Parkia biglobosa (Jacq.) Benth. Leaf Extract as an Antimicrobial Agent in Antiseptic Handwash Liquid Soap

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ABSTRACT

Synthetic antimicrobial agents, *e.g.,* methylchloroisothiazolinone and methylisothiazolinone, used in antiseptic handwash liquid soaps are now associated with both environmental and health concerns; therefore, it is pertinent to search for alternatives. Plant extracts are promising in this regard. Using saponification, a handwash liquid soap was formulated with *Parkia biglobosa* (Jacq.) leaf extract as an antimicrobial agent. The soap inhibited the growth of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* to the same degree ($p > 0.05$) as the extract alone. The results also compared favorably ($p > 0.05$) with those of Dettol handwash liquid soap which contains both methylchloroisothiazolinone and methylisothiazolinone as antimicrobial agents. From the foregoing, *P. biglobosa* (Jacq.) Benth. leaf extract can be used as an antimicrobial agent in antiseptic handwash liquid soaps rather than the noxious synthetic methylchloroisothiazolinone and methylisothiazolinone.

Keywords: Antimicrobial agent, Phytochemical, Antiseptic soap, Plant extract, Foam

INTRODUCTION

Regular washing of hands with antiseptic soap is important in preventing the spread of germs and curbing the spread of infectious diseases, which are transmitted by cross-contamination with hands, food and surfaces (Gibson et al., 2002). An antiseptic soap normally contains synthetic antimicrobial agents or chemical molecules that either kill the germs (biocidal) or impede their growth or reproduction (biostatic) (Holah et al., 1998). While this is beneficial, there are emerging evidence that these chemicals have adverse effects on the environment. For example, research has shown that triclosan and triclocarban, which are common antimicrobial agents in many commercial handwash liquid soaps are toxic, allergenic and

carcinogenic (Kwon and Xia, 2012). In addition, they are not easily degradable and thus persist in the environment (Higgins *et al*., 2011), accumulating in aquatic lives like fish and toad, with a disruptive effect on their endocrine system. Antibiotic resistance has also been traced to triclosan and triclocarban (Schweizer, 2001). As a result, the US Food and Drug Administration has banned products that contain triclosan and triclocarban (FDA-1975-N-0012), but there are still other synthetic antimicrobial agents in use (e.g., Methyl chloroisothiazolinone and methyl isothiazolinone) with serious health and environmental concerns. Methyl chloroisothiazolinone alone, or in combination with methylisothiazolinone, is often used as an antimicrobial agent (or preservative) instead of triclosan and triclocarban. However, like triclosan and triclocarban, methyl chloroisothiazolinone and methylisothiazolinone are cytotoxic and allergenic, causing allergic contact dermatitis (Lorente-Lavirgen et al., 2019). They can also disrupt enzymatic activity, damage DNA and the central nervous system (Du et al., 2002). Furthermore, residues of methyl chloroisothiazolinone and methylisothiazolinone have been reported in estuaries and coastal environment, meaning they are not easily degradable (Voulvoulis et al., 2002). Because of the profound adverse effects of these chemicals on humans (Levy, 2002), aquatic lives (Capkin et al., 2017) and the environment, it is imperative to search for effective antimicrobial agents that are not harmful and are environmentally benign.

Meanwhile, phytochemicals (i.e., plant-derived chemicals) have been reported to demonstrate significant antimicrobial activities against a wide range of microorganisms (Simões et al., 2009). Consequently, phytochemicals may be explored as potential antimicrobial agents in handwash liquid soaps and related products. Plants use these chemicals as a defence mechanism against pathogens and pests (Manickam *et al*., 2021). Unlike their synthetic counterpart, phytochemicals are less harmful, reasonably biodegradable and environmentally benign (Manickam *et al*., 2021). Even though this is well-known, their use as antimicrobial agents in antiseptic soaps and related products is limited and only beginning to gain pace, with studies concentrated on mushroom extract in cream formulation (Taofiq et al., 2016). Although these studies are revolutionizing the cosmetic and related industries, mushroom-based phytochemicals have some drawbacks. For instance, some of them are prone to rapid oxidation and degradation once exposed to air and light; therefore, require microencapsulation, before incorporating into a formulation (Taofiq *et al*., 2018). Nonetheless, microencapsulation is time-consuming and expensive. Also, commercialization of these formulations will be hampered by the availability of mushroom species with non-toxic and potent antimicrobial chemicals. Therefore, there is need to explore extracts of other plant species especially those whose phytochemicals are stable to air and light. Herein, the aim is to explore the possibility of using the leaf extract of the African locust bean, *Parkia biglobosa* (Jacq.) Benth., as an antimicrobial agent in handwash liquid soaps. *P. biglobosa* (Jacq.) Benth. is a Mimosaceae plant found in the tropical and subtropical regions of Africa like Nigeria (Tringali *et al*., 2000). The plant is not buttressed, 7 to 20 m tall, low-branching and has a large wide-spreading crown (Banwo et al., 2020). Previous studies indicate that the aqueous and alcoholic extracts of *P. biglobosa* (Jacq.) Benth. stem, stem-bark and root are antimicrobial (Abioye *et al*., 2013; Quansah *et al*., 2019), but harvesting these parts can adversely affect the plant's life. By contrast, harvesting the leaves will have little effect on the plant's life because they can easily regenerate.

