



Chemical and biological evaluations of potent antiseptic cosmetic products obtained from *Momordica charantia* seed oil

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ABSTRACT

The Principles of Green Chemistry was employed for the preparation of organic antiseptic soaps with improved skin sensitivity, reduced skin toxicity and improved biodegradability. Non-conventional and lesser known tropical seeds of *Momordica charantia* were used as the source of oil for the saponification processes. The fatty acid methyl esters of the oil were prepared via transesterification and subjected to GC–MS analysis to obtain the fatty acid composition of the oil and saponified products were subjected to various physicochemical and antimicrobial evaluations. The results indicated that stearic acid (18:0) and eleostearic acid (18:3) were the most abundant fatty acids making up 37.60% and 39.16%, respectively. Palmitic acid (16:0) 12.36%, oleic acid (18:1) 8.71%, linoleic acid (18:2) 0.67% and gamolenic acid (18:3) 1.50% were present in smaller amount in the oil. The oil is a viable source of polyunsaturated fatty acid which is required as essential fatty acids in the human body. The prepared soaps containing only natural additives (such as honey and shear butter) and components showed appreciable degree of hardness comparable to commercial Dudu Osun and the synthetic Lux soaps used as standards. This work demonstrates the probability of the preparation of green antiseptic soaps from the underutilized tropical seed of *M. charantia*. The seed oil and the allied products obviously have great potential for further advancement, development, research and applications in health and cosmetics sectors. The adopted approach could also assist maintaining a safe and healthy biotic and abiotic environment.

1. Introduction

Seeds are major source of oil with many applications such as dietary, paints, soap, oleochemicals in everywhere in the world. Apart from food and biochemical potentials, fats and oils are raw materials for coatings, paints, pharmaceuticals, soaps, detergent and the cosmetics industries (Ayode and Amoo, 2015). The biological activity of several seeds has been reported (Muhammed et al., 2012; Aremu et al., 2015). Biological activity of a seed plant entails its ability to successfully slow or prevent the growth of disease pathogens (antimicrobial), ability to mop off free radicals in the body (antioxidant) and ability to keep cells from bursting (anti-inflammatory). Application of bioactive seeds in the making soap might confer on the soap several biological properties. The presence of natural antioxidants in oil could help in the management of diseases conditions such as skin cancers, rashes, skin dryness and other

skin infections (Dillard and German, 2000).

The physicochemical characteristics of fatty acid compositions of fats and oils, besides their value for reference purposes, serve as useful guides to oil and fat analysts in determining the possible uses (Nagré et al., 2011).

Research has indicated that synthetic antioxidants induce allergic reactions in human, a disadvantage minimized in natural antioxidants found in seed oil (Joshi and Pawal, 2015; Suzuki, 2010). Similarly, development of microbial resistance had been credited to some modern commercial soaps that contain synthetic antimicrobial chemicals agents such as triclosan, trichlorocarbanilide and chloroxylenol, most of which are also reported to be carcinogenic and generate allergic reactions. Previous research efforts had indicated the viability of obtaining NaOH and KOH (lye) from wood ash for the production of natural soaps (Atolani et al., 2016; Zauro et al., 2016; Atiku et al., 2014).

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Soaps produced from the natural lye and underutilized bioactive oil using green chemistry principle offers products that are easily adaptable to human skin, biodegradable, environmental friendly, inhibit the growth of disease pathogens, scavenge of free radicals in the body, mild on cells, economically valuable, sustainable and environmentally friendly.

The production of soaps from completely natural means will significantly address one of United Nations goals of environmental sustainability pertaining to persistent environmental pollution caused by huge non-biodegradable soaps in the market. This will also enable less developed nations to reduce or eliminate the use and generation of hazardous substances (Lancaster, 2002; Anastas and Eghbali, 2010). Soap is a mixture of sodium or potassium salts of naturally occurring fatty acids (Gunstone, 2012). The physicochemical characteristic of soap depends on strength and purity of alkali, the composition of the oil used and degree of saponification. Physicochemical characteristics which include pH, solubility, hardness, washing efficiency and free alkalinity are used in determining the overall quality of the soap (Roila et al., 2001).

