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Formulation and Evaluation of Antimicrobial Topical Creams from Ethanol Extract of *Vernonia ambigua* Leaves

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases as well as ameliorating some of the untoward effects that are often associated with synthetic antimicrobials.

Objective: This study was conducted to formulate and investigate the antimicrobial property of ethanol extract of *Vernonia ambigua* topical cream formulation.

Method: The ethanol extract of the dried leaves was prepared by maceration and the filtrate obtained was concentrated to dryness. It was evaluated for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* as test organisms. The ethanol extract was formulated into a cream and evaluated for physicochemical and antimicrobial properties.

Result: Percentage yields for the ethanol extraction was 15.38% w/w. The pH range of the formulated creams was between 4.56 and 6.89; the viscosity ranges between 16500 and 51300 mPas. All formulated creams were stable. The three bacteria used were sensitive to both the ethanol extract and the formulated creams, though their zones of inhibition differ slightly. The ethanol extract was not effective against *Candida albicans*. The minimum inhibitory concentration (MIC) when the ethanol extract was applied was 100 mg/mL, 100 mg/mL, and 12.5mg/mL for *E. coli*, *P. aeruginosa*, *S. aureus*, respectively.

Conclusion: The study revealed that the ethanol extract and the formulated cream have antimicrobial properties and there was no loss of activity on formulation of the extract into a cream.

Keywords: *Vernonia ambigua*, Creams, Antimicrobial, Microorganisms, Ethanol extract

INTRODUCTION

Creams that are used for medical purposes are usually semi solid or very viscous liquid emulsions that contain medicaments dissolved or dispersed in the emulsion and are meant to be used externally [Barry, 1999; Okorie and Ofoefule, 2002]. Medicated creams are preferred to ointments because they are usually less greasy, spread quickly and the inflamed tissues are soothed due to evaporation of water [Barry, 1999; Okorie and Ofoefule, 2002]. The incorporated medicament could be synthetic, semi synthetic or natural product.

Natural products are predominantly obtained from plants, although animals and soil materials may also be used. Plants contain wide range of chemical constituents that are of therapeutic importance. They contain a lot of secondary metabolites and one plant may contain enormous spectrum of biologically salient active compounds [Koteshwasr, 2017].

Infectious diseases despite the discovery of new antimicrobial agents still pose a great threat to humans

with regards to morbidity and mortality, especially in developing countries [Shehu et al., 2016]. This may be due to poor access to drugs in developing countries, limited spectrum of activity, side effects, irrational use of these antibiotics and the emergence of multidrug-resistant strains of bacteria, virus, and fungi [Ofokansi et al., 2013]. This has led to the search for new, safe, alternative and more effective antimicrobial agents especially from natural sources [Shehu et al., 2016]. Medicinal plants have been identified and used throughout human history and have recently found its way as a drug source or lead in modern medicine since they are termed to act by stimulating and supplementing the body's healing forces. Vast arrays of antimicrobial substances are present in plants and have remained, hitherto unexplored. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases as well as ameliorating some of the untoward effects that are often associated with synthetic antimicrobials [Ofokansi et al., 2013; Hossain et al., 2014; Chen et al., 2016; Shehu et al., 2016; Ordu et al., 2018]. These active substances from plants are isolated as crude extracts, purified and incorporated into the desired dosage forms.

Vernonia ambigua Kotschy and Peyr is called Orungo by the Yorubas and Tabtaba/Tattaba by the Hausas in Nigeria. It is used traditionally as remedies for cough and fever. It belongs to the family Asteraceae. It is an annual herb that grows up to 65 cm high, occurring throughout the drier parts of tropical West Africa. It has a green erect stem that is very hairy (long and dense). It grows as a weed in Nigeria and it is of no economic importance [Kunle and Egharevba, 2009]. The antimalarial potential of the plant has been evaluated [Builders et al, 2011]. A study conducted using decoction from the whole plant showed that it contains alkaloids, flavonoids, tannins, saponins, sterols, phenols and reducing sugars and a moderate antioxidant activity [Builders et al, 2011; Kunle et al, 2010]. The results of the phytochemical screening and antibacterial activities of *Vernonia ambigua*, *Vernonia blumeoides* and *Vernonia oocephala* (Astraceae) showed the presence of steroids/terpenes, saponins, flavonoids, alkaloids, tannins and glycosides. The antibacterial activity was concluded to probably be due to the presence of flavonoids, saponins or sesquiterpene lactones [Aliyu et al., 2011].