MATERIALS AND METHODS

Materials

Plant sample, vegetable oils, water and other

chemicals

P. biglobosa (Jacq.) Benth. leaves were obtained (November 2018) from the Benue State University botanical garden and authenticated by a botanist (Joshua Waya). Three vegetable oil samples (100% pure), namely: laser pomace olive oil (Sun Mark Ltd., UK), coconut oil (KTC Ltd., UK) and castor oil (KTC Ltd., UK) were used as received. Water was ultrapure with resistivity 18 MΩ cm. KOH (85% pure, BDH Chemicals) was used as lye. Potato dextrose agar and nutrient agar (98% pure, T.M. Media) were, respectively, used for antifungal and antibacterial analyses of the handwash liquid soap. This was compared with Dettol antibacterial handwash liquid soap which contains two synthetic antibacterial agents (methylchloroisothiazolinone and methylisothiazolinone) and is commercially available. Other chemicals used in this work are listed in Table 1. These chemicals were used for phytochemical screening of *P. biglobosa* (Jacq.) Benth. leaves or phytochemical estimation.

Microorganisms

American Type Culture Collection (ATCC) bacteria, obtained from the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria, were used for antibacterial analysis. These bacteria were *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 29953. Antifungal analysis was with *Candida albicans,* isolated from the Benue State University Teaching Hospital,

Table 1. Source and purity of chemicals used for qualitative or quantitative Phytochemical analyses of *P. biglobosa* (Jacq.) Benth. Leaves.

Chemical	Source	Purity/%
NaOH	Loba Chemie Pvt Ltd	98
HCl	Loba Chemie Pvt Ltd	35
H_2O_2	G.G. Chemical & Pharmaceuticals Pvt Ltd	30
H_2SO_4	May and Baker Ltd	98
NH_4OH	Tianjin Kermel Chemical Reagent Co. Ltd	28
KI	Biomedicals LLC	99
$FeCl_3 \cdot 6H_2O$	Loba Chemie Pvt Ltd	97
Glacial acetic acid	May and Baker Ltd	99.5
Methanol	Fisher Scientific	98
Ethanol	JHD Chemical Reagent Co. Ltd	98
Tannic acid	Loba Chemie Pvt Ltd	98
Fehling's solutions A and B	Prepared in our laboratory	
Acetic anhydride	Merck & Co	99.5
n-Butanol	May and Baker Ltd	90
Diethyl ether	Merck & Co	98
Chloroform	Loba Chemie Pvt Ltd	99

Makurdi, Nigeria. The bacteria and fungus were maintained on nutrient agar and potato dextrose slants (4 °C), respectively.

Methods

Phytochemical screening of P. biglobosa (Jacq.) Benth. leaves

The leaves were washed with hydrogen peroxide to rid of microorganisms and dried (18 days) in air at ambient conditions in the laboratory. Once dried, the leaves were pounded into powder using a local mortar. The powdered leaves were then screened for the presence of tannins, phlobatannins, saponins, steroids, terpenoids, flavonoids, alkaloids, anthraquinones, carbohydrates, reducing sugars and cardiac glycosides according to standard procedures (Harborne, 1998).

Test for tannins (ferric chloride test)

The powdered leaf sample (0.3 g) was weighed into a test tube followed by addition of water (30 cm^3) and then boiled over a water bath (Clifton unstirred bath, Nickel-Electro Ltd., England) maintained at 100 °C for 10 min. The mixture was allowed to cool to 30 ± 1 °C and then filtered using a filter paper (125 mm-Whatman No.1, Thomas Scientific, US). The water bath and filter paper were similarly used throughout this work. The filtrate (5 cm^3) was measured into a test tube followed by addition of three drops of aqueous $FeCl_3$ solution (0.1 %) and observed for the formation of a blue-black (drops) to brownish green (excess) coloration which indicates the presence of tannins.

Test for phlobatannins

The powdered leaf sample (0.3 g) was weighed into a test tube. Water (30 cm^3) was added and filtered (24 h) using a filter paper. Aqueous HCl solution (1%, 5 cm^3) was added to the filtrate (10 cm^3) in a test tube. The mixture was boiled (10 min) over a water bath maintained at 100 °C and observed for the formation of a reddish precipitate which indicates the presence of phlobatannins.

Test for saponins (frothing test)

Water (30 cm^3) was added to the powdered leaf sample (0.3 g) and boiled (10 min) over a water bath maintained at 100 °C. The mixture was filtered using a filter paper. Water (5 cm^3) was added to a portion (10 cm^3) of the filtrate in a test tube, then corked, shaken vigorously and observed for the formation of a stable and persistent foam which indicates the presence of saponins. Three drops of olive oil were added to the mixture, shaken vigorously and observed for emulsion formation which confirms the presence of saponins.

Test for steroids and terpenoids (Salkowski's test)

Ethanol (20 cm^3) was added to the powdered leaf sample (0.3 g) in a beaker and filtered after 2 h using a filter paper. Acetic anhydride (2 cm^3) and concentrated H_2SO_4 (2 cm^3) were added to a portion (5 cm^3) of the filtrate in a test tube and then observed for a violet coloration which indicates the existence of steroids or a reddish-brown coloration at the interface which indicates the presence of terpenoids.

Test for flavonoids (alkaline test)

The powdered leaf sample (0.3 g) was weighed into a beaker, water (30 cm^3) was added and the mixture was filtered after 2 h using a filter paper. Aqueous ammonia solution (1 M, 5 cm^3) and concentrated H_2SO_4 were added to a portion (10 cm^3) of the filtrate in a test tube. The mixture was observed for the formation of a yellowish coloration which marks the presence of flavonoids.

