alkali is usually recommended in order to ensure complete saponification of the oil/fat and to retain the antibacterial effect of the alkalis (Kirk et al., 1954). The concentrated extract was heated to 60 to 70°C and 15 g of oil was gradually charged into the pot. The temperature was maintained at 70°C and 5 ml of double distilled water was added intermittently with continuous stirring until the mixture was semi solid and creamy in color, 10 ml of brine was charged into the beaker content and the soap was homogenized. The soap was scooped from the upper layer when the content of the beaker had cooled and the lye discarded. The soap was washed by pouring water on it.

Analysis of soap produced

Determination of total fatty matter (TFM)

The TFM was determined by the petroleum spirit extraction method. Soap (1 g) was dissolved in 10 ml of warm water and transferred to a separating funnel. Two drops of methyl orange indicator were added, followed by 4N H_2SO_4 until the indicator color changed from orange to pink. Petroleum spirit 1mL was added and the separating funnel shaken vigorously for 30 s. The solution was then allowed to settle for a few minutes until the fatty acid liberated from the soap formed a clear layer on top. The soap was skimmed off, washed with distilled water and dried to constant weight in an oven at 60°C. The percent total fatty matter was determined from the weight obtained for the fat and the soap.

Determination of total alkali

The total alkali was determined by titrating excess acid contained in the aqueous phase with standard volumetric NaOH solution. Five millilitre of ethanol was added to 1g of finished soap after which 0.5 ml of 1N H_2SO_4 solution was added to the mixture and heated till the soap sample dissolved. Test solution was titrated against 1 N NaOH using phenolphthalein as indicator. The total alkali was obtained following AOAC (1990).

Foamability test

About 0.5 g of the soap was added to a 100 ml standard flask containing 100 ml of double distilled water. The mixture was shaken vigorously two minutes to generate foams. The flask was allowed to stand for 10 min. The height of the foam in the solution was noted.

Antimicrobial activity assays

Test organisms

Microorganisms used include reference and clinical isolates comprising of Gram positive and Gram negative bacteria and fungi strains. These include *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas fluorescens* (clinical strains), *Shigella flexinerii* (clinical strain), *Klebsiella pneumonia* (clinical strain), *Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* NCIB 3610. *Candida albicans* ATCC 24433 and *Candida pseudotropicalis* NCYC 6 were the fungi strains used. The strains were from stocks of culture collections maintained in the Pharmaceutical Microbiology Laboratory of the Department of Pharmaceutics, Faculty of Pharmacy, Obafemi Awolowo University where the experiments were performed.

Agar diffusion tests: Disc diffusion and cup plate methods

The disc diffusion test was used for the pure oils and the soaps while the cup plate test was used for their dilutions. The oil samples and their soap preparations were dissolved in MeOH/H_2O to give varying concentration of 2.5, 5.0, 10.0, 20.0 and 40.0 mg/ml.

Surface plating of the organisms were done for the 20 ml oven dried Mueller Hinton Agar used for the overnight grown bacteria and the Sabouraud Dextrose Agar used for the fungi strains. For the dilutions, holes of diameter 9 mm were made in the agar plates using a sterile metal cup-borer. Two drops of each dilution and control were put in each hole under aseptic condition, kept at room temperature for 1 h to allow the agents to diffuse into the agar medium and incubated accordingly. For the pure oil and soap, each of these were used to soak sterile 6 mm Whatman paper discs and subsequently placed on the agar plates, allowed for diffusion and incubated. Streptomycin (1mg/ml) and acriflavine (6.3 mg/ml) were used as positive controls for bacteria and fungi respectively. MeOH/H_2O and Tween 80 were the negative controls. The plates were incubated at 37°C for 24 h for the bacterial strains and at 25°C

Figure 1. Seed oil and peel oil.

for 72 h for the fungal strains. Antimicrobial activity was evaluated by noting the zone of inhibition against the test organisms.

RESULTS AND DISCUSSION

The percent yield of the oils was 38 and 30% for seed oil and peel oil, respectively. Using same extraction process but different solvent Nwobi et al. (2006) got 36% yield for the orange seed oil, this is close to the value in this study.

The oil of orange seed and peel showed acidic pH values (4.2 and 5.2). These values were however higher than 3.69 reported by Nwobi et al. (2006) for the peel oil. The pH values indicated that the seed oil is more acidic than the peel oil probably due to presence of more fatty acids in the seed oil.