M. charantia (Karela) commonly known as Bitter melon or Bitter gourd is tropical and subtropical climber of the family Cucurbitaceae. It is commonly found in China, Malaysia, India and tropical Africa. The fruits extract is used in tradition medicines as medication to cure various diseases like: rheumatism, gout, worms, colic, liver disease (Agrawal and Kamal, 2004).

M. charantia contains an array of biologically active phyto-compounds such as triterpenes, proteins, steroids, alkaloids, saponins, flavonoids and fatty acids responsible for the antifungal, antibacterial, antiparasitic, antiviral, antifertility, antitumor, hypoglycemic and anticarcinogenic properties (Beloin et al., 2005; Grover and Yadav, 2004).

M. charantia leaves, stem and bark are known to contain compounds which includes momordicin, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, α -stearic acids, erythrodilol, galacturonic acids and gentisic acid (Braca et al., 2008).

This present study aimed at adopting the principles of green Chemistry for the sustainable production of natural cosmetic soaps from the underutilized tropical seed of *M. charantia* with improved antiseptic properties ability to ameliorate natural beauty, attractiveness and appearance of skin and hair.

2. Materials and methods

2.1. Collection of plant material

The seed of *M. charantia* were collected within Ilorin metropolis, Kwara State, Nigeria. The plant material was identified at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria where voucher specimen numbers UILH/003/916 was obtained.

The seeds were dried at ambient temperature, de-shelled, pulverized and kept in a cool dry place for further work.

2.2. Extraction of Oil from *M. charantia* Seed

The pulverized *M. charantia* seed (300 g) was subjected to Soxhlet extraction using n-hexane as the extracting solvent at 60 °C for approximately 3 h. The crude extract was concentrated via distillation using rotatory evaporator to obtain the oil (Islam et al., 2015).

2.3. Determination of the physicochemical parameter of the oil

The physicochemical parameters of the oil were determined using standard procedures with slight modification where applicable (AOCS, 2007; Gerpen, 2005; Ibeto et al., 2012). Odor, color and physical state were determined by sensory evaluation.

2.4. Preparation of fatty acids methyl ester (FAMES)

One g of the seed oils was weighed into a round bottom flask. 20 mL of 0.1 M methanolic KOH was added. The mixtures were refluxed for 1 h. After refluxing, supernatant obtained was cooled, transferred to a separating funnel and extracted with n-Hexane twice. The oil layer was concentrated in warm water bath and the oils derived kept in a vial and refrigerated until analysed using GC-MS (Atolani et al., 2016).

2.5. GC-MS analysis of the oils

The fatty acid composition of the transesterified oil was analysed using an Agilent Technology 7890A gas chromatograph GC-FID, equipped with a fused silica capillary column HP-5MS (30 m by 0.32, 0.5 μ m film thickness) on ultra-pure helium gas and coupled to a mass selective detector (mass spectrometer). The chemical compositions of the product obtained was identified by comparison of their MS spectral with data obtained from National Institute Standard and Technology (NIST, 2008) database. The relative percentages of the constituent compounds were percentages from the GC peak areas based on the total ion chromatogram.

2.6. Lye preparation

The wood ash was collected from a local bakery in Ilorin, Kwara State metropolis, sieved and weighed. 30 kg of the wood ash was soaked in 75 L of warm water for 24 h and then filtered using clean white layered clothe to obtain the lye, a brown colored solution. The solution was later concentrated to 4 l prior to use in soap making (Atolani et al., 2016).

2.7. pH, Conductivity and turbidity tests

The pH was determined using pH meter (827 pH lab model), conductivity of the lye solutions was determined using an EC 214 Conductivity Meter while the turbidity was determined using 2100N Turbidity Meter (Atolani et al., 2016).

2.8. Standardization of the lye using flame atomic absorption spectrometric

The extracted lye (100 mL) solution was filtered using a Whatman No. 1 filter paper into a 250 mL beaker, 2 mL concentrated HCl and 5 mL concentrated nitric acid were added and digested on a hot plate to 20 mL. The digested solution was filtered using a Whatman No. 1 filter paper into a 100 mL volumetric flask and adjusted to the mark with deionized water. The amount of Na^+ and K^+ ion in the weighted ash was determined by Flame Atomic Absorption Spectrometric method using the Agilent 240 FS double-beam AA spectrometer.

