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A COMPARATIVE STUDY OF THE PHYSICOCHEMICAL PROPERTIES AND ANTIMICROBIAL QUALITIES OF ABUAD MORINGA SOAP WITH CONVENTIONAL MEDICATED SOAPS

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ABSTRACT

The study was aimed at assessing the physicochemical properties and antimicrobial qualities of 'ABUAD moringa soap', a herbal soap produced with *Moringa oleifera* leaf by 'ABUAD Farm', Afe Babalola University, Ado Ekiti, Nigeria. The physicochemical properties and antimicrobial qualities of ABUAD moringa soap on some selected bacteria and fungi were evaluated and compared with those of some conventional medicated and herbal soaps commonly used in Nigeria, such as Dettol, Tetmosol, Tura, Septol, Delta and Dudu Osun (herbal), as well as Lux, which serves as a control soap. The results of the physicochemical analyses revealed that all the soaps fall within the pH range of 8.83 and 9.83. All the soaps possess low values of free caustic alkali and detectable free fatty acid, as well as moderate values of total fatty matter. *In-vitro* antibacterial and antifungal activities of the soaps were investigated against microbial agents commonly found in association with skin infections, using the well-agar diffusion technique. The bacteria tested were *Staphylococcus aureus* ATCC 25923 and *Proteus mirabilis* (ATCC 12453), as well as four clinical isolates namely, *Escherichia coli, Leutococcus sanguinis, Corynebacterium accolens* and *Burkholderia cepacia*. The fungi were *Candida albicans* ATCC 10231, *Malassezia furfur* ATCC 44349, and *Cryptococcus neoformans* ATCC 23645. All the soaps, with exeption of lux, produced varied degrees of antibacterial activities, but ABUAD Moringa soap and Dudu Osun indicated superior effectiveness against the bacteria tested. Antifugal activities were produced by ABUAD moringa and Dudu Osun soaps only, on the fungi tested. ABUAD Moringa produced significantly higher antifungal activities on *Malassezia furfur* ATCC 44349 and *Candida albicans* ATCC 10231 than Dudu Osun, but no significant difference was observed between the two soaps on their activities against *Cryptococcus neoformans* ATCC 23645. The study showed that ABUAD Moringa soap possesses high therapeutic potentials against agents of bacterial and fungal skin infections.

Keywords: antibacterial; antifungal; moringa soap; skin infections

INTRODUCTION

The skin is an important organ of the body that serves as an agent of protection against infection by microorganisms and shields delicate underlying tissue from injury, most of the microflora found on the human skin are harmless and some are beneficial. The normal flora also serves as a defence against invading microorganisms, and therefore should not be adversely eliminated. However, it should be noted that every organism is a potential pathogen, because microorganisms may cause infections particularly if the skin is broken, causing skin diseases and could enter the blood system creating life-threatening diseases particularly in immunosuppressed people **(Alam et al., 1989; Cogen et al. 2008; Calleweat et al., 2013)**. Some of the normal microfloras of the skin include *Staphylococcus aureus, Candida albicans, Malassezia furfur* etc. **(Speers et al., 1965)**.

The use of soaps plays a significant role in human's skin hygiene and as a consequence promotes health. Interestingly, soaps commonly called medicinal soaps are known to possess antimicrobial properties which make them useful for the treatment of various skin condition caused by microorganism while the regular soaps are used to wash of dirt.

Soap is a cleaning or emulsifying agent made by reacting animal or vegetable fats or oils with potassium or sodium hydroxide. Soap often contains colouring matter and acts by emulsifying grease and lowering the surface tension of water so that it readily penetrates to remove dirt. Medicated soaps contain additional ingredients, usually for the treatment of skin disorders. Bathing with soap and water has been considered a measure of personal hygiene **(Eckburg et al., 2005)**.