Test for glycosides, cardiac glycosides (Keller Killiani's test) and reducing sugars (Fehling's test)

The powdered leaf sample (2 g) was mixed with water (20 cm^3) in a 150 cm^3-conical flask, boiled on a hot plate (IKA® RCT 5, Imlab, France) for 5

min and then filtered using a filter paper. Chloroform (3 cm^3) was added to a portion (2 cm^3) of the filtrate in a test tube, stoppered and shaken vigorously. The chloroform layer (2 cm^3) was decanted into a test tube followed by addition of aqueous ammonia solution (10%). The mixture was subsequently observed for the formation of a pink coloration which marks the presence of glycosides. For cardiac glycosides, glacial acetic acid (1 cm^3) was added to a portion of the filtrate (2 cm^3) in a test tube followed by three drops of $FeCl_3$ (0.1%) and concentrated H_2SO_4. Thereafter, the mixture was observed for a green-blue coloration which marks the presence of cardiac glycosides. In the case of reducing sugars, a mixture of Fehling's solutions A and B (4 cm^3) was added to a portion (4 cm^3) of the filtrate and heated (10 min) on a water bath maintained at 100 °C. Afterwards, the mixture was observed for the formation of an orange to brick-red precipitate which marks the presence of reducing sugars.

Test for alkaloids (Wagner's test)

Two grams of the powdered leaf sample was placed into a 250 cm^3-conical flask, 20 cm^3 of H_2SO_4 solution (5%), prepared in an ethanol-water (1:1) mixture, was added and boiled (2 min) using a Bunsen flame. Thereafter, the mixture was filtered using a filter paper. The filtrate was placed into a separating funnel followed by addition of 5 cm^3 of aqueous ammonia solution (28%) and chloroform (5 cm^3). After shaking the mixture vigorously, the chloroform layer was decanted into another separating funnel followed by addition (5 cm^3) of 1 M H_2SO_4. The mixture was shaken vigorously and allowed to stand (30 min) where the acid and chloroform layers separated out. The acid layer was removed and replaced with a fresh H_2SO_4 solution (5 cm^3). Again, the mixture was shaken vigorously and allowed to stand for 30 min so that the acid and chloroform layers separate out. A portion of the acid layer was removed into a test tube followed by adding Wagner's reagent (0.5 cm^3) and observing whether or not an orange precipitate forms in the mixture.

Test for anthraquinones (Borntrager's test)

The powdered leaf sample (2 g) was weighed into a 150 cm^3-conical flask containing methanol (40 cm^3) and boiled (20 min) over a water bath maintained at 100 °C. The mixture was filtered after 24 h using a filter paper and the filtrate was concentrated by heating on a water bath maintained at 100 °C. Chloroform (5 cm^3) was added to a portion (0.5 g) of the concentrate, stoppered, shaken vigorously and filtered. Two-centimetre cube (2 cm^3) of ammonia solution (10%) was added to a portion of the filtrate and observed for the formation of a pink-violet or red coloration which indicates the presence of anthraquinones.

Quantitative estimation of phytochemicals

The quantity of crude saponins, alkaloids, flavonoids and terpenoids in *P. biglobosa* (Jacq.) Benth. leaves were estimated from the dried powdered leaf sample using standard procedures (Harborne, 1998).

Estimation of saponins

The powdered leaf sample (5 g) was dispersed in 100 cm^3 of aqueous ethanol solution (20%) in a beaker and heated (55 °C) over a hot plate for 2 h with continuous stirring. The mixture was filtered and the residues were re-extracted again with 200 cm^3 of aqueous ethanol solution (20%) using the same procedure. The filtrates were combined and concentrated to 40 cm^3 by heating on a water bath maintained at 90 °C and then transferred into a 250 cm^3-separatory funnel. Diethyl ether (20 cm^3) was added followed by vigorously shaking. After allowing the mixture to stand for 30 min, the diethyl ether phase that separated out was removed. *n*-Butanol (60 cm^3) was added to the aqueous phase left in the funnel, shaken vigorously and allowed to stand for 30 min. The

aqueous phase that separated out from the *n*-butanol phase was similarly removed. Aqueous (10 cm^3) NaCl solution (5%) was added to the *n*-butanol phase left in the funnel, shaken vigorously and allowed to stand (30 min) where it separated out from the *n*-butanol phase. The salt solution phase was removed from the *n*-butanol phase and discarded while the *n*-butanol phase was concentrated by heating over a water bath maintained at 90 °C for 30 min and dried to a constant mass in an oven to obtain crude saponins. The quantity of crude saponins in the sample was expressed as (mg/g):

Estimation of alkaloids

The powdered leaf sample (2.5 g) was mixed with 200 cm^3 of acetic acid solution (10%), prepared in ethanol, in a 250 cm^3-conical flask. The mixture was filtered after 4 h and the filtrate was concentrated by heating over a water bath maintained at 90 °C. A crude alkaloids sample was precipitated by adding 15 drops of concentrated NH_4OH to the concentrated filtrate and waiting for 3 h. The crude sample was filtered, washed with 20 cm^3 of NH_4OH solution (0.1 M), dried to a constant mass in an oven (MINO/30, Genlab, UK) and expressed as (mg/g):

$$\text{Crude saponins} = \frac{\text{mass of crude saponins (mg)}}{\text{Mass of sample used (g)}} \quad (1)$$

Estimation of flavonoids

The powdered leaf sample (2.5 g) was weighed into a 250 cm^3-beaker, 50 cm^3 of aqueous methanol solution (80%) was added and thoroughly mixed. After 24 h, the supernatant was removed and replaced with ethanol (50 cm^3). The mixture was filtered after 24 h and the residues were re-extracted with ethanol (50 cm^3). The combined filtrate was evaporated to dryness using a water bath maintained at 90 °C to obtain crude flavonoids. The crude sample was cooled in a desiccator and the quantity was expressed as (mg/g):

$$\text{Crude alkaloids} = \frac{\text{mass of crude alkaloids (mg)}}{\text{mass of sample used (g)}} \quad (2)$$