2.9. Saponification reaction

The extracted oil was saponified using hot process since the cold process produced no instant saponification. 5 mL of the oils were heated to boiling in a beaker and 50 mL of lye solution was added to the boiling oil with constant stirring. A thick semi-solid mass of soap was obtained, allowed to cool and set for some weeks following standard procedure (Warra et al., 2009; Ogunsuyi and Akinnawo, 2012). Additives which include 0.3 mg of Shear butter fat (for skin nourishment) and 0.4 mg of honey (for skin rejuvenation) were added after completing the saponification process but before the hardening of the semi-solid matter.

2.10. Soap characterizations

The saponified product was characterized for its pH, foaming ability, hardness, cleaning effectiveness, solubility and total alkalinity whilst comparing their values with commercial soap samples using

standard procedure (Ameh et al., 2013). Commercial bathing soaps which include Lux soap was used as standard for comparison.

2.11. Determination of pH of soap

The pH of the prepared soap was determined using a pH meter (827 pH lab model). 1 g of the soap shavings was weighed and dissolved in deionized water in a 100 mL volumetric flask. This was made up to form 10% soap solution. The electrode of the pH meter was inserted into the solution. The pH was recorded as described by (Umar, 2002).

2.12. Foaming ability test

2 g of the soap (shavings) was added to a 500 mL measuring cylinder containing 100 mL of deionized water. The mixture was shaken vigorously so as to generate foams. After shaking for about 2 min, the cylinder was allowed to stand for about 10 min. The height of the foam in the solution was measured and recorded (Atolani et al., 2016).

2.13. Solubility tests

0.2 g of the soap was added to a 100 mL measuring cylinder containing 10 mL of distilled water. The duration of the dissolution of the soap after continuous shaking was recorded.

2.14. Free alkalinity test

The total unreacted alkali left after the saponification reaction in the soaps and commercial soaps used as standards were determined by titration with 0.5 M HCl. 1 g of the soap and that of the commercial soaps used as standards was dissolved in 100 mL deionized water, 25 mL of each soap solution was then titrated against 0.5 M HCl in triplicates. The volume of acid used was recorded and the total free alkalinity was calculated using the formula (Ameh et al., 2013):

$$\text{Total Alkalinity} = \left(\frac{\text{vol. of acid used}}{\text{vol of soap solution}(25\text{ml})} \right) \times 100\%$$

2.15. Test for cleansing efficiency

A drop of oil was placed on four separate sheets of filter paper. The filter papers with the oil spot were immersed in a separate test tubes containing soap solution (2 g soap shavings/100 mL) each was vigorously shaken for 2 min. The filter papers were removed and rinsed with distilled water and the degree of cleanliness in each filter paper physically observed.

2.16. Determination of oil and soap antimicrobial sensitivity assay

Antimicrobial sensitivity of the oil of *M. charantia* seed and the soap produced from it with the commercial soaps used as standard was studied using selected bacteria: *Streptococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae* and fungi: *Candida albicans*, *Penicillium notatum*, *Rhizopus stoloniter* and *Aspergillus niger*. 500 mg of the oil was weighed and dissolved in 1.0 mL of Tween 20 to give concentration of 500 mg/mL. Serial dilutions to obtain concentrations 250, 125, 62.5, 31.25 and 15.625 mg/mL of the oil was made. 20 mg of each soap samples were equally weighed and dissolved in 10 mL Tween 20, to obtain 2.0 mg/mL concentration and later used to prepare 1.0, 0.5, 0.25, 0.125 and 0.0625 mg/mL following serial dilutions. An overnight culture of each organism was prepared by taken a full organism from stock and inoculate into a sterile nutrient broth of 5 mL each for 18–24 h at 37 °C. Out of the overnight culture, 0.1 mL of each organism was taken and put into 9.9 mL of sterile distilled water to get 1:100 (10^{-2}) of the

dilute solution of the organism. For fungi, pour plate method was used and surface plate method was used for bacteria. Using a sterile cork borer of 8 mm, the wells were made according to the number of graded concentrations of the sample. This was done in duplicates. The plates were allowed to stay on the bench for 2 h to allow pre-diffusion. The plates were incubated uprightly in the incubator for 24 h at 37 °C., for bacterial and 48 h at 28 °C for fungal. The zone of inhibition was measured thereafter using a ruler (Ameh et al., 2013).