Some common medicated soaps with their active ingredients are: Tura (Triclosan, Allantoin, Vitamin E, Tollowate, Sodium palmkernelote, Aqua, Parfum, C112940 (pigment Red), C177266 (carbon Black), C174160 (pigment Blue 15); Tetmosol (Soap base, Monosulfiran, B.P 5% and Citronella oil), Septol (Soap base, colour (yellow) Fragrance, water, Iragasan Dp300 (0.3%), EDTA (0.15%), Vitamin E (0.20%); Dettol (Soap base, Fragrance, Colour, Antibacterial deodorant, Chloroxylerol (0.3%); Delta (Sodium palmitate, Sodium palmkernelate, Stabiliers, Tricholorocarbanilide, Parfum, Colourant C.1.77891 **(Eckburg et al., 2005)**.

Herbal soaps are soaps made with plants and natural ingredient that have medicinal value used to promote healthy skin **(Soap History, 2014)**. Example of herbal soap include Dudu Osun (Pure honey Shea butter, Palm kernel oil Cocoa Pod ash, Lime juice, Aloe vera, Palm bunch ash, sulphur, Water and Fragrance.

Regular soap is designed to decrease water's surface tension and to lift dirt and oils off the surfaces so that it can be easily rinsed away. Though regular soap does not contain added antibacterial chemicals, it is effective in getting rid of bacteria and other germs **(Lalan, 2010)**. An example of a regular or toilet soaps include Lux (Sodium Tallowate, Sodium Palmate, Aqua, Sodium Palm Kernate,Glycerin, Parfum, Sodium Sulphate,Titanium Dioxide, Phosphoric acid,Tetrasodium EDTA, Disulfonate, Hexyl Cinnamal, Benzyl Salicylate, Buttylphenyl, Methylpropional, Citronellol, Gerniol, Coumarin, Limoene, Cl 74160).

The physicochemical properties of soaps determine their quality and cleansing efficacy. Such physiochemical characteristics include pH, total fatty matter, free caustic alkali, moisture content, and free fatty acid among others. The qualities of alkali and oil used as well achieving complete saponification also have significant contribution to soap quality **(Viorica et al., 2011; Vivian et al., 2014)**.

This study was aimed at investigating the physicochemical and antimicrobial qualities of ABUAD Moringa Soap® (ABUAD farm, Teaching and Research Farm of Afe Babalola University, Ado -Ekiti, Nigeria) and comparing these qualities with those of conventional medicated soaps that are in high demand in Nigerian market, namely, Dettol, Delta, Tura, Septol, and Tetmosol soaps.

MATERIAL AND METHODOLOGY

Collection of samples

ABUAD moringa soap was obtained from ABUAD soap factory, Afe Babalola University, Ado Ekiti, Nigeria. ABUAD moringa soap (Plate 1) is a herbal soap (soap base, palm kernel oil, coconut oil and leaf extract of *Moringa oleifera* Lam as major constituents) is produced and marketed by ABUAD farm, the Teaching and Research Farm of Afe Babalola University, Ado Ekiti, Nigeria. DuduOsun (herbal soap), five medicated soaps namely, Delta, Tura, Tetmosol, Septol and Dettol, as well as Lux (a regular soap) were purchased from Oba market in Ado Ekiti. All the soaps were anaylsed in the Science laboratories of Afe Babalola University for their physicochemical and antimicrobial qualities.

Physiochemical analysis

The physicochemical analyses of the soaps, namely determinations of pH, total fatty matter, free caustic alkali and free fatty acid, were carried out as described by **AOAC (2010)**

Determination of pH

A 10% aqueous solution of each soap was prepared and used for pH determination using Hanna Instrument pH Meter (HI 2210), that was previously calibrated with buffer solutions 7 and 10.

Determination of Total Fatty Matter test

A quantity of 5 grams of soap was weighed into 250 mL conical flask and 100 mL of distilled water was added, warmed completely until the soap dissolved. A few drops of methyl orange were added, then dilute H_2SO_4 was added until the solution turn acidic and 5 mL of H_2SO_4 was added in excess. With a small funnel placed unto the flask, the soap solution was heated to a temperature not exceeding 60 ^oC until the fatty acids separated as a clear layer. A volume of 50 mL saturated sodium chloride was added to the solution and was allowed to cool before quantitatively transferring into a separating funnel and the aqueous layer carefully separated. The separated aqueous layer was washed with three portions of 50 mL of ethyl ether. The ether extract was combined with the fatty acid in the first separating funnel. The fatty acid/ether solution was washed with 3 × 10 mL of water portion. The water portion used for washing was shaken with 20 mL ether and the ether portion was added to fatty acid/ether solution. The ether portions was collected into a weighed beaker and evaporated on a steam bath. After evaporation of the ether, the beaker was placed in the oven at a temperature of 90 ^oC for 10 minutes. It was then removed from oven and cooled in the desiccators and weighed. The fatty matter residue was weighed and calculated.

