FORMULATION AND EVALUATION OF ANTIMICROBIAL HERBAL CREAMS FROM AQUEOUS EXTRACT OF *MORINGA OLEIFERA* LAM SEEDS

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Accepted 21st May, 2020

This study was carried out to evaluate the antimicrobial activity of ethanolic extract, aqueous extract and topical creams from *Moringa oleifera* seeds against some bacteria and fungi. *M. oleifera* seeds were dried, powdered and macerated for 24 and 48 h in water and ethanol respectively. The filtrates were concentrated and stored properly. Antimicrobial sensitivity tests were conducted on the aqueous and ethanolic extracts using standard agar diffusion method. The minimum inhibitory concentrations of both extracts were determined against various test organisms. Creams were formulated using the aqueous extract. They were evaluated based on organoleptic properties, pH, viscosity and antimicrobial activities. The ethanolic extract was inactive against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The aqueous extract was active against *S. aureus*, *C. albicans*, *Trichophyton spp*, *Aspergillus flavus* but inactive against *E. coli*, *P. aeruginosa, Microsporium* spp, and *Rhizopus* spp. The MIC for sensitive organisms was achieved with 2.5 to 5%w/v concentration of the aqueous extract. Positive results were also obtained with the cream formulations. The pH of the cream formulations ranged from 4.17 to 4.33.The study showed that the antimicrobial property of the aqueous extract was retained by the cream formulations and both the aqueous extract and cream formulation could respectively be used to treat skin diseases caused by *S. aureus, C. albicans, Trichophyton* spp and *A. flavus*.

Key words: *Moringa oleifera* seeds, aqueous extract, antimicrobial, creams.

INTRODUCTION

Moringa oleifera is a plant which has been used extensively to treat certain infections; and as food supplement. It has been reported that due to the presence of a very wide range of vital antioxidants, antibiotics and nutrients including vitamins and minerals, *M. oleifera* has important medicinal uses. Almost all parts from *M. oleifera* can be used as a source for nutrition with other useful values (Abdul Razis et al., 2014). Extracts from different parts of *M. oleifera* such as leaves, stem bark, root bark, seeds cotyledon and seeds coat, have been shown to have antimicrobial activities (Arora et al., 2013). Aqueous extract of pods' husks of *M. oleifera* showed antimicrobial activity against gram positive, gram negative pathogenic bacteria and yeast strains (Onsare et al., 2013). The antibacterial activity of *M. oleifera* seed was reported by several authors (Olsen et al., 1987; Bukar et al., 2010). The presence of different phytochemicals, especially a short polypeptide named 4 (ά – L -rhamnosyloxy) benzyl-isothiocyanate, is responsible for the antimicrobial activity of *M. oleifera* seed (Eilert et al., 1981; Bukar et al., 2010).

Herbal extracts can be prepared from plant parts and incorporated into different skin care cosmetic creams, lotions and ointments. Herbal cosmetics can prevent the skin from developing different skin conditions, skin allergic reactions and skin diseases. They are preferred to synthetic cosmetics because they have fewer side effects, safe on skin, effective, and of higher quality,

economical and affordable to patients (Chandrasekar et al., 2018). There are numerous herbs available naturally that have different uses in cosmetic preparations for skincare, hair care and as antioxidants (Venkatachalam et al., 2019). Currently, herbal extracts are used in cosmetic preparations for augmenting beauty and attractiveness. Herbal cosmetics are classified on the basis of dosage form like cream, powder, soaps, solutions, etc. and according to part or organ of the body which requires the application such as skin, hair, nail, teeth and mouth etc. (Akhtar et al., 2011; Seema et al., 2017).

Creams are dosage forms which are applied externally. They are usually semi-solid emulsions or viscous liquids. Creams can be oil-in water (o/w) or water-in-oil (w/o) (Barry, 1999; Block, 2005). They are usually opaque in appearance unlike ointments that are translucent (Idson and Lazarus, 2009). Pharmaceutical creams contain one or more medicinal agents dissolved or dispersed in either the emulsion or in another type of water - washable base. When oil-in-water cream, also known as 'vanishing cream' is rubbed on the skin, water which is the dispersion medium evaporates and the concentration of the water-soluble drug that it contains increases in the adhering film. An o/w cream does not leave a continuous film of water-impermeable liquid and therefore is non-occlusive (Barry, 1999). Creams are mainly used in topical skin products and in products that are applied to the vagina or rectum. Due to ease of spread and removal, creams are more frequently used compared to ointments by many patients and medical practitioners (Allen et al., 2011).

