World Journal of Biology Pharmacy and Health Sciences, 2020, 02(03), 019–029

World Journal of Biology Pharmacy and Health Sciences

e-ISSN: 2582-5542, Cross Ref DOI: 10.30574/wjbphs

Journal homepage: http://www.wjbphs.com

(RESEARCH ARTICLE)

Antibacterial profiling of methanolic leaf extracts and herbal cosmetic cream formulations containing the leaf extracts of *Urtica dioica*, *Amaranthus viridis* and *Aloe vera*

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Publication history: Received on 03 May 2020; revised on 14 June 2020; accepted on 16 June 2020

Article DOI: https://doi.org/10.30574/wjbphs.2020.2.3.0020

Abstract

Synthetic antibacterial drugs are not only expensive but often associated with toxicity, adverse reactions and resistance. There is therefore the need for drugs of natural origin with little or none of the problems associated with synthetic drugs. This study evaluated the antibacterial potency of the methanolic leaf extracts and herbal cosmetic cream formulations containing the leaf extracts of, *Urtica dioica, Amaranthus viridis and Aloe vera* which were extracted with methanol by cold maceration method. The antimicrobial susceptibility of some organisms to the leaf extracts and their cream formulations was determined by agar-well diffusion method using working concentrations of 100 mg/mL, 200 mg/mL and 400 mg/mL of the extracts with distilled water as the negative control. Neomycin® in the concentrations of 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, and 50 µg/mL was used as the positive control for the leaf extracts while 2% w/v mupirocin (Supirocin®) cream and penicillin® ointment were used as positive control for herbal cream evaluation. The MIC of the leaf extracts was determined by agar dilution method using various concentrations (20 mg/mL to 210 mg/mL). The leaf extracts and the formulated creams exhibited antimicrobial activities comparable with the standard antimicrobial compounds and formulations. *Urtica dioica* exhibited the highest antibacterial activity. The cream formulation containing a combination of 20% each of *Urtica dioica* and *Aloe vera* appeared the most potent against the bacteria used. These results suggest that herbal topical formulation containing the leaf extracts of these plants has potentials in the treatment of dermal bacterial infections.

Keywords: Antibacterial, Methanolic extract, Inhibitory, Maceration and potent

1. Introduction

Herbal therapy is the main element in traditional and alternative medicine practiced in the developing and the developed countries [1, 2]. Despite the availability of modern medicine, herbal medicines continue to maintain popularity in some communities for historical and cultural reasons, in addition to their efficacy and cheaper cost [3]. According to the World Health Organization, medicinal plants can provide the best alternative source(s) for obtaining a variety of drugs, since they possess a variety of bioactive principles known as phytochemicals [4, 5, 6] which make them potential sources of antimicrobial agents [7, 8, 9]. These phytochemicals include alkaloids, flavonoids, terpenoids, glycosides, tannins and saponins [3, 10].

In many developing countries, including Nigeria, 80% of patients use indigenous herbal remedies to treat infectious disease [11, 12]. Herbal medicine is now globally accepted as a valid alternative system of therapy in the form of pharmaceuticals, functional foods e.t.c, a trend recognized and advocated by the World Health Organization [13, 14].

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Herbal medicines represent sources of potentially important new pharmaceutical substances since all parts of a plant, from roots to seed heads and flowers, are employed in traditional remedies and can, therefore, act as sources of lead drug compounds [15, 16]. The use of herbal medicines and phytonutrients continues to expand rapidly across the world with many people resorting to these products for treatment of various health challenges in different national healthcare settings [17].

Urtica dioica (*U. dioica*) L., Figure 1, commonly known as Stinging Nettle is an herbaceous perennial plant that grows in temperate and tropical wasteland areas around the world. It belongs to Urticaceae family [18]. It has been used in folklore medicine for a long time as a diuretic agent and to treat arthritis and rheumatism. It is an important medicinal herb, consumed as a component of the human diet in Africa due to its phytonutrients: minerals, chlorophyll, amino acids, lecithin, carotenoids, vitamins, flavonoids, tannins and sterols [19].