Estimation of terpenoids

One hundred milligrams of the powdered leaf sample were mixed with ethanol (9 cm^3) and filtered (24 h) into a separating funnel containing petroleum ether (10 cm^3). The mixture was shaken vigorously and left undisturbed (30 min) to separate into both layers. The ether layer was subsequently separated into a pre-weighed crucible where it evaporated in air and then in an oven (40 °C) to a constant mass. The quantity of crude terpenoids was expressed as (mg/g):

$$\text{Crude flavonoids} = \frac{\text{mass of crude flavonoids (mg)}}{\text{mass of sample used (g)}} \quad (3)$$

Crude extraction of *P. biglobosa* (Jacq.) Benth. Leaves

P. Biglobosa (Jacq.) Benth. leaf extract was obtained by "cold" (30±1°C) maceration extraction. The powdered leaf sample (200 g) was thoroughly mixed with ethanol (1000 cm^3). After 72 h, the mixture was filtered and the filtrate was concentrated by heating over a water bath maintained at 40 °C where ethanol evaporated to give the crude extract. The crude extract was subsequently dried in a desiccator to a constant mass and the yield was expressed as (%):

$$\text{Crude terpenoids} = \frac{\text{mass of crude terpenoids (mg)}}{\text{mass of sample used (g)}} \quad (4)$$

Soap preparation

A vegetable oil mixture containing olive oil (80.0 wt.%), coconut oil (14.3 wt.%) and castor oil (5.7 wt.%) was saponified with aqueous KOH solution (22.5 wt.%) in a stainless-steel pot. This oil mixture was used so as to obtain a soap that

exhibits a combined characteristics of the various characteristics of soaps obtained from the individual oils (Spitz, 2016). The vegetable oil mixture was heated on a hot plate to 160 °C, cooled to 95 °C, aqueous KOH solution was added and then heated to 200 °C for 1 h with intermittent stirring using a hand-held electric stick blender (Logik stick

$$\text{Extract yield} = \frac{\text{mass of crude extract (g)}}{\text{mass of sample used (g)}} \times 100\% \quad (5)$$

blender, Masstores Ltd., China). During this process, the mixture transitioned from a liquid to a gel, making stirring with the blender impossible. At this point, a wooden stirrer was used instead of the electric blender until a paste formed. After cooling to room temperature, the soap paste was transferred into plastic bags and stored in a refrigerator (10 to 14 °C). The soap yield was expressed (%) as:

The "actual yield" was the mass of the soap paste obtained while the "theoretical yield" was estimated from the saponification reaction (Figure 1).

The fatty acid composition of olive, coconut and castor oils are summarized in Table 2 (Gunstone, 2002). The most abundant fatty acids in these oils are (Table 2) oleic acid (71%, olive oil), lauric acid (45-52%, coconut oil) and ricinoleic acid (89.5%, castor oil). Because these fatty acids and acylglycerols of these oils are ~100% saponifiable (Gutfinger and Letan, 1974), their corresponding homotriacylglycerols were used to estimate the

$$\text{Soap yield} = \frac{\text{actual yield of soap paste (g)}}{\text{theoretical yield of soap paste (g)}} \times 100\% \quad (6)$$

theoretical yield. Therefore, the oils were considered to contain 71% trioleoylglycerol and 13% tripalmitoylglycerol (olive oil), 45% trilauroylglycerol and 16% trimyristoylglycerol (coconut oil) and 89.5% triricinoleoylglycerol (castor oil) (Salimon *et al.*, 2010). Microsoft Excel spread sheet was used to obtain the theoretical yield, *vis-à-vis* these triacylglycerols,

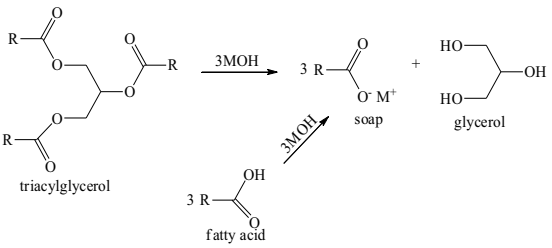


Figure 1. Saponification reaction of a triacylglycerol or fatty acid with three lye molecules to soap, where a triacylglycerol molecule or three fatty acid molecules give(s) a glycerol molecule and three soap molecules. "R" stands for an acyl group while "M" stands for potassium.

using Equation (7):

with X as the theoretical yield when considering an n triacylglycerol ($n = 1, 2, 3, \ldots, N$.)

Subsequently, the soap paste was dissolved in water (30 ± 1 °C) at a paste:water ratio of 2:3 in the presence of different concentrations (0 to 23.5 wt.%) of *P. biglobosa* (Jacq.) Benth. ethanolic leaf extract to obtain the antiseptic handwash liquid soap.