The seed oil has a golden-yellowish color (Figure 1); similar result was obtained by Nwobi et al. (2006) while the peel oil has a brownish-yellow color, similar to the yellow color obtained in Okunowo et al. (2013) grape peels oil. The yellow color may be an indication of carotenoids, a fat, soluble in humans due to the presence of long unsaturated aliphatic chains as in some fatty acids. Carotenoids are known as provitamin A. They act as precursors to the production of vitamin A in the body which performs several biological functions within the body. They also act as antioxidants (Sommer and Vyas, 2012).

Refractive Index decreased with unsaturation and molecular weight of the fatty acids. The refractive index of the seed oil and peel oil was 1.46 and 1.47, respectively. This corroborates the findings of Nwobi et al. (2006) and Ezejiofo et al. (2011). This indicates that the seed oil, compared with the peel oil, has a lower unsaturation and a lower molecular weight of fatty acids. The lower molecular weight of fatty acid is suggestive of its higher saponification value since saponification value is inversely proportional to the mean molecular weight of fatty acids (Dimberu et al., 2011). The smoke point, flash point and free fatty acid content of the oils have a linear relationship. The higher the free fatty acid content of an oil, the lower the smoke point. The smoke point and flash point of the seed oil were 140 and 150°C, respectively while that of the peel oil were 149 and 160°C, respectively. Nwobi et al. (2006) obtained 149°C in orange seed oil which is still within the values found in seed and peel oils of this study.

The seed oil has a specific density of 0.997 g/cm^3 while the peel oil has a value of 0.788 g/cm^3. The value obtained from Ezejiofor et al. (2011) study lies within the range of density of the oils in this study. Density of seed oils depends on their fatty acid composition, minor components and temperature (Table 1).

Acid value accounted for the presence of free fatty acids in the oils as an indicator of the presence and extent of hydrolysis of lipolytic enzymes and oxidation and it is used as an indicator of edibility of an oil. The values indicated that the oils were non edible because it was above the limit of 10 mg KOH/g of oil and found to be unsuitable for dietary purposes (Barkatullah et al., 2012) and 0.6 mg KOH/g FAO/WHO (1993), as the peel oil contain higher fatty acid contents.

The free fatty acid content in seed oil which is 11.86% (as oleic acid) is lower than that of peel oil which is 12.61% (as oleic acid). This indicates that the oils could readily react with metal salt to generate soaps since the FFA were far above the 2.5 and 1.376% FAO/WHO recommended for coconut and palm oil respectively (FAO/WHO, 1993). High FFA nullified their edibility.

Peroxide value serves as a common indicator of lipid oxidation. Orange seed oil has a peroxide value of 18.00 millieq/kg while the peel oil has a value of 5.40 millieq/kg. This indicates that the seed oil has undergone primary oxidation than the peel oil since peroxide value gives a measure of the extent to which an oil sample has undergone primary oxidation. The peroxide value of the peel oil is within the acceptable range of 10.00millieq/kg FAO/WHO (FAO/WHO, 1993) while that of seed oil was above indicating that lipid oxidation had occurred.

The saponification value of the seed oil is found to be 222.58 mg KOH/g while it is 41.25 mg KOH/g for the peel oil. The higher saponification value of the seed oil shows the presence of lower molecular weight fatty acids in the oil and it may therefore be regarded as more edible than the peel oil.

The Iodine value of the seed oil is 78.00 gI_2/100 g which is lower than that of the orange peel oil which is 120.10 gI_2/100 g indicating that orange peel oil is rich in unsaturated fatty acid (70.05%). This implies that orange seed oil has a lower amount of double bond (59.76% unsaturated fatty acid) thus lowering the susceptibility of such oil to oxidative rancidity. Triglyceride oils are divided into three groups depending on their iodine values: drying, semi-drying and non-drying oils. The iodine value of a drying oil is higher than 130. This value is between90 and 130 for semi-drying oils. If the iodine value is smaller than 90, oil is called non-drying oil (Guner et al., 2006).

This classifies orange seed oil as a non-drying oil and

Table 1. Physico-chemical parameters of the oil samples.