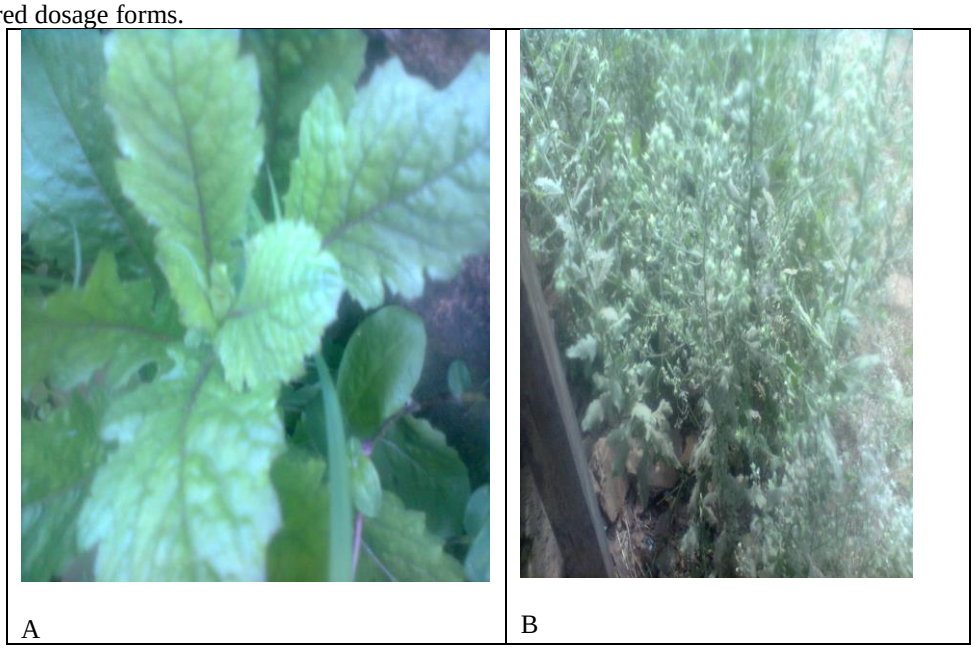

Fig. 1: Vernonia ambigua, (A) Young plant (B) Matured plant with flowers

METHODOLOGY

Materials

Emulsifying wax, white soft paraffin, liquid paraffin, absolute ethanol (May & Baker, Dagenham, England), nutrient agar (Titan Biotech, India), Sabourand Dextrose Broth (Titan Biotech, India), Mueller Hinton's agar (Titan Biotech, India), gentamicin (Yanzhou Xier Kangtai, Shandong, China) and ketoconazole (Hovid Bhd, Ipoh, Malaysia).

The test organisms used are *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli* (Gram-negative bacteria), *Pseudomonas aeruginosa* (Gram-

negative bacteria), *Candida albicans* (Fungi).

They were all obtained from the stock preparation of Pharmaceutical Microbiology Laboratory of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Delta State University, Abraka, Nigeria.

Collection and identification of plant material

Leaves of *Vernonia ambigua* were obtained from Enugu, Enugu State, Nigeria and dried under shade for one week. It was authenticated by Mr. Felix Nwafor, a taxonomist in the Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria. The plant was deposited in the department's herbarium and was assigned voucher number PCG/UNN/0319. The dried leaves were ground to fine powder using corona hand grinding mill.

Preparation of *Vernonia ambigua* leaves extracts

A 50 g quantity of the powdered dried leaves of *Vernonia ambigua* was weighed and transferred into a 1000 mL beaker. A 500 mL quantity of 70 % v/v ethanol was added and stirred properly. It was allowed to macerate for 48 h. The macerate was filtered using muslin cloth and the filtrate obtained was concentrated by heating at 70 ± 0.5°C in a water bath. The concentrated ethanol extract was stored in porcelain dish wrapped in aluminum foil. The percentage yield was determined.