Amaranthus viridis (*A. viridis*) as shown in Figure 2 belongs to Amaranthaceae family. *Amaranthus*, communally known as Green amaranth is a multinational genus of herbs. It is generally known as "never-fading flower" in Greek. They are generally used as leaf vegetables, cereals and ornamentals [20].

Aloe vera (*A. vera*), Figure 3 is of Liliaceae family. It is a cactus (leaves) like plant with green, dagger- shaped leaves that are fleshy, tapering, spiny, and filled with a clear viscous gel [21]. It is used locally in treating stomach ailments, gastrointestinal problems, skin diseases, constipation, wound healing, ulcer and diabetes [21].

Figure 1 *Urtica Dioica*

Figure 2 *Amaranthus viridis*

Figure 3 *Aloe vera*

Topical antimicrobials are the mainstay for the treatment of skin infections caused by bacterial, fungal and viral organisms and these antimicrobials are available as creams, ointments, powders and sprays [5, 15]. With the problem of increasing resistance, high cost and side effects associated with synthetic topical antibacterial creams available in the market, it is necessary to formulate an herbal cream which would be affordable, potent and safe for use. This study was therefore designed to investigate the antibacterial potency of methanolic extracts of the leaf of *U. dioica, A. viridis, A. vera* and the herbal cream formulations containing the extracts.

2. Material and methods

2.1. Materials

Mueller Hinton agar (Oxoid U K), Methanol, neomycin®, Supirocin® cream (Glenmark Pharmaceuticals, Andheri (E), Mumbai India), Penicillin® ointment (Drugfield Pharmaceuticals, Nigeria), Shea butter, cetyl alcohol, almond oil, coconut oil, geranium oil, jasmine oil, tea tree oil, honey and methyl (Lagos, Nigeria).

2.2. Collection and authentication of plant materials

Fresh leaves of *U. dioica*, *A. viridis* and *A. vera* were collected from Isibor Botanical Garden, Surulere, Lagos State, Nigeria in July, 2019. The leaves were identified and authenticated by a taxonomist, Dr. Nadoza G. I at the Herbarium of the Department of Botany and Microbiology, University of Lagos. The specimen of each of the leaves assigned with Voucher numbers LUH8408, LUH8414 and LUH8410 respectively were deposited in the herbarium for future reference.

2.3. Test microorganisms

Clinical isolates of *Staphylococcus aureus, Staphylococcus albus, Bacillus subtilis, Escherichia coli, Proteus myrabilis, Klebsiella oxytoca, Enterococcus faecalis* and *Pseudomonas aeruginosa* were obtained from the Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy of the University of Lagos, Nigeria.

2.4. Extraction of the leaves of *U. dioica, A. viridis* and *A. vera*

Mature, healthy and freshly collected leaves of *U. dioica, A. varidis* and *A. vera* were washed with distilled water and air dried. The dried leaves were shred to coarse powder using a laboratory mill (Christy and Norris Ltd, Chelmsford, England). The milled leaves, 750 g of each of the plants were extracted with 7.5 L of 70 % methanol for *A. vera* and 80 % methanol for *U. dioica* and *A. viridis* using cold maceration method [22, 23]. The maceration process was repeated after 4 days for each of the leaves. The extracts were clarified by filtration using a fine pored muslin cloth. The filtrate was concentrated in a rotary evaporator (Buchi V-801), dried in an oven at 37 ˚C [24, 25] and stored at 4 °C for further analysis.