This study was conducted to determine the antimicrobial activity of the aqueous and ethanolic extracts of *Moringa oleifera seeds* against selected microorganisms; and also to evaluate creams formulated with the more antimicrobial active extract using an emulsifying ointment as cream base.

MATERIALS AND METHODS

The materials used were of analytical grade and they include: absolute ethanol (JHD, China), ciprofloxacin, ketoconazole, emulsifying wax, white soft paraffin, liquid paraffin, cetostearyl alcohol (Loba Chem, India), nutrient agar (Titan, India), Sabouraud dextrose agar, Sabouraud dextrose broth, nutrient broth, Mueller Hinton agar (Titan, India). Microorganisms were obtained from the Pharmaceutical Microbiology laboratory of the Faculty of Pharmacy, Delta State University, Abraka and they include *Staphylococcus aureus, Escherichia coli, Candida albicans, Pseudomonas aeruginosa, Trichophyton spp, Aspergillus flavus, Microsporium* spp, and *Rhizopus* spp.

Collection of plant materials

Seeds of *M. oleifera* were purchased from Main market in Onitsha, Anambra State, Nigeria. The seeds were identified by Dr. Emmanuel Ikpefan, the Head of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, Delta State University, Abraka.

Preparation of plant materials: The *M. oleifera* seeds were de-shelled and air dried for about 2 weeks before grinding. The seeds were powdered using a clean blender and stored in a closed plastic container.

Aqueous extraction: A 100 g quantity of the powdered dried seeds was weighed and transferred into an air tight container. A 250 ml quantity of distilled water was added to the powder to macerate for 24 h with occasional agitation. The mixture was filtered using a muslin cloth and the filtrate was concentrated and stored in an air tight container.

Ethanolic extraction: A100 g quantity of the powdered dried seeds was weighed and put in an airtight container. A 350 ml quantity of 70% v/v ethanol was added to the powder and allowed to stand for 48 h with occasional agitation. The mixture was then filtered and evaporated to dryness by heating at 60 ± 0.5°C in a water bath.

Preparation of bacterial isolates: Nutrient agar slants were prepared according to Manufacturer's instruction, while aseptic rules were strictly observed, to obtain sterile media in slants. Stock cultures of the bacteria isolates were sub-cultured into the agar slants, labeled and incubated at 37°C

for 24 h.

Preparation of fungal isolates: A 6.8 g quantity of Sabourand dextrose agar was prepared according to Manufacturer's instruction, while aseptic rules were strictly observed. Sterile agar slants were prepared and inoculated with different fungal isolates, labeled and incubated at 37°C for 36 h.

Preparation of broth cultures: Following Manufacturer's instruction, nutrient broth and Sabourand dextrose broth were prepared aseptically. The bacterial and fungal isolates were sub-cultured respectively, from the above agar into the broth media and the tubes were incubated at 37°C for 24 h and at room temperature for 36 to 72 h, respectively.

Antimicrobial sensitivity testing

Antimicrobial sensitivity was done using the agar well diffusion technique. Mueller Hinton agar was prepared, following Manufacturer's instruction, autoclaved, dispensed (20 ml) aseptically into each Petri dish and allowed to solidify. Doubling dilutions of each of the extracts were made to obtain the following concentrations: 10, 5, 2.5, 1.25, 0.63 and 0.31 (%w/v).

Plates were inoculated in triplicates, with the bacterial isolates, by spread plate technique. Using a sterile cork borer, wells were bored into each agar plate. Afterwards, each dilution for all the extracts was placed in each well with a micropipette, and labeled appropriately. The control, Ciprofloxacin 2%w/v, was also added into one of the wells in every plate. The plates were incubated at 37°C for 24 h. The zones of inhibition were measured with a millimeter ruler and recorded.

Sabourand dextrose agar was sterilized by autoclaving, having followed Manufacturer's instruction and allowed to cool. Twenty milliliters was dispensed aseptically into each Petri dish and allowed to solidify. Fungal isolates were tested using the method for antibacterial sensitivity above but the control used is 2%w/v ketoconazole. Incubation was at room temperature for 36-72 hours. The zones of inhibition were measured in millimetres and recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The method employed for the determination of MIC is the pour plate technique. Petri dishes were appropriately labeled and 1 ml of the various concentrations of the ethanolic and aqueous extracts was transferred into the labeled Petri dishes. Then, 19 ml of sterilized Mueller Hinton agar for the bacteria isolates and 19 ml of sterilized Sabourand dextrose agar for fungal isolates were poured into the Petri dishes and mixed thoroughly. The plates were then allowed to solidify and upon solidification, the plates were inoculated with the different bacteria and fungi isolates by streak plate technique. The plates were incubated at 37°C for 24 h and room temperature for 36-72 h, for bacteria and fungi, respectively. The minimum inhibitory concentration was determined.