Preparation of Agars and Overnight Broth

A 2.8% w/v freshly prepared nutrient agar sterilized at 121°C and 100 kPa for 15 minutes was poured respectively into three sterile bottles under aseptic condition and the bottles were slantly placed on a wooden rack to solidify. Upon solidification, *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* obtained from stock culture were inoculated on the respective agar. The agar bottles containing the organisms were then incubated at 37°C for 24 hours. Bottle inoculated with *Candida albicans* was incubated at 37°C for 72 hours.

Antimicrobial sensitivity of the extracts

This was done using the agar diffusion method. The prepared agar, Mueller Hinton (for bacteria) and Sabourand Dextrose (for fungi) were sterilized and then aseptically poured into eight Petri dishes to solidify. The respective organisms were spread on the surface of the solidified agar with the aid of sterile swab sticks. Six holes were bored into each of the agar plates with a 6 mm cork borer. The holes were aseptically filled respectively with 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL concentration of the ethanol extracts and standard drugs (40 mg/ mL of Gentamicin or 20 mg/mL of ketoconazole). Those inoculated with the bacteria were incubated at 37 ^{0}C for 24 hours, after which, zones of inhibition were measured. Those containing fungi were wrapped with aluminum foil and then kept under room temperature for 72 hours to grow. After 72 hours, zones of inhibition were then measured to the nearest millimeter along two axes 90° to each other and the mean of the two reading was calculated.

Determination of Minimum Inhibitory Concentration (MIC)

Petri dishes containing agar were demarcated into three equal parts with a marker and labeled *E. coli*, *P. aeruginosa* or *S. aureus* respectively. Mueller Hinton's agar was prepared by dissolving 7.6 g of the agar powder in 200 mL of water and sterilized. Two-fold dilutions of the ethanol extract of *Vernonia ambigua* was made and the concentrations obtained were 200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL respectively. One milliliter of each of the dilutions of the ethanol extract was put into respective Petri dishes and 19 mL of the prepared agar was poured into the respective plates containing the extracts. The plates were rocked to ensure uniform mixing and were allowed to set. Using a wire-loop, the plates were inoculated with overnight broth culture of the respective organisms, and were incubated at 37 °C for 24 h.

Preparation of emulsifying cream base

The emulsifying cream base was prepared according to the formula on Table 1. Nine grams of emulsifying wax was weighed and melted in a water bath at 60 °C ± 0.5 using a porcelain dish. Fifteen grams of white soft paraffin was added and also melted. Thereafter, 6.98 mL of liquid paraffin was incorporated into the melted-mix and stirred properly. The porcelain dish was removed from the water bath and with continuous stirring the content was allowed to solidify.

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

Ingredients	Formulae	Amount used
Emulsifying wax	300 g	9 g
White soft paraffin	500 g	15 g
Liquid paraffin	200 g	6 g (6.98 mL)

Preparation of *Vernonia ambigua* topical antimicrobial cream

The topical antimicrobial cream was prepared using the formula on Table 2. Six grams of the emulsifying

cream base was transferred into a porcelain dish. It was melted in a water bath at 60 ± 0.5 ^{0}C and the required amount of the ethanol extract of *Vernonia ambigua* was incorporated into it with continuous stirring until it was evenly mixed. Sufficient quantity of warm distilled water to produce 20 g of the topical creams was incorporated in portions into the mixture prior to stirring until a uniform blend was obtained, which was then allowed to cool before it was transferred into the cream jar and then labeled.