Characterization of soap

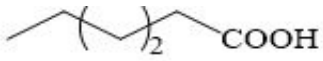
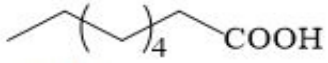
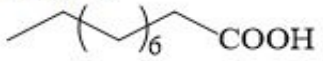
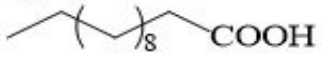
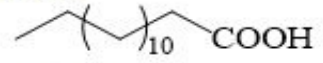
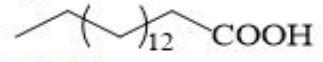
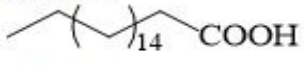
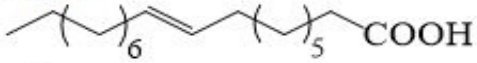
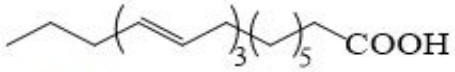
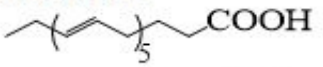
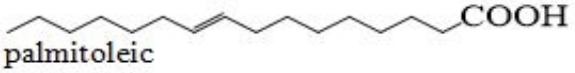
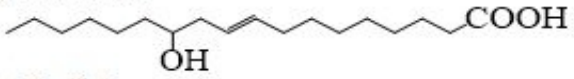
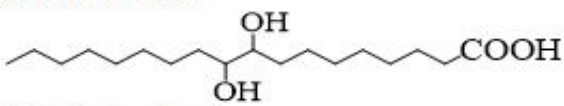
The characterization of soap samples was in terms of pH, foaming, cleaning and antimicrobial abilities. These were compared with those of a Dettol handwash liquid soap containing synthetic Antimicrobial agents (Methylchloroisothiazolinone and Methylisothiazolinone).

$$\text{Soap yield} = \frac{\text{mass of soap paste (g)}}{\sum_{n=1}^{N} X_n(\text{g})} \times 100\% \quad (7)$$

pH

The pH of soap solutions, prepared by dissolving 3 g of soap in water (60 g), was measured using a pH meter (HI9024, Hanna Instruments, UK) after

Table 2. Fatty acid composition (%) of olive, coconut and castor oils (Gunstone, 2002) .

Name and structure of fatty acid	% Composition		
	Olive oil	Coconut oil	Castor oil
 caproic		0.5-1	
 caprylic		5-10	
 capric		4-8	
 lauric		45-52	
 myristic		16-21	
 palmitic	13	7-10	1
 stearic	3	2-4	1
 oleic	71	5-8	3
 linoleic	10	1-3	4.2
 α-linolenic	1	0.2	0.3
 eicosanoic/arachidic	1		0.3
 palmitoleic	1		
 ricinoleic acid			89.5
 dihydroxystearic			0.7

calibration with suitable buffers. Three pH values were measured for each soap sample to obtain the average and standard deviation values.

Foaming

Soap solutions, prepared by dissolving 1 g of soap in water (20 g) in the presence of *P. biglobosa* (Jacq.) Benth. leaf extract (0 to 23.5 wt.%), were aerated at a speed of 13000 rpm (2 min) in 50 cm^3-Pyrex glass measuring cylinders using a high-speed rotor stator (Ultra-Turrax® T25, IKA®-Werke, Germany). Photographs and the volume of foam produced were obtained immediately after foaming. The photographs of foams and others reported here, were obtained with a digital camera (4.3 V Power Shot SX220 HS, Canon, Japan). For each sample, three foam volumes were measured to obtain the average and standard deviation values.

Cleansing ability

A white piece of fabric (4 cm × 4 cm), Figure 2, was placed (30 min) in a mixture of stearic acid, paraffin wax, olive oil, coconut oil (all 21.44 wt.%) and hexane (14.24 wt.%). The fabric was dried (60 °C) to a constant mass (M_1) in an oven, placed (30 min) in a soap solution (4.76 wt.%), dried in air to a constant mass and then in an oven at 60 °C (M_2). The difference between M_1 and M_2 was expressed (%), with respect to M_1, as a measure of the cleansing ability. The experiment was repeated thrice to obtain the average and standard deviation and these values were compared with those obtained using the Dettol handwash liquid soap solution or only water.

Antimicrobial analysis

Stock cultures of the bacteria (*S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 29953) and the fungus (*C. albicans*) were sub-cultured onto petri dishes containing freshly prepared nutrient and potato dextrose agars (20 cm^3), respectively, maintained at 55 °C. After 24 h, suspensions of these microorganisms were prepared in sterile normal saline with the turbidity adjusted to that of a 0.5 McFarland standard which is equivalent to ~1.5×10^8 CFU/mL. Petri dishes containing fresh media were evenly inoculated (streaked) with these suspensions using a sterile cotton swab. Wells were made on the inoculated-agar media using a sterile cork-borer (diameter ~ 9 mm). The ethanolic extract of *P. biglobosa* (Jacq.) Benth. leaves was diluted with water to a concentration of 0 to 1.18 wt.% and filled (50 µL) into the wells. Similarly, soap samples (1 g), containing the plant extract (0 to 23.5 wt.%), were diluted with water (2.5 g) to obtain samples with extract concentrations 0 to 1.18 wt.% and filled (50 µL) into the wells of separate petri dishes. All the plates were incubated (37 °C) for 24 h (bacteria) or 48 h (fungus) and diameters of zones of growth inhibition were measured using an electronic digital calliper (Powerfix® Profi +, OWIM GmbH & Co. KG, Germany).

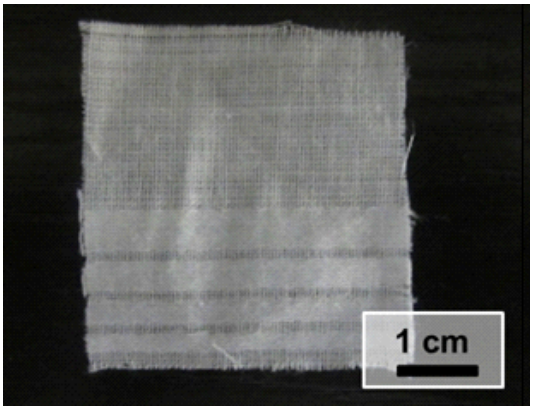


Figure 2. Photograph of a white fabric (4 cm × 4 cm) used for testing the cleansing ability of soap samples.