2.5. Evaluation of the antimicrobial activity of the extracts

The antimicrobial activities of the methanolic extracts of *U. dioica, A. viridis* and *A. vera* leaves on clinical isolates of *Staphylococcus aureus, Staphylococcus albus, Bacillus subtilis, Escherichia coli, Proteus myrabilis, Klebsiella oxytoca, Enterococcus faecalis* and *Pseudomonas aeruginosa* were evaluated using the agar-well diffusion method [26]. A 25 mL volume of sterile Mueller-Hinton agar, prepared according to the manufacturer's instruction was poured in sterile petri-dishes containing 1mL of 10^6 cfu of overnight cultures of the microorganisms in saline broth respectively, swirled gently for proper mixing and allowed to solidify. Wells were bored on the seeded agar using a sterile cork-borer (12 mm in diameter) and filled with 0.5 mL each of the graded concentrations of the leaf extract (100 mg/mL, 200 mg/mL and 400 mg/mL) and two-fold dilution of neomycin® standard solution (6.25 – 50 µg/mL) prepared with sterile water. A blank well in each of the culture plates was filled with 0.5 mL sterile water which served as the negative control. The cultures were incubated for 24 h at 37˚C and the zones of inhibition recorded [27].

2.6. Determination of the minimum inhibitory concentration of the leaf extracts

The minimum inhibitory concentration (MIC) of the leaf extracts on susceptible microorganisms (obtained from the antimicrobial activity evaluation) were determined using the Agar dilution method as described by Adeniyi and Ayepola, 2008 [28]. Different concentrations (0.8 mg/mL, 1.6 mg/mL, 3.2 mg/mL, 6.4 mg/mL, 12.8 mg/mL, 25.6 mg/mL, 51.2 mg/mL, 102.4 mg/mL, 204.8 mg/mL and 409.6 mg/mL) of the various leaf extracts were prepared using sterile water as the diluent. A volume of 1 mL from each of the leaf extract concentrations was mixed with 25 mL of Mueller Hinton agar seeded with 1 mL of 2 x 10^6 cfu of overnight clinical cultures of the microorganisms, swirled, allowed to set and incubated at 37 ˚C for 24 h. Different concentrations (20 mg/mL to 210 mg/mL) of the leaf extract from the highest concentration at which there was growth to the least concentration that showed no growth were sub-cultured, incubated at 37 ˚C and the least concentration at which there was no growth for the extracts on each microorganism was recorded as the MIC as shown in Table 4.

2.7. Preparation of herbal cosmetic cream formulations

A total of six different formulations (F1 – F6) of herbal cosmetic cream as shown in Table 1, containing the leaf extracts and other ingredients at various concentrations, were prepared using the method of Gupta *et al*., 2015 [29]. The formulations were oil in water (O/W) emulsion-based cream containing the leaf extracts of *U. dioica, A. viridis* and *A. vera,* an emulsifier (*Shea butter*), essential oils (*Jasmine oil, Tea tree oil, Geranium oil, Almond oil* and *Coconut oil*), antioxidant (*honey*), diluent (*Cetyl alcohol*), water and preservative (*Methyl paraben*).

The emulsifier (*Shea butter*) was mixed with the oil soluble ingredients (*Cetyl alcohol, Almond oil, Coconut oil, Geranium oil, Jasmine oil* and *Tea tree oil*), which was the oil phase and heated to 70 °C. The water-soluble components, Honey and plant extract(s) were mixed together as the aqueous phase and also heated to 70 °C. The oil phase was added in portions to the water phase with continuous stirring to form a homogenous dispersion of the oil phase in the aqueous phase. The mixture was continually stirred until the desired emulsion was formed, allowed to cool to room temperature. The produced emulsion was then transferred into a sterilized cream jar and sealed. The composition and the quantity of ingredient used to make 10 g of the herbal cream for each formulation is given in Table 1

2.8. Evaluation of the antimicrobial activity of the formulated herbal cosmetic creams containing the leaf extracts

The antimicrobial assay of the herbal creams formulated with the leaf extracts of *U. dioica, A. viridis* and *A. vera* (F1-F6) were evaluated by agar diffusion method [26] using clinical isolates of *S. aureus, S. albus, B. subtilis, E. coli, P. myrabilis, K. oxytoca, E. faecalis and P. aeruginosa.* Briefly, 25mL of sterile Mueller-Hinton agar, prepared according to the manufacturer's instruction was poured into sterile petri-dishes, each containing 1 mL of 10^6 cfu of overnight culture of the microorganism in saline broth, swirled gently for proper mixing and allowed to solidify. Wells were bored on the seeded agar using a sterile cork-borer (12mm in diameter) and filled with 1 mL each of herbal cream formulations (F1-F6). A blank well in each of the culture plates was filled with 1ml of formulated cream without the leaf extracts which served as negative standard control. Supirocin® cream and penicillin® ointment were used as positive standard antibiotic controls. [27]