2.16.1. Statistical analysis

All experiments were performed in triplicate except and the results presented as a mean of the three values with the standard error of mean otherwise indicated. The data obtained were subjected to statistical analysis using Graphpad prism 7 (San Diego, USA). The Graphpad prism 7 was also used to determine statistical significance as differences at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Physicochemical analysis of oil

Physicochemical characteristics of the oil of *M. charantia* obtained via Soxhlet extraction is given in Table 1 below. The oil was obtained as a creamy solid at ambient temperature (25 °C). The oil exhibited a high iodine value of 126.55 ± 0.49 , projecting a high degree of unsaturation of the fatty acids, which was confirmed in the GC-MS analysis. The total unsaturated fatty acids were found to be 50.04% (Table 2). The acid value of 14.52 ± 0.89 mgKOH/g was higher than 4 mgKOH/g recommended value for edible oil by FAO/WHO indicating the unsuitability of the oil for consumption as food. Seed has a high yield of 25.49% oil confirming its potential for commercial exploration as in soap making. The low peroxide value of the oil (8.03 ± 0.78) indicates that soaps produced from it could possess the ability to scavenge reactive chemical species that are generated in human skin as a result of metabolic activities and radiation of ultraviolet light on the skin. Low peroxide value has been attributed to the presence of natural antioxidant (Nagré et al., 2011). The oil has saponification value of 77.67 ± 0.66 compare to 181.40 ± 2.60 mgKOH/g recommended value for edible oil. The low free fatty acid ($8.40 \pm 0.78\%$ free oleic acid) which is within recommended range of 5.78–7.28% by WHO/FAO portends the oils could have a high shelf life.

3.2. Fatty acids composition of the oils

Fatty acids composition of the oil of *M. charantia* which was determined by subjecting the transesterified oil to GC-MS analysis is as shown in Table 2. Stearic acid (18:0) and α -eleostearic acid (18:3) are the most abundant fatty acids making up 37.60% and 39.16% respectively. Palmitic acid (16:0) 12.36%, oleic acid (18:1) 8.71%, linoleic acid (18:2) 0.67% and gamolenic acid (18:3) 1.50% were also found in limited proportions in the oil. The result is in agreement with previous

Table 1
Physicochemical parameter of the oil seed.

Parameter	<i>Mormodica charantia</i>	WHO/FAO
% Yield	25.49	NS
Saponification value (mg KOH/g)	77.67 ± 0.66	181.4 ± 2.60
Acid value (mg KOH/g)	14.52 ± 0.89	4
% Free fatty acid	8.40 ± 0.78	5.78–7.28
Peroxide value (meq kg ⁻¹)	8.03 ± 0.18	10
Iodine value (g of I ₂ /100 g of oil)	126.55 ± 0.49	80–108
Color	Creamy	NS
Physical state at ambient temperature (25 °C)	Solid	NS

Values are average of triplicates sample; NS – None Specified.

Table 2
Fatty acids composition of the oil.

Peak no.	Retention Time (min)	Fatty acids	Saturation	% Composition
1	12.55	Palmitic acid	16:0	12.36
2	13.32	Gamolenic Acid	18:3	1.50
3	13.81	Linoleic acid	18:2	0.67
4	13.93	Oleic acid	18:1	8.71
5	14.13	Stearic acid	18:0	37.60
6	14.93	α -Eleostearic acid	18:3	39.16
Total Saturation				49.96%
Monounsaturate				8.71%
Polyunsaturate				41.33%
Total Unsaturate				50.04%

work on the seed in Bangladesh and India where α -eleostearic acid was reported to be the major fatty acid (Ali et al., 2008; Arora and Chaudhary, 2012). The results indicated that the oil is a rich source of essential fatty acids (α -eleostearic acid and gamolenic acid) required by the body. Human body requires essential fatty acid for various biological processes and seed oil are recommended sources (Maclean et al., 2004). Evidence of health effect of essential fatty acids such as α -eleostearic acid and gamolenic acid in fighting diabetes and other heart related diseases have been reported by Kris-Etherton et al. (2002) and Li et al. (2003) (Figs. 1 and 2)