Variable	Orange seed oil	Orange peel oil
pH	4.2	5.2
Colour	Golden-yellowish	Brownish -yellow
Percentage yield	38%	30%
Specific density	0.997 g/cm^3	0.778 g/cm^3
Refractive Index	1.46	1.47
Smoke point	140°C	149°C
Flash point	150°C	160°C
Cloud point	13°C	16°C
Pour point	7°C	10 °C
Viscosity 100°C	3.8185cst	0.9622cst
Viscosity 40°C	11.968cst	1.9766cst
Acid value	23.6 mgKOH/g	25.1 mgKOH/g
Peroxide value	18.00 mgKOH/g	5.40 mgKOH/g
Free fatty acid	11.86% as oleic acid	12.61% as oleic acid
Saponification value	222.58 mgKOH/g	41.25 mgKOH/g
Ester value	178.24 mgKOH/g	28.96 mgKOH/g
Iodine value	78.83 I_2g/100 g	120.10 I_2g/100 g

Table 2. Fatty acid composition of the seed oil.

Saturated fatty acid (relative abundance, %)	Monounsaturated fatty acid (relative abundance, %)	Polyunsaturated fatty acid (relative abundance, %)
Palmitic acid C16:0 (31.1)	Palmitoleic acid C16:1 (0.34)	Linoleic acid C18:2n6c (35.13)
Stearic acid C18:0 (4.97)	Oleic acid C18:1n9c (24.95)	Dihomo-linolenic acid C20:3n6 (0.04)
Arachidic acid C20:0 (3.68)	-	-
Heneicosylic acid C21:0 (0.32)	-	-
Tricosylic acid C23:0 (0.16)	-	-
Total = 40.23	Total = 25.29	Total = 35.17

the peel oil as a semi-drying oil. The peel oil will be more applicable in varnishes and paint industry while the seed oil will be useful in soap industry. The seed oil contains 59.76% unsaturated fatty acid, peel oil has 70.05% unsaturated fatty acid, 24.59% mono-unsaturated fatty acid and 35.17% polyunsaturated fatty acid (Table 2) while the peel oil has 70.05% unsaturated fatty acid, 31.83% mono-unsaturated fatty acid and 38.22% polyunsaturated fatty acid (Table 3). This implies that the peel oil is more unsaturated than the seed oil thereby confirming the reason for its higher iodine value, smoke point and higher refractive index. This also predicts the more oxidation stability of the seed oil and its possibility of serving as edible oil.

From the fatty acid profiles represented in Tables 2 and 3), it indicates that the peel oil has a high proportion of fatty acids with high molecular weight and this explains its low saponification value (Table 1). This is because they have relatively fewer numbers of carboxylic functional groups per unit mass of the oil. Thus it is regarded non-edible and may not be suitable for soap making. The higher percentage of unsaturation (mono and polyunsaturation) in peel oil makes it more reactive and useful in industrial application such as surface coating applications for example, paints, vanishes, printing and writing inks. The seed oil contains one out of the two families of essential fatty acid which is linoleic acid (omega-6) and it is the most abundant unsaturated fatty acid with a relative abundance of 35.13%. The peel oil contains the two families of essential fatty acid which is linoleic acid (omega-6) 18.63% and -linoleic acid 3.62%. Palmitoleic acid (omega-7) is the most abundant unsaturated fatty acid with a relative abundance of 22.78% in the peel oil.

The peroxide value of the orange seed oil exceeds the permitted maximum peroxide value for edible oil, which is 10 mequivalent of oxygen/kg of the oil (FAO/WHO, 1993) and its high acid value, coupled with high percentage of saturated fatty acid indicate that the orange seed oil may not be good for consumption but useful in industrial applications such as the cosmetics industry which includes soap making, perfumes and unguents.

The metal analysis (Table 4) of the peel ash showed metals of varying concentrations. Although the soap produced from the ash-derived alkalis was softer than bar

Table 3. Fatty acid composition of peel oil.

Saturated fatty acid	Relative abundance %	Monounsaturated fatty acid	Relative abundance %	Polyunsaturated fatty acid	Relative abundance %
Undecylic acid C11:0	10.83	Palmitoleic acid C16:1	22.78	Linoleic acid C18:2n6c	18.63
Lauric acid C12:0	0.88	Oleic acid C18:1n9c	9.05	-Linolenic acid	3.62
Palmitic acid C16:0	0.75	-	-	-	-
Stearic acid C18:0	2.61	-	-	Dihomo-linolenic acid C20:3n6	7.46
Eicosanoic acid C20:0	1.69	-	-	Arachidonic acid C20:4n6	1.63
Behenic acid C22:0	0.48	-	-	Cis-13,16- docosadienoic acid C22:2	6.88
Tricosylic acid C23:0	8.41	-	-	-	-
Lignoceric acid C24:0	4.32	-	-	-	-
Total	29.97	-	31.83	-	38.22

Table 4. Concentrations and percentage compositions of ash derived alkali from peels.