2.9. Physicochemical evaluation of the cream formulations

2.9.1. Determination of the pH of the cream formulations

A 0.5 g of the herbal cream was weighed and dispersed in 50.0 mL distilled water; the pH of the resulting emulsion was determined using a digital pH meter [30].

2.9.2. Determination of wetness, type of smear and emolliency of the cream.

The cream was applied on the skin surface and the smear was checked if it was greasy or non-greasy [31]. Emolliency, slipperiness, and residue left after the application of each cream formulation was checked. The ease of removal of the cream applied was examined by washing the applied part with tap water [31].

2.10. Determination of emulsion type of the cream formulations

2.10.1. Dilution test

Dilution test determines the type of emulsion formed, whether it is oil in water (O/W) or water in oil (W/O) emulsion. The herbal cream was diluted with water and oil and the type of emulsion determined. An O/W emulsion remains stable when diluted with water but cracks when diluted with oil since water is the dispersion medium. The W/O emulsion is stable when diluted with oily liquid and cracks upon addition of water since oil is the dispersion medium. O/W emulsion can easily be diluted with an aqueous solvent, whereas water in oil emulsion can be diluted with an oily liquid [31].

2.11. Irritancy test of the formulations

A $1cm^2$ area was marked on the left-hand dorsal skin surface. The herbal cosmetic cream was applied to the specified area which was examined every 3 hours for irritancy, erythema, and edema for 24 h [32]

2.12. Spreadability test of the formulations

Spreadability of the formulations was determined by measuring the spread diameter of 2.0 g of the cream sample between two horizontal glass plates (10 cm × 20 cm) after 1 minute. The herbal cream was placed at the center of the lower glass plate after which the second glass plate was placed on the lower plate containing the cream. The plates were pressed together by placing a 1 kg weight on the plates for 1 minute to obtain a film of uniform thickness. After 1 minute, the weight and the upper plate were gently pulled out and the diameter of the cream was measured [33].

2.13. Determination of homogeneity and appearance of the formulations

The formulations were tested for homogeneity by their visual appearance and by touch. The appearance of the cream was characterized by its color, coherence and roughness.

2.14. Statistical analysis

Student's t-test was used to determine the statistical significant difference. The difference was regarded as significant when $P < 0.05$. Every data was expressed as mean ± standard deviation of the mean.

3. Results

3.1. Antimicrobial activity of the extracts

The methanolic extracts of the three leaves (*U. dioica, A. viridis* and *A. vera*) exhibited activity against *S. aureus* as shown in Table 2. Of the three leaf extracts, *U. dioica* showed the broadest antibacterial spectrum with activity against *S. aureus, S. albus, B. subtilis, E. coli, P. myrabilis, E. faecalis and P. aeruginosa.* (Table 2). It also exhibited maximal activity against *E. coli* and *S. albus* at low concentration of 30 mg/mL which compared favorably with the standard, Neomycin® as shown in Table 3. None of the extracts showed any activity against *P. myrabilis* and *K. oxytoca*. The antibacterial activity of the extracts was concentration dependent i.e. the activity increased as the concentration increased. The minimum inhibitory concentrations (MICs) of each of the extracts on various susceptible organisms are presented in Table 4.