Statistical analysis

An average and standard deviation of three separate measurements is reported for all the experiments as average ± standard deviation. The differences between values were analyzed using independent two-tailed *t*-test or ANOVA, at 95% confidence level, using IBM SPSS statistics

programme version 20 (IBM Corp., Armonk, NY, USA) for Windows. ANOVA was accompanied by Tukey's-b post-hoc multiple comparison to separate the averages

RESULTS AND DISCUSSION

Phytochemical screening

P. biglobosa (Jacq.) Benth. leaves were screened for the presence of tannins, phlobatannins, saponins, steroids, terpenoids, flavonoids, alkaloids, glycosides, cardiac glycosides, reducing sugars and anthraquinones. Of these phytochemicals, tannins, saponins, steroids, terpenoids, flavonoids and alkaloids were present while phlobatannins, glycosides, cardiac glycosides, reducing sugars and anthraquinones were absent. These phytochemicals have also been found in the leaves of *Acacia nilotica* (L.) Del. (Sadiq *et al.*, 2015) and Astragalus species (Jaradat *et al.*, 2017) which are in the same family (fabaceae) with *P. biglobosa* (Jacq.) Benth. This can be compared to the stem-bark which contains alkaloids, tannins, saponins, flavonoids, steroids, glycoside and sugars (Abioye *et al.*, 2013). Because saponins (Güçlü-Üstündağ and Mazza, 2007), terpenoids (Mathabe *et al.*, 2008), flavonoids (Cushnie and Lamb, 2011) and alkaloids (Cushnie *et al.*, 2014) are responsible for antimicrobial properties of plant extracts, their presence in *P. biglobosa* (Jacq.) Benth. leaf extract means it will have antimicrobial properties.

Estimation of phytochemicals

Because saponins, alkaloids, flavonoids and terpenoids possess antimicrobial properties and they are present in *P. biglobosa* (Jacq.) Benth. leaf extract, as evidenced by the phytochemical screening experiment, we estimated their amount. Based on this experiment, *P. biglobosa* (Jacq.) Benth. leaf extract contains 101 ± 4.24 mg g^{-1} saponins, 106 ± 2.83 mg g^{-1} alkaloids, 110 ± 2.24 mg g^{-1} terpenoids and 541 ± 43.28 mg g^{-1} flavonoids. These values are statistically similar ($p > 0.05$) for saponins, alkaloids and terpenoids, but different ($p < 0.05$) for flavonoids. They are also about three times higher than those reported for the leaf extracts of *Acacia nilotica* (L.) Del. (Sadiq *et al.*, 2015) and Astragalus species (Jaradat *et al.*, 2017) which are in the same family with *P. biglobosa* (Jacq.) Benth.

Soap with crude *P. biglobosa* (Jacq.) Benth. leaf extract and antimicrobial activity

The crude ethanolic extract (60 g) was obtained from 200 g of powdered *P. biglobosa* (Jacq.) Benth. leaves while 870 g of soap paste was obtained from a mixture of olive oil (400 g), coconut oil (71.5 g) and castor oil (28.5 g). The yield in the former and the latter – estimated from Equations (5) and (7) – are 30% and ~98.5 %, respectively. The antimicrobial property of the extract was investigated on three skin pathogenic microorganisms (Byrd *et al.*, 2018), namely: *S. aureus* (Gram-positive bacteria), *P. aeruginosa* (Gram-negative bacteria) and *C. albicans.* Different extract concentrations (0-1.18 wt.%) were used and the average diameter of zones of inhibition are plotted against extract concentration in Figure 3(i).

For each extract concentration, the zones of inhibition of the microorganisms were compared using ANOVA ($p = 0.05$). Results of this analysis show that the zones of inhibition of *P. aeruginosa* are significantly higher ($p < 0.05$) than those of *S. aureus and C. albicans* at extract concentrations of 0.25, 0.73 and 1.18 wt.%. This is in contrast to extract concentrations of 0.45, 0.92 and 1.07 wt.% where there is no significant difference ($p > 0.05$) between the zones of inhibition. Overall, the inhibition zone diameters are consistent with those reported for other plant species (Ertürk, 2006), but higher for certain plant extracts (Herman et al., 2013).

Because the extract exhibited antimicrobial properties, it was incorporated (0-23.5 wt.%) into the prepared handwash liquid soap. The soap was