Determination of free caustic alkali

A quantity of 5 grams of soap sample was weighed in a 250 mL conical flask and 25 mL of distilled water plus a few drops of phenolphthalein solution were added such that the solution turn pink, indicating an alkaline solution. The solution was titrated against 0.1 M of HCl until a pink coloration was discharged. The burette reading was used to calculate the free caustic alkali.

Determination of free fatty acid

A quantity of 5g of soap sample was weighed into a conical flask and set aside. 25 mL of each of diethyl ether and ethanol was measured into a conical flask and neutralized with NaOH solution using Phenolphthalein indicator and warmed on a water bath. Then the mixture was added into conical flask containing weighed soap sample and was titrated against 0.1 M NaOH until colour change. The titre value was used to calculate the free fatty acid.

Collection of test organisms

All microorganisms, six bacteria namely, Proteus mirabilis (ATCC 12453), Luteococcus sanguinis,

Burkholderia cepacia, Escherichia coli, Corynebacterium accolens and Staphylococous aureus ATCC 25923, as well as three fungi namely, Malassezia furfur ATCC 44349, Candida albicans ATCC 10231, were obtained from the stock culture of the Department of Biological Sciences, Afe Babalola University, Ado-Ekiti.

Preparation of standard Inoculum

Bacteria isolates were suspended in nutrient broth in comparison to 0.5 McFarland standards and Fungi isolates were suspended into potato dextrose broth in comparison to 1.0 McFarland standards.

Sterilization Methods

Materials such as conical flask, universal bottles and beaker were sterilized by using the oven at 160 ^{o}C for 1 hour. Distilled water and Media such as Mueller Hinton agar, Nutrient broth and potato Dextrose broth were sterilized in autoclave (model type; ST 19T) at 121 ^{o}C for 15 minutes.

Preparation of Soap Concentration

A stock solution of 500 mg.mL^{-1} of the soap was prepared by adding 5 g of the soap into 10 mL of sterile water in a universal bottle and warmed to dissolve the soap into solution in a water bath at 60 ^{o}C. Using the stock solution, various concentrations of the soap; 250, 125, 100 and 50 mg.mL^{-1} were obtained.

Antimicrobial Susceptibility Test.

The antimicrobial susceptibility investigations for the soaps were done using agar well diffusion method as described by **CLSI (2012, 2013)**. Each organism was inoculated onto the surface of already prepared sterile Muller Hinton agar, a sterile swab stick was used to carpet spread the inoculums on the agar. A sterile cork-borer was used to bore 5 wells into the agar gel, while the different soap concentrations were dispensed into the five wells and were allowed to diffuse for 1 hour. Incubation was carried out at 37 ^{o}C for 24 hour for bacterial and 3 to 5 days at room temperature for fungi.

GC-MS analysis of oil of Moringa oleifera leaf

Hexane extract of dried leaves of *Moringa oleifera* was analyzed for the chemical constituents of the essential oil by Gas Chromatography Mass Spectrometry (GC-MS) technique as described by **Sengupta et al. (2015)**.

Statistical Analysis

Statistical analyses were determined with Analysis of Variance (ANOVA) using SPSS window 20.0.

RESULTS AND DISCUSSION

Physicochemical analysis of soaps

The results obtained from analyses of different physicochemical parameters of the soaps tested are presented in Table 1. All the soaps fall within the pH range of 8.83 and 9.83. All the soaps possess low values of free caustic alkali and detectable free fatty acid, as well as moderate values of total fatty matter.