3.2. Evaluation of antimicrobial potency and physicochemical parameters of the herbal cosmetic cream

S. aureus was susceptible to all the herbal cosmetic cream formulations (F1 to F6) and formulation 2 which contained 20 % *U. dioica* was active against all the organisms used as shown in Table 5. Commercial brands of two antibacterial creams were used as positive standards and they are: Supirocin® (2 % Mupirocin) Glenmark Pharmaceuticals Ltd., Lagos, Nigeria and Penicillin® ointment (Drugfield Pharmaceuticals, Sango Ota, Ogun state, Nigeria. The physicochemical parameters of the cream are represented in table 6.

Table 1 Cream formulations (F1 - F6) containing herbal extracts and other excipients

INGREDIENT	F1	F2	F3	F4	F5	F6
Jasmine oil (mL)	1.1	1.1	1.1	0.6	0.6	0.4
Tea tree oil (mL)	0.6	0.6	0.6	0.3	0.3	0.2
Geranium oil (mL)	1.0	1.0	1.0	0.7	0.7	0.5
Almond oil (mL)	1.6	1.6	1.6	1.0	1.0	0.7
Coconut oil (mL)	1.1	1.1	1.1	0.7	0.7	0.4
Shea butter (g)	1.0	1.0	1.0	1.0	1.0	1.0
Cetyl alcohol (g)	1.0	1.0	1.0	1.0	1.0	1.0
Honey (mL)	0.1	0.1	0.1	0.1	0.1	0.1
Urtica dioica (g)	-	2.0	-	2.0	2.0	1.0
Amaranthus viridis (g)	2.0	-	-	2.0	-	1.0
Aloe vera (g)	-	-	2.0	-	2.0	1.0
Methyl Paraben (g)	0.02	0.02	0.02	0.02	0.02	0.02
Water (mL)	3.0	3.0	3.0	3.0	3.0	3.0

F = Formulation

Table 2 Antibacterial activity of the leaf extracts

ORGANISM	Leaf extracts and their concentration (mg/mL)								
	Urtica doica			*Amaranthus viridis*			*Aloe vera*		
	100	200	400	100	200	400	100	200	400
	Zone of inhibition (mm)								
S. aureus	-	11.20±0.40	13.67±0.47	-	12.67±0.47	14.67±0.47	-	12.50±0.50	14.50±0.50
S. albus	11.86±0.74	13.30±0.47	17.00±0.50	-	-	-	-	-	-
B. subtilis	-	10.70±0.65	12.70±0.47	-	12.67±0.47	14.00±0.82	-	-	-
E. coli	13.49±0.58	15.30±0.47	18.70±0.47	-	-	-	-	-	-
P. myrab.	-	-	-	-	-	-	-	-	-
K. oxytoc.	-	-	-	-	-	-	-	-	-
E. faecal	-	13.00±0.00	17.50±0.50	-	-	-	-	-	-
P. aerugi.	-	14.00±36	16.50±0.50	-	-	-	-	-	-

S. aureus = Staphylococcus aureus, S. albus = Staphylococcus albus, B. subtilis = Bacillussubtilis, E. coli = Escherichia coli, P. myrab. = Proteus myrabilis, K. oxytoc. = Klebsiella oxytoca, E. faecal. = Enterococcus faecalis, P. aerugin. = Pseudomonas aeruginosa

Table 3 Antibacterial activity of the standard, neomycin®

Concentration/zone of inhibition (mm)				
ORGANISM	6.25µg/mL	12.5µg/mL	25µg/mL	50µg/mL
S. aureus	-	-	11.30±0.36	14.67±0.44
S. albus	-	-	11.30±0.41	14.30±0.23
B. subtilis	-	11.00±0.01	11.30±0.24	13.30±0.34
E. coli	-	-	14.30±0.27	18.30±0.45
P. myrabilis	-	-	12.30±0.34	15.30±0.25
K. oxytoca	-	14.30±0.47	15.67±0.40	18.30±0.47
E. faecalis	-	-	11.30±0.47	13.30±0.43
P. aeruginosa	-	-	-	14.30±0.33

S. aureus = Staphylococcus aureus, S. albus = Staphylococcus albus, B. subtilis = Bacillus subtilis,
E. coli = Escherichia coli, P. myrab. = Proteus myrabilis, K. oxytoc. = Klebsiella oxytoca,
E. faecal. = Enterococcus faecalis, P. aeruginosa. = Pseudomonas aeruginosa
- = No zone of inhibition.