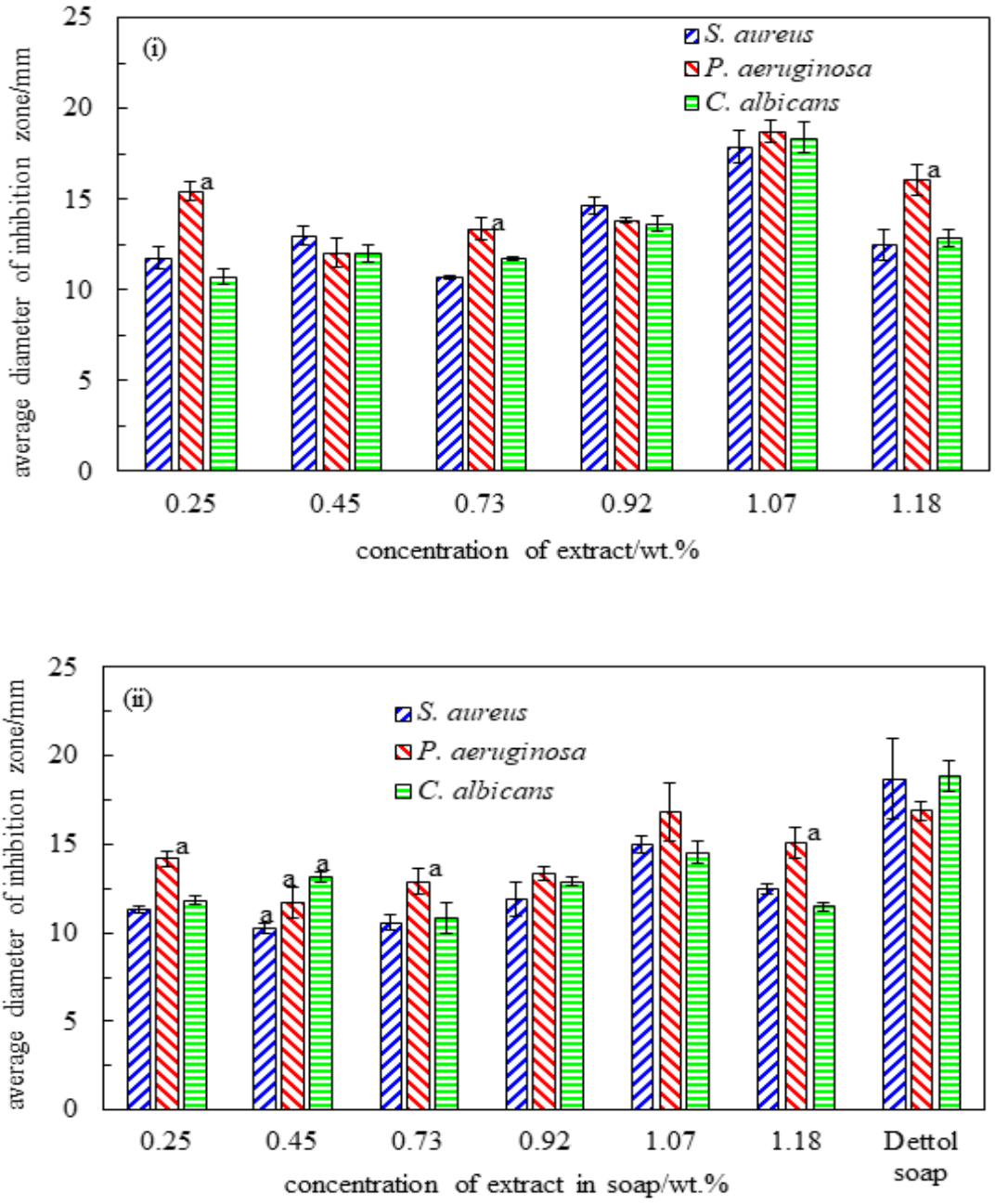


Figure 3. Average diameter of inhibition zone versus (i) concentration of extract alone and (ii) concentration of extract in soap sample in comparison to that of Dettol soap. The error bars are standard deviations of three separate inhibition zones while "a" represents zones that are significantly ($p < 0.05$) different from the rest

subsequently diluted to obtain soap solutions containing different extract concentrations (0-1.18 wt.%) and then characterized in terms of antimicrobial activity and compared with Dettol handwash liquid soap which contains synthetic antimicrobial agents (methylchloroisothiazolinone and methylisothiazolinone). Figure 3(ii) shows a plot of average diameter of zone of inhibition against concentration of extract in the prepared handwash liquid soap for the three microorganisms (*S. aureus, P. aeruginosa and C. albicans*) in comparison to that of Dettol handwash liquid soap. Although maximum inhibition is observed at 1.07 wt.% for both the extract and soap solutions (Figure 3), there appears to be no clear relationship between extract concentration and zones of inhibition. Further comparison of the inhibition zones of the soap samples with those of the extract alone, using independent two-tailed *t*-test at 95% confidence level, revealed that there is no significant difference ($p > 0.05$) between them. However, ANOVA revealed significant differences ($p < 0.05$) between inhibition zones of *P. aeruginosa,* which is more susceptible to the extract, and those of *S. aureus* and *C. albicans.* Finally, *t*he inhibition zones of the prepared soap samples were further compared with those of the Dettol soap using independent two-tailed *t*-test at 95% confidence level, but there was no significant difference ($p > 0.05$) between them. This indicates that the prepared soap samples and Dettol soap have similar antimicrobial properties for these microorganisms which means the extract can be used instead of the synthetic antimicrobial agents (methylchloroisothiazolinone and methylisothiazolinone) used in Dettol soap. The prepared soap was further characterized in terms of pH, foaming and cleansing properties.

pH, foaming and cleansing ability

Incorporating *P. biglobosa* (Jacq.) Benth. leaf extract (black) into the prepared soap changed its color from white to black (Figure 4). Irrespective of the amount of extract incorporated, the soap samples have a pH of 9.26 ± 0.03 (basic), consistent with that of many commercial liquid soaps (Baranda *et al.*, 2002; Gibson *et al.*, 2002), This is different from that of the Dettol soap which is 4.15 ± 0.04 (acidic) like the human skin pH (Ali and Yosipovitch, 2013).