Table 2: Composition of Vernonia ambigua topical antimicrobial creams

Formulations	Ethanol Extract (%w/w)	Emulsifying ointment (g)	Distilled water to 20 g
BF1	4	6	Q.s
BF2	3	6	Q.s
BF3	2	6	Q.s

Physical Evaluation of the *Vernonia ambigua* Cream

Homogeneity: The herbal creams formulated were evaluated for homogeneity by visual inspection and were ranked as follows: Excellent = +++, Very Good = ++, Good = +, Poor = - (Sahoo et al, 2006; Ashish et al, 2013)

Determination of pH: The pH meter was calibrated using a standard buffer solution. About 2 g each of the formulations were collected in a beaker and then the pH was read using a model HI 2211 digital pH/ORP meter (Hanna Instruments).

Organoleptic Test: Organoleptic properties like appearance, texture, and odor were evaluated (Kandarp et al, 2014). They were determined using various sense organs such as eyes, nose, etc (Ashish et al, 2013).

Ease of Removal: This was determined by taking a finger tip unit of each of the creams and these units were applied consecutively on the skin. By washing the applied part with tap water, the creams were ranked according to their ease of removal as follows:

Excellent = +++, Very Good = ++, Good = +, Poor = - (Ashish et al, 2013).

Emolliency: A fingertip unit of the formulated creams was applied on the skin and checked for emolliency and greasiness.

Determination of Viscosity: The viscosity of the formulated creams was determined using a Brook-field viscometer with spindle #4 at speed 6.

Stability of the Formulations: Accelerated stability studies were carried out on the herbal creams by keeping them in a stability chamber at 45^0 C and 75 RH for 3 months. The parameters checked were pH, homogeneity and ease of removal at different time intervals (Sahoo et al., 2006).

Antimicrobial Susceptibility of the formulated creams

Zone of inhibition

The zone of inhibition of the test organisms by the formulated creams was determined using the disc diffusion method. Mueller Hinton agar was prepared and poured into a Petri dish and allowed to solidify. Test organisms from overnight broth were uniformly spread over the surface of the sterile agar plate with a sterile swab stick. Paper discs were smeared with respective formulations of the herbal cream and disc smeared with gentamicin served as control. The plates were then incubated at 37 ^{0}C for 24 h. The diameter of the zone of inhibition was measured. Clear zones of inhibition show susceptibility of the organisms, while absence of such zones indicated resistance.

RESULTS AND DISCUSSION

Percentage Yield of the Extract

The percentage yield of the ethanol extract obtained from powdered dried leaves of *Vernonia ambigua* was 15.38%w/w.

Antimicrobial sensitivity of the extracts

The result of the sensitivity pattern of the various *Vernonia ambigua* leave extracts on test organisms is shown in Table 3.

It showed that the ethanol extracts of *Vernonia ambigua* leaves at different concentrations were effective against test bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) but were ineffective against *Candida albicans*.

Higher zones of inhibition against all the test bacteria were produced by higher concentrations of the ethanol extracts. The zone of inhibition against *Pseudomonas aeruginosa* was the highest when compared to the

other test organisms at same concentration of the extracts.

The positive control (ketoconazole for the fungi and gentamicin for the bacteria) used for the sensitivity pattern determination gave higher zones of inhibition respectively compared to the ethanol extract. No zone of inhibition was observed with the negative control (distilled water).

Table 3: Zones of inhibition of the *Vernonia ambigua* extract on the test organisms

Drug	Concentration (mg/mL)	Zones of Inhibition For The Test Organisms (mm)			
		Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Candida albicans*
Ethanol Extract	100	5.00	4.00	10.00	NIL
	50	5.00	4.50	6.00	NIL
	25	3.50	4.00	5.00	NIL
	12.5	3.00	3.00	4.00	NIL
	6.25	2.00	2.50	2.50	NIL
Gentamicin	40	28.5	36.0	35.0	N.A.
Ketoconazole	20	N.A.	N.A.	N.A.	22.5

NIL = No zone of inhibition, N.A. = Not applicable

Table 4: Determination of Minimum Inhibitory Concentration (MIC in mg/mL) of ethanol extract of *Vernonia ambigua* leaves on different microorganisms

Concentration of Extract (mg/mL)	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
200	–	–	–
100	–	–	–
50	–	+	+
25	–	+	+
12.5	–	+	+
6.25	+	+	+

- indicates no growth, + indicates growth

Minimum inhibitory concentration

As shown in Table 4, the MIC of the *Vernonia ambigua* leaves extract against *Escherichia coli* and *Pseudomonas aeruginosa* was 100 mg/mL while that of *Staphylococcus aureus was* 12.5 mg/mL.