Table 4 Minimum inhibitory concentration (MIC) of the extracts

		Concentration of the extract (mg/mL)																	
EXTRACT	ORGANISM	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190
U. dioc	*S. aureus*	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
	S. albus	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	B. sub	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
	E. coli	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E. faec	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
	P. aeru	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
A. virid	*S. aureus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
	B. sub	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
A. vera	*S. aureus*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-

S. aureus = Staphylococcus aureus, S. albus = Staphylococcus albus, B. sub = Bacillus subtilis, E. coli = Escherichia coli, E. faec = Enterococcus faecalis, P. aeru. = Pseudomonas aeruginosa, U. dioc=Urtica dioica, A. Virid= Amaranthus viridis, A. Vera=Aloe vera

+ = Growth, - = No Growth

Table 5 Antibacterial activity of the cosmetic herbal cream and the standards (Supirocin® & Penicillin®)

	ZONE OF INHIBITION IN MM							
BACTERIA	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**SUPIROCIN®**	**PENICILLIN®**
S. aureus	20	22	12	25	23	21	20	13
S. albus	-	11	-	-	-	-	20	12
B. subtilis	15	14	-	-	14	-	34	16
E. coli	-	15	-	-	-	-	25	-
E. faecalis	-	13	-	-	-	-	21	-
P. aeruginosa	-	16	-	11	23	-	40	30

S. aureus = Staphylococcus aureus, S. albus = Staphylococcus albus, B. subtilis = Bacillus subtilis, E. coli = Escherichia coli, E. Faecalis = Enterococcus faecalis, P. aeruginosa = Pseudomonas aeruginosa.

The physicochemical parameters of the creams are represented in Table 6. The creams formulations were non-greasy, smooth and oil-in-water type of emulsion

Table 6 Evaluation of physical parameters of the herbal cosmetic cream

PARAMETERS	F1	F2	F3	F4	F5	F6
Color	Dark-green	Brown	Dark-brown	Black	Golden-brown	Brown
pH	5.8	5.5	6.0	6.2	5.9	5.6
Dilution	O/W	O/W	O/W	O/W	O/W	O/W
Smear type	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy	Non-greasy
Emollience	No residue	No residue	No residue	No residue	No residue	No residue
Removal	Easily	Easily	Easily	Easily	Easily	Easily
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous	Homogenous
Appearance	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Irritancy	-	-	-	-	-	-
Erythema	-	-	-	-	-	-
Edema	-	-	-	-	-	-
Spreadability	4.0cm	3.8cm	4.1cm	3.9cm	4.0cm	3.5cm
Wetness	Moisturizes	Moisturizes	Moisturizes	Moisturizes	Moisturizes	Moisturizes

- = None observed, O/W = oil in water

4. Discussion

This study evaluated the antimicrobial activities of the methanolic leaf extracts of three plants (*U. dioica, A. viridis* and *A. vera*) and formulated herbal creams containing their extracts against eight bacteria *S. aureus, S. albus, B. subtilis, E. coli, P. myrabilis, K. oxytoca, E. faecalis and P. aeruginosa.*

Each of the leaf extracts and their cosmetic cream formulations (F1 – F6) showed strong antibacterial activity against *S. aureus* as shown in Tables 2 and 5 which was an interesting result because *S. aureus* is notorious for its ability to develop resistance to antibiotics. Infections of such antibiotic-resistant strains often lead to epidemic waves initiated by one or a few successful mutants [34]. *S. aureus* is commonly found on the skin and is not easily eliminated especially from the deeper skin layers, sweat glands, sebaceous glands and the hair follicles by routine washing and scrubbing even with some antiseptics [35] The cream formulations of these extracts could therefore be a veritable source of antibacterial drugs used in the treatment of skin infections involving *S. aureus* thereby avoiding the undesirable side effects of synthetic drugs [36] and the resistance that could be associated with them.