3.3. Characterization of the prepared lye solution

The lye solution prepared from wood ash was characterized using Atomic Absorption Spectrophotometer, pH meter, Conductivity meter and Turbidity meter.

3.3.1. pH, conductivities and turbidity of the lye solution

The extracted lye solution had a high pH value of 11.2, indicating a strong alkaline solution. The conductivity and turbidity (Table 3) before concentration were 20 ± 1.41 NTU and 15.0 ± 0 mS respectively and 179.5 ± 0.71 NTU and >4000 mS respectively after concentrating from 75 L to 3 L.

3.3.2. Determination of Na^+ and K^+ in Lye solution using AAS

The extracted lye (and blank sample of deionized water) was characterized using Atomic Absorption Spectrophotometric (AAS) to determine the concentration of K^+ and Na^+ . The blank sample shows a concentration of 6.33 mg/L of K^+ and 24.56 mg/L of Na^+ while the lye

shows a $[\text{K}^+]$ and $[\text{Na}^+]$ of 7250.03 mg/L and 807.56 mg/L respectively (Table 4). The result supports the production of soft and skin-tender soap since the lye solution had higher concentrations of K^+ that is almost tenfold the concentration of Na^+ . K^+ is known for formation of soft soaps for toilet use (Warra et al., 2011).

3.4. Antimicrobial activity of the oil

The antimicrobial activity of the oil of *M. charantia* is given in Table 5. There was however a dose dependent inhibition at concentrations between 125 and 500 mg/mL. The oil exhibited highest activities against *S. typhi* and *K. pneumone* with minimum inhibitory concentration (MIC) of 15.63 mg/mL. The oil shows a high antibacterial inhibitory value of 21.0 ± 1.41 mm against *S. typhi*, 18.0 ± 0 mm against *E. coli* and 17.0 ± 1.41 mm against *K. pneumone* and *S. aureus*. The highest antifungal inhibition was observed against *C. albicans* (17.0 ± 1.41 mm), *R. stoloniter* (15.0 ± 1.41 mm) and *A. niger* (15.0 ± 1.41 mm). The result obtained indicated that the oil has a higher antibacterial activity with an MIC value of 15.63–62.50 mg/ compared to 62.50–125 mg/L recorded against fungi.

3.5. Mixing ratio of the oil/fat for the saponification

The mixing ratio of soap, color and washing efficiency of the soaps are shown in Table 6. The soap made from *M. charantia* had a deep brown coloration. Honey and shear butter were added for rejuvenation and protection of the skin. The soap shows washing efficiency that is comparable to commercial soaps (Dudu Osun and Lux) used as standards (Atolani et al., 2016).

3.6. Physicochemical characterization of the prepared soaps

The pH value (Table 3) of the prepared soap was determined at two different stages which include the day the soap was saponified and after the soap had cured (a month later). The pH value of the cured soap and the commercial soaps used as standards fell within acceptable range (7–10) that is permitted by regulating agency in Nigeria. The pH was also in agreements with several literatures (Atolani et al., 2016; Vivian et al., 2014; Warra et al., 2010.). The near neutral pH value of the soap (7.40 ± 0) indicates high degree of the saponification process and that less unreacted alkali exist. The application of lye derived from wood ash used in this work produced less alkaline soap which would be more body friendly. The use of soaps with high pH value has been reported to increase the pH of the skin which has been attributed to the cause of