Physical Evaluation of antimicrobial cream

The result of the physical evaluation of the antimicrobial cream formulations is shown in Table 5.

Table 5: Physical Evaluation of the Antimicrobial cream Formulations

Formulations	BF1	BF2	BF3
Appearance	Black	Black	Black
Odour	Aromatic	Aromatic	Aromatic
pH	6.89	4.59	4.56
Viscosity (mPas)	18000	16500	51300
Homogeneity	++++	++++	++++
Ease of removal	++++	++++	++++
Emolliency	++++	++++	++++

Organoleptic properties: The different formulations of the antimicrobial cream (BFI to BF3) were black in

colour on visual examination. The black colouration was impacted by the black colour of the ethanol extract. Herbal creams unlike orthodox creams are usually coloured. The presence of colour (green, brown or black) reinforces the confidence of the patient that the cream is obtained from natural source. The creams had aromatic odour when subjected to olfactory examination. They all had smooth texture when felt between the thumb and index finger.

pH: The antimicrobial cream formulations had pH values that range from 4.56 to 6.89 which is slightly acidic. This indicates that they can be applied on the skin without having irritating effect since they are within the normal pH range of the skin surface (4.0 to 7.0). The fairly acidic nature of the skin helps it to ward off the advances of harmful bacteria and fungi [Lambers et al., 2006]

Homogeneity: The homogeneity of the creams was evaluated by visual inspection and they were ranked to be excellent.

Ease of removal: The creams were ranked as excellent in terms of ease of removal. They were easily washed off from the skin with tap water. Oil–in–water creams are easily washable because their continuous phase are composed of water and mix easily with water.

Emolliency: The formulated creams on application left no residue on the skin; thus were ranked as excellent. Oil-in-water creams are usually non-occlusive because they do not leave a continuous film of water-impervious liquids. They are said to be emollient creams when they deposit lipids and other moisturizers on and in the stratum corneum, thereby preventing the skin from drying out by restoring its hydration ability (Barry, 1999); Okorie and Ofoefule, 2002).

Viscosity: The viscosity of the various formulations of the antimicrobial creams was within 16500-51300 mPas (milliPascal second) which is an indication of their high viscosity.

Stability: The stability evaluation of the formulated creams showed that there were no changes in the physical appearance of the different cream formulations when evaluated after 3 months. The pH values for formulations BF1, BF2 and BF3 were 6.85, 4.60 and 4.54, respectively. The pH values were comparable to the values obtained when the creams were freshly prepared. The homogeneity and ease of removal were still ranked as excellent.

Antimicrobial susceptibility of the formulated creams: As shown in Table 6, all the antimicrobial cream formulations inhibited the growth of all the test organisms. The extent of inhibition was comparable to that of the respective extract shown on Table 4. The similarity between the zone of inhibition produced by the extracts and that of the cream formulations showed that the antimicrobial activity of the extract was not reduced when it was incorporated into the cream formulations. The excipients used in the cream formulation did not affect the extracts antimicrobial activity. It was observed that the zone of inhibition produced by the control (gentamicin) was higher than that produced by the cream formulations for all the test organisms. However, increasing the concentration of the ethanol extract in the cream may result in creams that are equipotent to the control.

Table 6 Zone of inhibition of the antimicrobial cream formulations against the test organisms

Formulations	**Zone of inhibition (mm)**		
	Escherichia coli	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
BF1	5.00	4.50	6.00
BF2	4.50	4.50	5.50
BF3	3.50	4.00	5.00
Gentamicin	27.5	34.5	36.0

CONCLUSION

It was determined from the study that the crude ethanol extract of the powdered dried leaves of *Vernonia ambigua* has antimicrobial property against *S.aureus, P. aeruginosa , E. coli* but not against *Candida albicans.* There was no loss of antimicrobial activity on formulation of the extract into creams. The creams were stable.

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