Preparation of emulsifying ointment base

This was prepared according to the formula on Table 1. A 6 g quantity of emulsifying wax was weighed in a porcelain dish and melted at 70°C in a water-bath; 10 g of white soft paraffin was added to the porcelain dish and allowed to melt. This was followed by the incorporation of 4.65 ml of liquid paraffin into the mixture in the porcelain dish. The content of the porcelain dish was carefully removed from the water-bath and allowed to solidify with continuous stirring to achieve a homogenous mixture.

Table 1. Composition of the emulsifying ointment (The Pharmaceutical Codex, 1979).

Ingredient	Amount given	Amount used
Emulsifying wax (g)	300	6
White soft paraffin (g)	500	10
Liquid paraffin (g)	200	4 (4.65 ml)

Key: grams (g); millilitre (ml).

Preparation of creams: The herbal creams were prepared using the formula on Table 2. One gram of the emulsifying ointment base was weighed in a porcelain dish and melted at 70°C in a water bath. Two gram of cetostearyl alcohol was added and melted in the porcelain dish. The required amount of the aqueous extract of *M. oleifera* seeds was added into the porcelain dish and stirred until a uniform blend was obtained. A 0.02 g quantity of benzoic acid was added as a preservative. Appropriate quantity of water was added gradually with stirring to make up the weight of the cream to 20 g. This was removed from the water-bath and allowed to cool before being transferred into a cream jar.

Table 2. Composition of the *M. oleifera* cream formulations.

F	Emulsifying ointment (g)	Cetostearyl alcohol (g)	*M. oleifera* extract (%w/w)	Benzoic acid (g)	Distilled water to 20 g
F1	1.0	2.0	5.00	0.02	q. s
F2	1.0	2.0	2.50	0.02	q. s
F3	1.0	2.0	1.25	0.02	q. s

Key: F = formulation code; q.s = sufficient quantity.

PHYSICAL EVALUATION OF FORMULATED CREAMS

Organoleptic properties: The creams were evaluated based on their organoleptic (appearance, texture, and odor) and physicochemical properties.

Homogeneity: The formulated creams were evaluated for homogeneity by visual inspection and touch.

After feel: The creams were evaluated for emolliency and greasiness by applying a finger tip unit of the cream on the skin.

Determination of pH: The creams were transferred into a beaker and subjected to pH test using a pH meter and pH readings obtained were recorded.

Determination of viscosity: The viscosity of the formulated creams was determined using a Brookfield viscometer with spindle number 4 at speed 6 with a coefficient of 1000. The spindle was placed in a beaker containing the cream and rotated at 30 rotations per minute. The corresponding reading was recorded and multiplied by 1000.

Accelerated stability study: A 5 g quantity of the cream was kept in a stability chamber at 45°C and another 5 g cream was kept at room temperature (27 ± 2°C) for 4 weeks. Organoleptic properties (color, odor and texture), homogeneity and after feel were re – assessed after 4 weeks.

Evaluation of the antimicrobial activity of the formulated creams using the disc diffusion method

The prepared agar (Muller Hinton and Sabourand dextrose agar) were sterilized and aseptically poured into Petri dishes to solidify. Upon solidification, the agar were inoculated with the organisms (bacteria in Mueller Hinton agar and fungi in Sabouraud dextrose agar), using the spread plate technique. By means of a sterile forceps, a moistened sterile disc was placed into the different concentration of the formulated creams and impregnated on each agar plate. The plates were then incubated at 37°C for 24 h. The effect of the extract was assessed by measuring the diameters of zones of inhibition to the nearest millimeters.