The antibacterial activity of the extracts against the tested organisms were concentration dependent. Out of the three methanolic leaf extracts tested, *U. dioica* exhibited the broadest spectrum of activity, being active against *S. aureus, S. albus, B. subtilis, E. coli, P. myrabilis, E. faecalis and P. aeruginosa.* (Table 2). *U. dioica* also exhibited maximal activity comparable to the standard antibiotic, neomycin® against *E. coli* and *S. albus* even at low concentrations (Table 3). In this study, the cream formulation (F2) containing 20 % *U. dioica* showed better activity against *E. coli and E. faecalis* compared with penicillin®, the standard antibiotic ointment used as positive control which further supports the evidence of broad-spectrum activity of *U. dioica.* Formulation 5 containing 20 % *U. dioica* and 20 % *A.* vera produced the highest zones of inhibition which is an indication of possible synergistic effect amongst the two extracts, suggesting there may be value if the two plant extracts are combined together in a herbal cream formulation. Antibacterial effects due to extracted phytonutrients in *U. dioica* is in line with the findings of Huda *et al*, 2015 [37]. *U. dioica* plant extract is known to contain neophytadiene and other constituents such as alkaloids, phenols, flavonoids, tannins and saponins which have been claimed to be responsible for their antimicrobial activity [38]. The antibacterial activity of the alkaloidal constituents may be due to its ability to react with amino, carboxyl, sulfhydryl and hydroxyl groups in bacterial protein as well as nucleic acids. This highly reactive chemical compounds combine with proteins to give intermolecular cross-links and intercalate with DNA [38]. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them [39].

Evaluation of the physicochemical properties of the cream formulations showed a cosmetically appealing product. The pH of the skin should ideally be slightly acidic at the range of 4 to 7. With more acidity, the skin can combat harmful microbes and damaging free radicals that might increase the aging process while increase in pH causes an increase in dehydrative effect, irritability and increased microbial count [40]. An ideal product to be used on the skin should have similar pH with the skin. In this study, the herbal cream formulations with the leaf extracts had pH values of 5.5 to 6.2 as shown in Table 6 which fall within the pH range of normal healthy skin and ideal for enhanced antimicrobial activities in a topical cream.

The cream formulations were oil in water (O/W) type of emulsion which is easy to wash off from skin thus encouraging patient/customer compliance. The herbal cream formulations appeared smooth with appreciable odour, easily spreadable and homogenous. There was no residue felt from the cream, no skin irritation, no induced edema nor erythema. The presence of essential oils (*Jasmine oil, Tea tree oil, Geranium oil, Almond oil* and *Coconut oil*) and antioxidant (*honey*) must have contributed to the emolience, moisturizing, smoothness and glittering effects of the cream observed on the skin. The prepared herbal cosmetic cream thus compared favorably with the standards: Supirocin® and Penicillin®.

5. Conclusion

The methanolic extracts of the leaves of *U. dioica, A. viridis* and *A. vera* exhibited antimicrobial activities with *U. dioica* exhibiting the potentials of a broad-spectrum antibacterial agent. The cream formulations containing 20 % *U. dioica* exhibited broad spectrum activity comparable to the activities of Supirocin® and more potent than the standard, Penicillin®**.** The herbal cream formulation was thus potent as an antibacterial agent, safe for application on the skin and cosmetically appealing to enhance patient/customer compliance.

Compliance with ethical standards

Acknowledgments

Our thanks go to Mr. Abdulrahman Usman and Mr. Hosana Anibema for their technical assistance in the laboratory.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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How to cite this article

Igbokwe NH, Idowu AO, Abdulwahab UB, Oseni BA and Isibor K. (2020). Antibacterial profiling of methanolic leaf extracts and herbal cosmetic cream formulations containing the leaf extracts of *Urtica dioica*, *Amaranthus viridis* and *Aloe vera*. World Journal of Biology Pharmacy and Health Sciences, 2(3), 19-29.