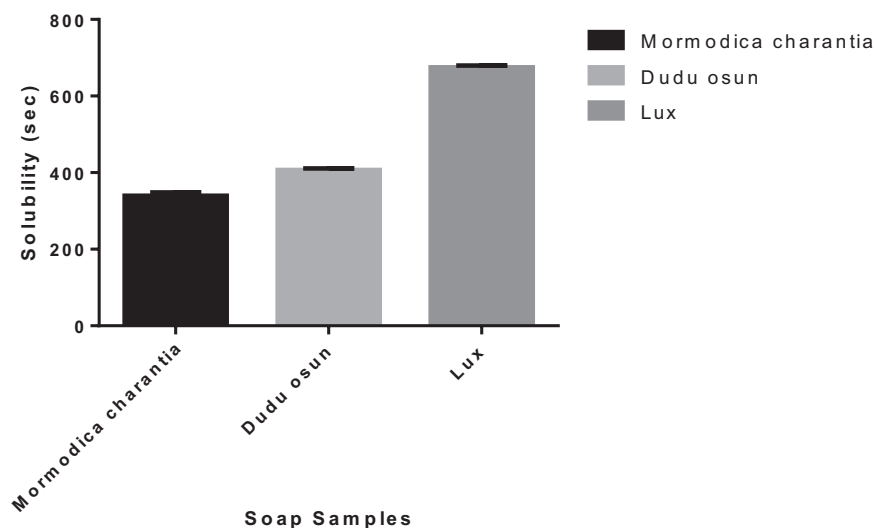


Fig. 1. Solubility test of the soaps.

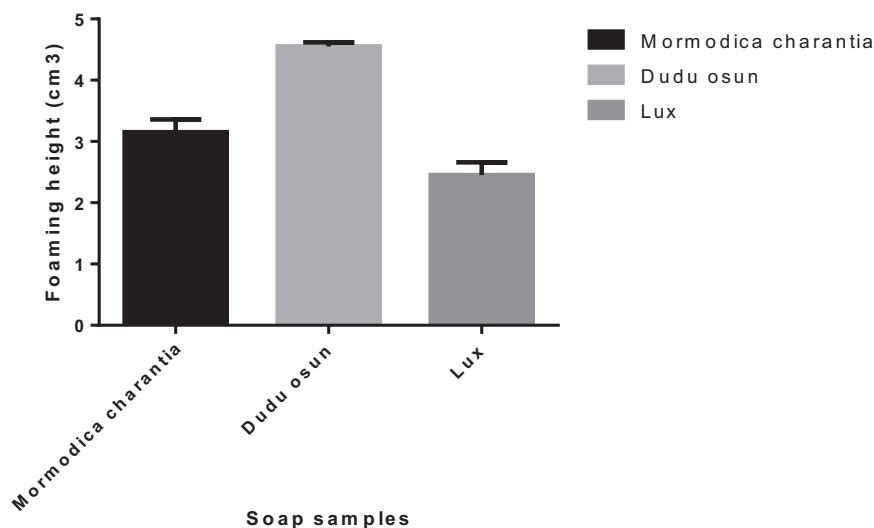


Fig. 2. Foaming ability of the soaps.

Table 3

pH, Conductivities and turbidity of the lye solution.

Parameters	Before Concentration of Lye (75 L)	After Concentration (3 L)
pH	11.20	ND
Conductivity (mS)	15.00 ± 0.00	179.50 ± 0.71
Turbidity (NTU)	20.00 ± 0.00	>4000.00

Values are average of duplicates sample; ND = not determined.

Table 4

Characterization of the lye solution using AAS.

Parameters	Blank (mg/L)	Lye Solution (mg/L)
K ⁺	6.33	7250.03
Na ⁺	24.56	807.56

increased dehydration, irritation and destruction of upper layer of the skin (Tarun et al., 2014). High alkaline soap can also neutralize the natural acidic condition of the skin thereby exposing the skin to infectious diseases (Mak-Mensah and Firemping, 2011). The soap shows appreciable degree of hardness comparable to Lux soap used as standards (Table 7). The hardness of the soap can be attributed to the degree of saturation of the oil. The solubility of soap is also comparable to

Table 6

Mixing ratio of the oil/fat used for the saponification.