RESULTS AND DISCUSSION

The ethanolic extract of the seeds of *M. oleifera* inhibited *S. aureus* at concentrations (%w/v) of 0.31, 0.63 and 2.50 with mean zones of inhibition ranging from 3.00 - 3.66 mm (Table 3). The control, ciprofloxacin inhibited all the bacteria in a manner incomparable to that of *S. aureus*. Ketoconazole, the control for fungi, considerably inhibited *C. albicans*. However, *E.coli, P.*

Table 3. Mean Zones of inhibition (mm) of the ethanolic extract of *M. oleifera* seeds on test organisms

Drug	Concentration (%w/v)	Zone of inhibition(mm)			
		Escherichia coli	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Candida albicans***
Ethanolic extract	10.00	NI	NI	NI	NI
	5.00	NI	NI	NI	NI
	2.50	NI	3.66	NI	NI
	1.25	NI	3.46	NI	NI
	0.63	NI	3.36	NI	NI
	0.31	NI	3.00	NI	NI
	PC	9.00	26.67	24.00	20.00

PC - Positive control (Ciprofloxacin for the bacteria and ketoconazole for the fungi); NI - No inhibition.

aeruginosa and *C. albicans* were resistant to the extract at all concentrations.

Table 4 shows that *Staphylococcus aureus* was susceptible to the aqueous extract of the seeds of *M. oleifera* at all the concentrations. The mean zones of inhibition (mm) had a range of 3.67 – 15.33. *P. aeruginosa* and *E. coli* were resistant to the extract at all concentrations but susceptible to the control, ciprofloxacin.

C. albicans is the only fungal isolate which was susceptible to the aqueous extract at all concentrations, with a range of zones of inhibition (mm) 2.33 – 6.00. It is interesting to note that *Trichophyton* spp and *Aspergillus flavus* at concentrations (%w/v) of 2.50, 5.00 and 10.00 were inhibited with larger zones of inhibition (11.00- 15.00) mm than *C. albicans*. Ketoconazole, the fungal control however, inhibited all the isolates.

Table 5 shows the result of the minimum

Table 4. Mean Zones of inhibition of the aqueous extract of *M. oleifera* on the test organisms.

Drug	Conc. (%w/v)	Zone of inhibition (mm)							
		E. coli	**S.A**	**P.A**	**C.A**	**T.P**	**A.F**	**Rhi**	**M.C**
Aqueous extract	10.00	NI	7.00	NI	6.00	11.00	13.00	NI	NI
	5.00	NI	10.00	NI	5.67	13.50	12.50	NI	NI
	2.50	NI	15.33	NI	5.33	15.00	12.50	NI	NI
	1.25	NI	10.67	NI	4.00	NI	NI	NI	NI
	0.63	NI	7.33	NI	3.33	NI	NI	NI	NI
	0.31	NI	3.67	NI	2.33	NI	NI	NI	NI
	PC	14.00	31.33	24.00	14.33	17.00	17.00	17.00	17.00

Conc. – concentration; PC- Positive control (Ciprofloxacin for the bacteria and ketoconazole for the fungi); NI - No inhibition; E.C- *Escherichia coli*; S.A- *Staphylococcus aureus*; P.A. – *Pseudomonas aeruginosa*; C.A- *Candida albicans*; T.P- *Trichophyton spp*.; A.F- *Aspergillus flavus*; Rhi- *Rhizopus spp*; M.C- *Microsporium* spp.

inhibitory concentration of the aqueous and ethanolic extract of *M. oleifera* seeds against *S. aureus, C. albicans, Trichophyton* spp, and *A. flavus*. From the result it can be seen that the ethanolic extract had no effect on *Candida albicans, Trichophyton spp,* and *A. flavus*; however, for *S. aureus*, MIC was achieved with 1.25%w/v extract concentration. The result also showed that for aqueous extract, MIC was achieved with 2.5%w/v extract concentration for *C.albicans, Trichophyton* spp, and *A.flavus* while that of *S. aureus* was at 5%w/v.

Table 6 shows the zone of inhibition of the formulated creams on the test organisms. The result shows that the formulated cream containing 5%w/v of the aqueous extract of *M. oleifera* gave the highest zone of inhibition on *S. aureus,* while the cream containing 2.5% of *M. oleifera* gave the least zone of inhibition. The formulated cream containing 10% of the aqueous extract of *M. oleifera* gave the highest zone of inhibition on *C. albicans* and *A. flavus*, while the cream

Table 5. Determination of the minimum inhibitory concentration of the aqueous and ethanolic extracts of *Moringa oleifera* seeds on test organisms.

Drug	Concentration (%w/v)	*Staphylococcus aureus*	*Candida albicans*	*Trichophyton* spp.	*Aspergillus flavus*
Ethanolic extract	10.00	-	+	+	+
	5.00	-	+	+	+
	2.50	-	+	+	+
	1.25	-	+	+	+
	0.63	+	+	+	+
	0.31	+	+	+	+
Aqueous extract	10.00	-	-	-	-
	5.00	-	-	-	-
	2.50	+	-	-	-
	1.25	+	+	+	+
	0.63	+	+	+	+
	0.31	+	+	+	+

- = indicates no growth; + = indicates growth.