The soap samples produced significant foam, with the volume increasing with extract concentration (Figure 5), reaching a maximum (39 cm^3) at 21.3 wt.% and then decreasing at 23.5 wt.%. The maximum foam volume corresponds to the volume produced by the Dettol soap. The increment in foam volume with extract concentration may be adduced to foam-producing phytochemicals (saponins) in the extract (Chen *et al.*, 2010). These chemicals are able to produce stable air bubbles at relatively low concentrations where they are monomeric, but lose this ability at relatively high concentrations where they form stable aggregates (micelles) (Azira *et al.*, 2008). In terms of cleaning, both the prepared and Dettol soap samples removed ~88 wt.% oil from the linen, compared to water which removed ~2 wt.%, implying that the prepared soap samples and Dettol soap have the same cleaning ability.

CONCLUSION

Unlike the previous paper on the stem-bark extract of *P. biglobosa* (Jacq.) Benth. (Abioye *et al.*, 2013), we show that the leaf extract inhibits the growth of skin pathogenic microorganisms like *S. aureus*, *P. aeruginosa* and *C. albicans.* Consequently, it was incorporated into a prepared handwash liquid soap and tested on these microorganisms. Alike, the soap inhibited the growth of *S. aureus*, *P. aeruginosa* and *C. albicans* to the same degree as the extract. These results compare favorably with those of a commercial handwash liquid soap (Dettol) which contains methylchloroisothiazolinone and methylisothiazolinone as antimicrobial agents. Therefore, given the numerous health and

environmental concerns posed by methyl chloroisothiazolinone and methylisothiazolinone, *P. biglobosa* (Jacq.) Benth. leaf extract can be used as an antimicrobial agent in handwash liquid soaps and other related formulations. Such soaps will have several advantages over those containing synthetic antimicrobial agents. For example, the plant-derived antimicrobial agents will be environmentally benign and less harmful (Manickam *et al.*, 2021) compared with their synthetic counterpart which are non-degradable,

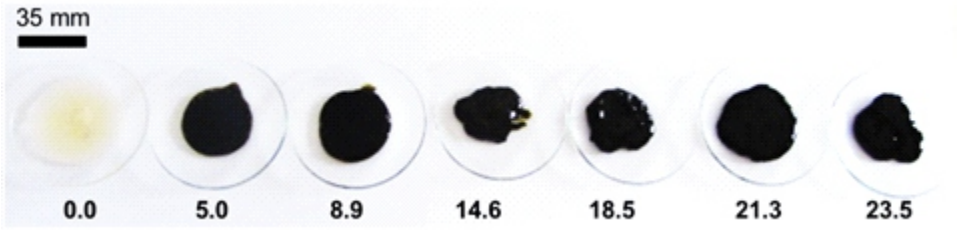


Figure 4. Photographs of petri dishes containing handwash liquid soap with different concentrations (wt.%, given) of *P. biglobosa* leaf extract.

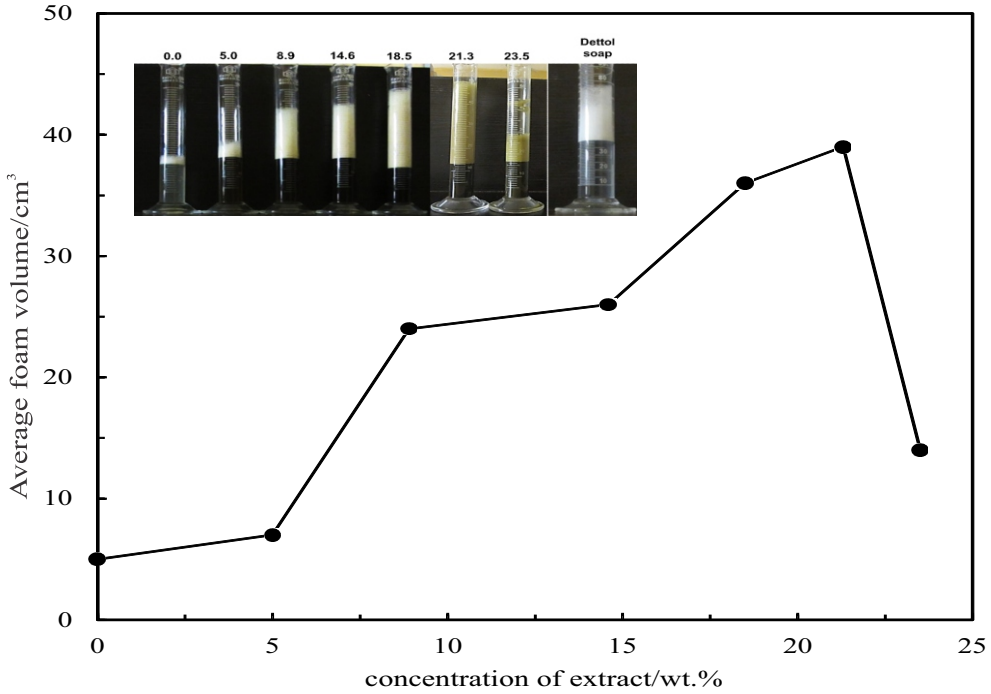


Figure 5. Plot of average foam volume against concentration of extract in soap sample. The error, standard deviation of three values, for each volume is less than 0.2 cm^3. (insert) Photographs (soon after aeration) of glass cylinders containing foams of aqueous solutions of the corresponding soap samples as well as that of the commercial Dettol soap.

cytotoxic, allergenic, carcinogenic and responsible for antibiotic resistance (Suller and Russell, 2000; Schweizer, 2001; Rodrigues-Barata and Conde-Salazar, 2014; Lorente-Lavirgen *et al.*, 2019; Capkin *et al.*, 2017).

Authors' contributions

MOO − Carried out the work. AAT Conceptualization, Supervision, Paper Drafting and Editing. MMT − Data Analysis. MT Paper Drafting and Editing. All authors have read and approved the manuscript.

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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