Soap Formulations	Oil/Fat and Additive mixing ratio	Mixing ratios	Soap Color	Washing efficiency
Momordica charantia oil Soap	Oil + honey + shea butter	0.95:0.025:0.025	Deep brown	Very good
Dudu Osun	–	–	Deep brown	Very good
Lux	–	–	white	Good

Table 7

Physicochemical characteristics of the soaps.

Soap Samples	Momordica charantia	Dudu Osun	Lux
pH before curing	9.85 ± 0.07	NS	NS
pH after curing	7.40 ± 0.00	8.4 ± 0.00	8.50 ± 0.00
Foam height (cm ³)	3.15 ± 0.21	4.55 ± 0.07	2.45 ± 0.21
Solubility (s)	339.50 ± 8.90	407 ± 4.20	675 ± 4.60
Total Alkalinity %	0.80%	0.80%	0.40%
Texture	Hard	Soft	Hard

Data were obtained in duplicate; NS = not specified.

Table 5

Inhibitory effect of Mormodica charantia seed oil against pathogens.

Concentrations (mg/mL)							
Pathogens	500	250	125	62.5	31.25	15.63	MIC (mg/mL)
	Diameter Zone of Inhibition (mm)						
<i>S. aureus</i>	17 ± 1.41	14 ± 0.00	14 ± 0.00	12 ± 0.00	* 10 ± 0.00	–	31.25
<i>B. subtilis</i>	15 ± 1.41	13 ± 1.41	12 ± 0.00	10 ± 0.00	* 10 ± 0.00	–	31.25
<i>E. coli</i>	18 ± 0.00	16 ± 0.00	14 ± 0.00	12 ± 0.00	* 10 ± 0.00	–	31.25
<i>P. aeruginosa</i>	16 ± 0.00	14 ± 0.00	12 ± 0.00	10 ± 0.00	–	–	62.50
<i>S. typhi</i>	21 ± 1.41	18 ± 0.00	16 ± 0.00	14 ± 0.00	* 12 ± 0.00	* 10 ± 0.00	15.63
<i>K. pneumoniae</i>	17 ± 1.41	14 ± 1.41	14 ± 0.00	12 ± 0.00	* 10 ± 0.00	* 10 ± 0.00	15.63
<i>R. stolonifer</i>	15 ± 1.41	13 ± 1.41	10 ± 0.00	–	–	–	125.00
<i>P. notatum</i>	14 ± 0.00	12 ± 0.00	10 ± 0.00	–	–	–	125.00
<i>C. albicans</i>	17 ± 1.41	14 ± 0.00	12 ± 0.00	10 ± 0.00	–	–	62.50
<i>A. niger</i>	15 ± 1.41	13 ± 1.41	11 ± 1.41	–	–	–	125.00

Data is a mean ± SEM; n = 2. *Statistically different (p < 0.05) compared to group across the groups. (–) means no activity, values are average of duplicates sample, *S. aureus* = *Streptococcus aureus*, *E. coli* = *Escherichia coli*, *B. subtilis* = *Bacillus subtilis*, *P. aeruginosa* = *Pseudomonas aeruginosa*, *S. typhi* = *Salmonella typhi*, *K. pneumoniae* = *Klebsiella pneumoniae*, *C. albicans* = *Candida albicans*, *P. notatum* = *Penicillium notatum*, *R. stolonifer* = *Rhizoptius stolonifer* and *A. niger* = *Aspergillus niger*.

Table 8
Inhibitory effect of *Mormodica charantia* Soap against pathogens.