Elements	Concentration (ppm)	Composition of elements (%)
K	151.97	68.77
Ca	39.7	17.96
Na	24.7	11.17
Mg	4.62	2.08
Total	220.97	

Figure 2. Soap from ashed peel alkali and seed oil.

Table 5. Physicochemical analysis of the soap produced.

Colour of soap	Yellow
pH	9.79
Total fatty matter	41.0%
Total alkali	4.65%
Foam height	7 cm^3
Solubility in water	Soluble
Texture	Soft

Figure 3. Soap foamability test.

soap in the market it could still be described as soft solid soap (Figure 2). This is expected as the percentage concentrations of K, Ca, Na, Mg in the peel were 68.77, 39.7, 24.7 and 4.62% respectively (Table 4) of the total metal ions analyzed in the sample. The solubility of soap in water increased with the size of the monovalent cation (base); an increase in the size of a divalent cation (Mg, Ca) results in a decrease in the foamability. Potassium soaps are more soluble in water than sodium soaps; hence, the soap produced was soluble and lather very well (Figure 3, Table 5). Potassium soaps in concentrated form are called soft/liquid soap. Potassium soaps require less water to liquefy because of their softness and greater solubility; thus can contain more cleaning agent than liquefied sodium soap and can be used as shampoos, shaving creams, cleaning of dirty floors and cooking utensils, in emulsion polymerization processes used in rubber and plastic industries and in such other

Table 6. *In-vitro* antimicrobial activity of the oil and soap of the seed and peel of *C. cinensis.*

Agent	Organisms	Concentration (mg/ml)	Diameter of zone of inhibition (mm)**
Peel oil	*P. mirabilis* (clinical strain)	Pure oil	16.0
	K. pneumonia (clinical)	Pure oil	2.0
	P. fluorescence (clinical strain)	40	4.0
	S. aureus (ATCC 29213)	20	6.0
		40	8.0
	Shigella flexinerii (clinical)	40	5.0
	C. albicans (ATCC 24433)	20	4.0
		40	7.0
	C. pseudotropicalis (NCYC 6)	40	10.0
Seed oil	*E. coli* ATCC 25922	Pure oil	6.0
		40	2.0
	B. subtilis (NCIB 3610)	Pure oil	3.0
		20	2.0
		40	8.0
	Proteus mirabilis (clinical strain)	Pure oil	14.0
	K. pneumonia (clinical strain)	Pure oil	5.0
		40	4.0
	Ps. aeruginosa ATCC 27853	10	2.0
		20	3.0
		40	5.0
	Ps. fluorescence (clinical strain)	40	4.0
	S. aureus (ATCC 29213)	2.5-5.0	6.0
		10	7.0
		20	8.0
		40	14.0
	Shigella flexineri (clinical strain)	20	4.0
		40	11.0
	C. albicans (ATCC 24433)	2.5	2.0
		5.0-10.0	4.0
		20	6.0
		40	10.0
	C. pseudotropicalis (NCYC 6)	10	4.0
		20-40	10.0
Seed oil soap	*Proteus mirabilis* (clinical strain)	Pure Soap	4.0
	Klebsiella pneumonia (clinical strain)	Pure Soap	9.0

Diameter of zone of inhibition of streptomycin (1 mg/ml) for each organism was: *E. coli* ATCC 25922, 14.0 mm; *P. aeruginosa* ATCC 27853, 14.0 mm; *P. fluorescence* (clinical strain), 14.0 mm; *S. aureus* ATCC 29213, 14.0 mm; *B. subtilis* NCIB 3610, 10.0 mm; *K. pneumonia* (clinical), 12.0 mm; *S. flexinerii* (clinical) 10.0 mm; *P. mirabilis* (clinical strain), 10.0 mm. Acriflavin (6.3 mg/ml) inhibition for the fungi was: *C. albicans* (ATCC 24433),18.0 mm, *C. pseudotropicalis* (NCYC 6), 21.0 mm. *The agents showed activities only against the organisms indicated. **Zone of inhibition less cup size.

similar uses. The presence of 24.7% sodium out of the total percent of the alkali increases the firmness of the soap which ought to be liquid or semi-solid. Calcium is the major ion that limits its foam ability because of 39.7% composition.