The pH values of all soaps analysed in this study agreed with the values obtained by **Habib et al. (2016)** for toilet soaps sold in Bangladesh; but were far higher than the pH 5.4 – 5.9 of normal human skin **(Tarun et al., 2014)**. The use of soaps with high pH values will cause the skin pH to increase, resulting in dehydration, and alteration of bacteria flora, and ultimately pathogenesis of microbial skin diseases **(Tarun et al., 2014)**. Most commercial soaps

Figure 1 Plate 1: ABUAD Moringa soap (with *Moringa oleifera* leaf shown on the package).

Table 1 Physicochemical analysis of soaps.

Soap	pH	Total Fatty Matter (%)	Free Caustic Alkaline (%)	Free Fatty Acid (%)
Moringa	9.32 ±0.02	70.3 ±2.02	0.65 ±0.12	0.60 ±0.01
DuduOsun	8.83 ±0.05	66.6 ±3.72	0.96 ±0.42	0.98 ±0.22
Tetmosol	9.44 ±0.55	70.7 ±2.41	0.35 ±0.06	0.20 ±0.02
Septol	9.56 ±0.13	68.0 ±1.02	0.25 ±0.02	2.80 ±0.25
Tura	9.61 ±0.20	77.1 ±1.02	0.37 ±0.23	1.19 ±0.11
Dettol	9.71 ±0.40	78.6 ±0.92	0.04 ±0.01	1.01 ±0.32
Delta	9.83 ±0.30	72.3 ±1.12	0.25 ±0.03	0.51 ±0.01
Lux	9.68 ±0.19	73.7 ±0.09	0.06 ±0.02	0.85 ±0.02

Table 2 Activities of soaps on bacteria showing zones of inhibition.

Soaps	Concentrations (mg.mL^{-1})	*Ecsherichia coli*	*Burkholderia cepacia*	*Corynebacterium accolens*	*Luteococcus Sanguinis*	*Staphylococus aureus* ATCC 25923	*Proteus mirabilus* (ACCT 12453)
Moringa	500	22	13	27	17	25	14
	250	20	6	20	10	23	13
	125	18^{a}	4^{adhj}	17^{a}	9^{ac}	17^{a}	11^{ad}
	100	6	0	10	4	9	9
	50	3	0	0	0	3	0
DuduOsun	500	28	13	18	18	14	12
	250	23	11	13	13	9	10
	125	18^{b}	8^{bi}	12^{b}	11^{b}	7^{b}	9^{bce}
	100	8	0	9	5	4	4
	50	0	0	6	0	0	0
Tetmosol	500	12	0	14	9	27	0
	250	9	0	9	5	23	0
	125	4^{c}	0^{dhjkl}	5^{c}	0^{bcdg}	18^{c}	0^{ab}
	100	2	0	0	0	15	0
	50	0	0	0	0	9	0
Septol	500	19	12	16	14	25	12
	250	15	10	12	10	17	6
	125	12^{d}	9^{d}	9^{d}	8^{d}	9^{d}	0^{ac}
	100	10	6	5	5	3	0
	50	6	0	0	0	0	0
Tura	500	12	14	9	17	19	14
	250	8	10	4	14	17	9
	125	6^{eh}	9^{ck}	0^{efg}	13^{e}	15^{e}	0^{bde}
	100	4	6	0	10	14	0
	50	0	0	0	0	9	0
Delta	500	16	13	14	10	10	13
	250	13	10	10	8	8	0
	125	11^{fh}	9^{ej}	8^{f}	5^{f}	5^{f}	0^{ab}
	100	8	6	5	3	3	0
	50	0	0	0	0	0	0
Dettol	500	15	0	12	14	14	0
	250	13	0	10	12	12	0
	125	10^{gh}	0^{hik}	8^{g}	9^{g}	9g	0^{ab}
	100	8	0	2	3	2	0
	50	0	0	0	0	0	0
Lux	500	0	0	0	0	0	0
	250	0	0	0	0	0	0
	125	0^{h}	0^{hik}	0^{a-h}	0^{a-g}	0^{g}	0^{ab}
	100	0	0	0	0	0	0
	50	0	0	0	0	0	0
F-values		2.971	3.799	3.262	2.214	5.407	3.682
P-values		0.016	0.004	0.010	0.059	0.002	0.013

Note: Values are in mm.