Table 6. Zones of inhibition of the formulated creams on test organisms.

Organism	Concentration of extract in cream (%w/v)	Zone of inhibition (mm)
Staphylococcus aureus	10.00	17.25
	5.00	17.75
	2.50	13.50
Candida albicans	10.00	13.50
	5.00	11.00
	2.50	11.00
Trichophyton spp.	10.00	15.00
	5.00	15.25
	2.50	15.75
Aspergillus flavus	10.00	13.52
	5.00	12.74
	2.50	11.68

containing 5 and 2.5% of the aqueous extract gave the least zone of inhibition. However, the formulated cream containing 2.5% of the extract gave the highest zone of inhibition on *Trichophyton* spp., while the 10% concentration gave the least zone of inhibition.

The physical evaluation of the different formulations of *M. oleifera* cream (Table 7) shows a white appearance and chocolaty odor, though F3 had a mild odor compared to F1 and F2; this is because it contains a smaller concentration of the extract. The pH of the formulations ranged between 4.17 and 4.33, which indicates that the different formulations were slightly acidic and can be applied on the skin. The pH of the different formulations falls within the normal pH range for skin surface (4.0 to 6.5). The fairly acidic nature of the skin helps it to ward off the advances of harmful bacteria and fungi. Resident bacteria floras are kept attached to the skin at skin pH (4.0 to 4.5) (Lambers et al., 2006). Creams were homogenous; thus they were ranked to be excellent. The viscosities of the formulated creams were evaluated and F1 was

Table 7. Physical evaluation of the formulated creams.

Formulations	F1	F2	F3
Appearance	White	White	White
Odor	Chocolaty	Chocolaty	Mildly chocolaty
pH	4.26	4.13	4.17
Viscosity (mpas)	20,000	58,000	59,833
Homogeneity	++++	++++	++++
Emolliency	++++	++++	++++

Keys: ++++ = excellent.

found to have a viscosity of 20,000 mPas which is an indication that F1 is less viscous; thus it is easily spreadable and can be packed in a collapsible tube. However, F2 and F3 had viscosities of 58,000 and 59,833 mPas respectively. This indicates that the two formulations were highly viscous and should be packed in a cream jar. The formulated creams were ranked excellent, since on application no residue was left on the skin. From Table 8, it can be seen that various concentrations of the formulated creams gave higher zones of inhibition against the test organisms compared to the aqueous extract. This may be due to the additional effect of benzoic acid that was used as preservative in the formulation of the creams.

Table 8. MIC for the pure aqueous extract and the formulated creams.

	Concentration (%)	Zone of inhibition (mm)			
		Staphylococcus aureus	***Candida albicans***	***Trichophyton spp***	***Aspergillus flavus***
Aqueous extract	10.00	7.00	6.00	11.00	13.00
	5.00	10.00	5.67	13.50	12.50
	2.50	15.33	5.33	15.00	12.50
Formulated cream	10.00	17.25	13.50	15.00	13.52
	5.00	17.75	11.00	15.25	12.74
	2.50	13.50	11.00	15.75	11.68

The result shows that the cream containing 5% of the extract gave the highest zone of inhibition on *S.aureus* compared to the aqueous extract which gave its highest zone of inhibition at 2.5% concentration. Also the cream containing 10% of the extract gave the highest zone of inhibition on *C. albicans* and *A. flavus* just like the aqueous extract. The formulated cream containing 2.5% of the extract gave the highest zone of inhibition on *Trichophyton* spp as did the aqueous extract.

There was no change in organoleptic properties such as color, odor and texture when the samples were re-assessed after 12 weeks of storage at 40^{o}C. Also, there was no change in the homogeneity or after feel of the creams and this showed that the stability of the creams was maintained.

Conclusion

This study showed that the aqueous extract of *M. oleifera* seeds exhibited higher antimicrobial activity than the ethanolic extract against the test organisms. Likewise, the aqueous extract when formulated into creams retained their antimicrobial activity. The *M. oleifera* herbal creams could be used for the treatment of various skin diseases caused by *S. aureus, C. albicans, Trichophyton* spp and *A. flavus*.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors appreciate the contributions of Mr.

Benedict Iwetan and Mr. Michael Oghenejobo, Technologists in the Department of Pharmaceutics and Industrial Pharmacy, and Department of Pharmaceutical Microbiology respectively, of the Faculty of Pharmacy, Delta State University, Abraka.

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