The yellowness of the oil was considerably reduced by bleaching, which gave the soap a cream colour. Spectrophotometry analysis of the metallic ions present in ashed samples solution (Table 4) showed that the alkali consist of ions that are essential diet components by contributing sodium, calcium, potassium and other essential nutritional elements.

Results of antimicrobial evaluation show that the two oil samples possess useful antimicrobial activities as anti-bacterial and antifungal inhibitory activities were obtained at concentrations of 40 mg/ml and below (Table 6). The

antimicrobial activities of grape fruit (*C. paradisi*) and grape peel oil had earlier been documented (Okunowo et al., 2013). Furthermore, the presence of metabolites with documented antimicrobial effects such as alkaloids, saponins, flavonoids, tannins and phenolic compounds in *C. sinensis* peel extract has been reported (Bocco et al., 1998; Hussain et al., 2015). Thus the antimicrobial activities obtained in this study have known scientific basis. The antimicrobial activities are broad spectrum against a wide range of Gram positive and Gram negative bacteria and the two candida strains, *C. albicans* and *C. pseudotropicalis*, screened. These organisms have been implicated in skin and mucous membrane infections with reports of morbidity and mortality (Mahmoud, 2001). Seed oil demonstrated better activities than the peel oil indicating that antimicrobial constituents are more concentrated in the seed oil.

Further studies are therefore needed to elucidate these constituents and their contributions to the antimicrobial effects. These results also indicate that free fatty acids, obtained at a higher content in the peel oil compared with the seed oil, do not contribute to the antimicrobial effects of *C. sinensis*. Inhibition zones were obtained for the seed oil against *S. aureus* and *C. albicans* at a concentration as low as 2.5 mg/ml. In some cases the activities of these oils were observed to be comparable to that obtained for the standard antibacterial agent, streptomycin, at the tested concentration. These cases include inhibitory activities obtained for the pure peel and seed oil against *Proteus mirabilis* (16 and 14 mm, respectively) compared with that for streptomycin [1 mg/ml] which was 10 mm.

Seed oil at 40 mg/ml also demonstrated similar inhibitory activity with streptomycin at 14 mm zone of inhibition. The activity of the seed oil against *P. aeruginosa* at a concentration as low as 10 mg/ml is especially noteworthy as this organism is notorious for its intrinsic resistance to most standard antibacterial agents. For the soap, antimicrobial activities were obtained only for the seed oil soap with activities demonstrated against *P. mirabilis* and *K. pneumonia* at 4.0 and 9.0 mm zone of inhibition respectively.

The antimicrobial activities of the pure seed oil and peel oil showed its usefulness in cosmetic and pharmaceutical industries in preparation of topical cream/gel against both gram -positive and gram negative bacteria and fungi infection. The activities of the seed oil soap further strengthen the usefulness of the seed oil for potential use in soap formulation against susceptible organisms. The peel oil will also find good use as antimicrobial agent in many infectious diseases especially against infections caused by *S. aureus*. It also has great potential as antifungal agents against the candida strains (Table 6).

Conclusions

From the physicochemical parameters and fatty acid composition of *C. sinensis* seed and peel oil analyzed, both oils are recommended for industrial applications, specifically the cosmetics industry. High composition of unsaturated fatty acid such as palmitoleic acid, linoleic acid, cis-13, 16- docosadienoic acid, alpha-linoleic acid and arachidonic acid in the peel oil makes it reactive and to have a semi-drying property as confirmed by its iodine value. Thereby making it suitable in the production of paints, inks and vanishes.

The presence of fatty acids such as linoleic acid, palmitoleic acid, oleic acid and other unsaturated fatty acid in the seed oil could function as emollient and thickening agents. They also serve as fragrance ingredient and cleansing agents. Linoleic acid is an anti-oxidant which could prevent ageing. Saturated fatty acids such as palmitic acid, stearic acid and arachidic acid fulfill the role of a fragrance ingredient, thickener or hardener when the oil is used in soap making.

The broad spectrum activities of the seed oil against strains of organisms responsible for many infectious diseases together with the favourable physicochemical properties obtained for this oil, which support its use in cosmetic and soap making, are actually synergistic and make this oil of tremendous potential for these industries. This study has shown that *C. sinensis* seeds and peels could be put to productive use in the cosmetic and pharmaceutical industries rather than continuing to constitute worrisome menace as environmental wastes and pollutants.

Conflict of interests

The authors have not declared any conflict of interests.

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