$^{a-k}$Values of zones of inhibition (mm) compared between soaps along column with no common superscript are significantly different (p <0.05).

do have high pH values. **Tarun et al. (2014)**, working on 66 different soaps (including bathing soaps) in India reported that only 5 of the soaps had pH <9.0; 53 soaps had pH 9.01 – 10.0; while the remaining 6 had pH value of 10.63 – 11.01. **Onyangu et al. (2014)** equally reported high pH values of 10.03 – 11.71 in soaps sold in Kenya. Since alteration in skin pH plays significant roles in pathogenesis of skin diseases, the use of cleansing agents with low pH values of about 5.5 has been advocated for prevention and treatment of skin diseases **(Tarun et al., 2014)**.

The free caustic alkali normally results from improper or incomplete saponification. The recommended value is 0.25% for laundry soap and 0.2% for toilet soap **(Snell, Ettre and Hilton, 2007)**. The free caustic alkali contents of tetmosol, septol, tura, dettol, delta and lux soaps are relatively low, while those of ABUAD Moringa and DuduOsun soaps were of higher values. Although the values obtained for the free caustic alkali contents of

Table 3 Activities of soaps on fungi showing zones of inhibition.

Soaps	Concentrations ($mg.mL^{-1}$)	*Malassezia furfur* ATCC 44349	*Candida albicans* ATCC 10231	*Cryptococcus neoformans* ATCC 23645
Moringa	500	14	16	14
	250	12	15	10
	125	11^{a}	14^{a}	9^{a}
	100	9	9	0
	50	0	0	0
Dudu Osun	500	13	12	14
	250	10	10	9
	125	7^{b}	6^{b}	8^{a}
	100	6	3	5
	50	0	0	0
Tetmosol	500	0	0	0
	250	0	0	0
	125	0^{c}	0 c	0^{b}
	100	0	0	0
	50	0	0	0
Septol	500	0	0	0
	250	0	0	0
	125	0^{c}	0^{c}	0^{b}
	100	0	0	0
	50	0	0	0
Tura	500	0	0	0
	250	0	0	0
	125	0^{c}	0 c	0^{b}
	100	0	0	0
	50	0	0	0
Delta	500	0	0	0
	250	0	0	0
	125	0^{c}	0^{c}	0^{b}
	100	0	0	0
	50	0	0	0
Dettol	500	0	0	0
	250	0	0	0
	125	0^{c}	0^{c}	0^{b}
	100	0	0	0
	50	0	0	0
Lux	500	0	0	0
	250	0	0	0
	125	0^{c}	0^{c}	0^{b}
	100	0	0	0
	50	0	0	0
F-values		0.675	0.692	0.898
P-values		0.614	0.602	0.426

Note: Values are in mm; $^{a\text{-}c}$Values of zones of inhibition (mm) compared between soaps along column with different superscripts are statistically different (p <0.05).

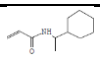
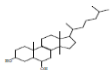
ABUAD Moringa and DuduOsun soaps agreed with the values reported by **Taiwo et al (2010)**, but were far lower that values reported by **Beetseh et al. (2013)**.

The total fatty matter of soap is a measure of its suitability for bathing and washing of materials **(Ogunsuyi and Akinnawo, 2012)**. The total fatty matter for all the reported in this compared favourably with the work of **Vivian et al. (2014)**, as well as the values of 71 – 84 obtained by **Viorica et al. (2011)**; but lower than values of 74 – 92 obtained by **Kuntom (1999)**.

In-vitro antimicrobial activities of soaps

The results of anti-bacterial activities of the soaps are presented in Table 2. All the bacteria tested were susceptible to ABUAD moringa soap, DuduOsun, Septol, Tura and Delta, while varied susceptibilities were obtained for Tetmosol and Dettol. Lux which served as negative control did not produce any antibacterial activity. ABUAD moringa and DuduOsun soaps indicated superior effectiveness against the bacteria tested compared to other soaps. However there was no significant difference

Table 4 Compounds identified in moringa leaf oil extract.