Concentrations (mg/mL)							
Pathogens	2.0 Diameter Zone of Inhibition (% of control)	1.0	0.5	0.25	0.125	0.0625	MIC (mg/mL)
<i>S. aureus</i>	58.1 ± 0.00	48.3 ± 0.00	48.0 ± 0.00	47.6 ± 0.00	–	–	0.25
<i>B. subtilis</i>	56.0 ± 0.00	54.5 ± 0.00	55.6 ± 0.00	–	–	–	0.5
<i>E. coli</i>	55.6 ± 1.41	56.5 ± 1.41	50.0 ± 0.00	–	–	–	0.5
<i>P. aeruginosa</i>	51.9 ± 0.00	43.5 ± 0.00	–	–	–	–	1.0
<i>S. typhi</i>	–	–	–	–	–	–	–
<i>K. pneumoniae</i>	–	–	–	–	–	–	–
<i>R. stoloniter</i>	62.5 ± 1.41	92.9 ± 1.41	83.3 ± 0.00	–	–	–	0.5
<i>P. notatam</i>	85.0 ± 1.41	87.5 ± 0.00	76.9 ± 0.00	–	–	–	0.5
<i>C. albicans</i>	–	–	–	–	–	–	–
<i>A. Niger</i>	–	–	–	–	–	–	–

Data is a mean ± SEM; n = 2. *Statistically different (p < 0.05) compared to group across the groups. (–) means no activity, values are average of duplicates samples, *S. aureus* = *Streptococcus aureus*, *E. coli* = *Escherichia coli*, *B. subtilis* = *Bacillus subtilis*, *P. aeruginosa* = *Pseudomonas aeruginosa*, *S. typhi* = *Salmonella typhi*, *K. pneumoniae* = *Klebsiella pneumoniae*, *C. albicans* = *Candida albicans*, *P. notatam* = *Penicillium notatum*, *R. stoloniter* = *Rhizoptus stoloniter* and *A. niger* = *Aspergillus niger*.

those of the commercial soaps used as standard, the results from Table 7 indicates the ability of *M. charantia* soap to last long when used due to its slowly dissolution in water. The soap equally shows appreciable foaming height of $3.15 \pm 0.21 \text{ cm}^3$ slightly higher than $2.45 \pm 0.07 \text{ cm}^3$ recorded for Dudu Osun.

3.7. Antimicrobial activity of the soaps

The antimicrobial activity of the soaps from the oil of *M. charantia* calculated as a percentage of the commercial soap (Lux Osun) used as standards is given in Table 8. *M. charantia* was active against both bacteria and fungi, with MIC value range of 0.25–1.0 mg/mL against most of the test organisms. The antimicrobial activity of the oil was retained in the soap produced at concentrations as low as 2.0–0.25 mg/mL compared to 500–15.63 mg/mL equivalent concentration of the oil against the same organisms. The soap inhibits *S. aureus* at MIC value of 0.25 mg/mL, *E. coli* at MIC value of 0.5 mg/mL and *P. aeruginosa* at MIC value of 1 mg/mL against the test bacteria. An MIC value of 0.5 mg/mL each against *R. stoloniter* and *P. notatam* was recorded against the tested fungi. Presence of natural antimicrobial agent in *M. charantia* soap may help in prevention of skin infections caused by these pathogens whilst reducing microbial resistance as a result of synergistic action of different natural antimicrobial agents in the oil and natural additive used in making the soap. The antimicrobial activity of both oil and soaps of *M. charantia* indicated that soaps made from bioactive oil can be used for making natural antiseptic soaps applicable in treating skin related diseases thereby enhancing, nourishing and rejuvenating the skin for improved skin outlook.

4. Conclusion

The fatty acids profile of underutilized seed of *M. charantia* and its potential for preparing antiseptic soaps was investigated. The results indicated that the oil can be beneficial medicinally in preventing skin related diseases, microbial infection, skin inflammation, aging of skin thereby beautifying human skins possibly due to the synergistic activity of oil components and natural additives. The result indicated that the seed can be exploited commercially in making natural antiseptic soaps due to its high yield. The high acid value makes it a good choice for soap production as such would not be recommended for food purposes. The fatty acids profile obtained via GC-MS characterization indicated that the oil can be explored as a plausible source of natural essential fatty acids required by the body. This research equally demonstrated that wood ash which is generally discarded as waste by many bakeries and restaurants in Nigeria can be a good source of natural lye (Alkaline

solution) applicable in making quality natural antiseptic soaps by adopting the Principles of Green Chemistry. The produced soap is free from all forms of synthetic substance such as alkaline, preservatives, colourants, antibiotics and stabilizers.

Conflict of interest

Authors declared that there is no conflict of interest.

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