Peak no	Retention Time	Peak Area (%)	Name of compound	Molecular weight	Formula	Structure
1	15.292	1.73	2-Propenamide, N-(1-cyclohexylethyl)-	181	C11H19NO	
2	15.409	39.18	Hexadecanoic acid, methyl ester	270	C17H34O2	
3	16.793	47.13	9-Octadecenoic acid (Z)-, methyl ester	296	C19H36O2	
4	16.985	10.26	Heptadecanoic acid, 10-methyl-, methyl ester	298	C19H38O2	
5	18.793	1.70	Cholest-8-ene-3,6-diol, 14-methyl-, (3.beta.,5.alpha)	416	C28H48O2	

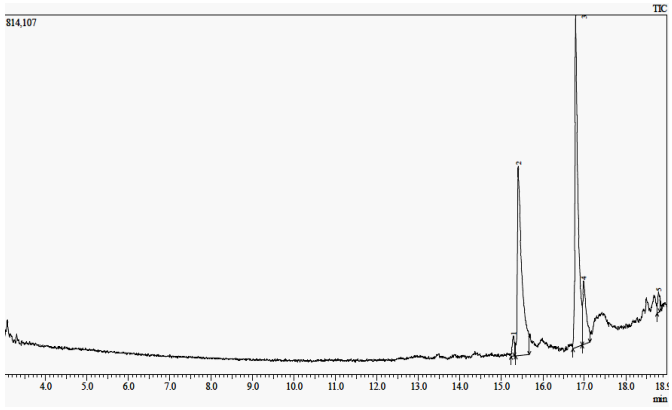


Figure 2 Spectra of GCMS Analysis of the Moringa leaf oil extract.

observed in antibacterial activities between ABUAD moringa and DuduOsun soaps.

The results of the antifungal activities of the soaps are presented in Table 3. Only ABUAD moringa and DuduOsun soaps were effective against all the fungi tested. ABUAD moringa soap indicated higher significant activities on *Malassezia furfur* ATCC 44349 and *Candida albicans* ATCC 10231 than DuduOsun. However there was no significant difference between the two soaps on their activities against *Cryptococcus neoformans* ATCC 23645.

The work of **Ekanola et al. (2012)** has previously established similar experimental evidence comparable to this work. **Ekanola et al. (2012)** investigated the role of soaps in human hygiene. In the work, the determination of the *in vitro* inhibitory activities of the various industrial soaps (Delta, Dettol, Lux, Septol, Tetmosol, and Tura), were carried out. It was discovered that none of the soaps showed inhibitory activity against *Candida*. Other researchers have also investigated the *in-vitro* inhibitory activities of soaps. According to **Ihuma et al. (2013)**, Delta soap retained more normal flora than Tetmosol or Tura. Therefore based on this study, ABUAD moringa and Dudu Osun soaps were observed to have better justification in terms of their physicochemical properties and antimicrobial quality for preventive and therapeutic soap measures for maintaining good hygiene. Other soaps in this study have also indicated some effectiveness against bacterial isolates but not sufficiently against antifungal isolates.

The antimicrobial efficacy of ABUAD moringa soap could be attributed to the content of *Moringa oleifera* leaf extract, as previous studies on *Moringa oleifera* leaf cultivated in ABUAD farm, used for the soap production, have been found to possess vital phytochemicals and high antibacterial activities **(Okiki et al., 2015a,b)**. In addition, coconut oil, which is known to possess antimicrobial and anti-inflammatory properties **(Fitzpatrick, 2012)**, used in the ABUAD Moringa soap production, could contribute to the soap's antimicrobial qualities.

GCMS Analysis of Moringa Oleifera leaf

Figure 1 shows the spectra of GCMS analysis of the Moringa leaf oil extract and the identified compounds in the oil are presented in Table 4. The presence of hexadecanoic, 9-octadecenoic, heptadecanoic and cholest-8-ene-3,6-diol may be useful as anti-inflammatory, immunostimulant, antitumour, antioxidant and flavour enhancers in cosmetic production **(Omotoso et al., 2014)**. This may serve as a plus to Moringa oleifera leaf saop.

CONCLUSION

ABUAD moringa soap was reported in this study to have potent antibacterial and antifungal activities. Antimicrobial soaps with high antimicrobial potency should be used only for therapeutic purposes and not for prophylaxis because its continuous use may eliminate both beneficial and harmful microflora of the skin.

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