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Research Article

Preparation and Evaluation of Herbal Topical Antimicrobial Formulation of *Alchornea Cordifolia* (Euphorbiaceae) Hydro-Ethanol Leaf Extract

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ABSTRACT

Herbal medicine has been used in the treatment of many diseases including those superficial infections on the skin. *Alchornea cordifolia* is widely distributed in the tropical region of Africa and is used traditionally as a remedy for healing wounds and skin infections. This study was carried out to prepare acceptable topical semisolid formulations containing the hydro-alcohol leaf extract of Alchornea cordifolia. The dried leaves of *Alchornea cordifolia* was macerated in 50 % v/v hydro-ethanol for 72 h, the resulting dried extract (ACLE) was incorporated at concentrations of 5 and 10 %w/w into an ointment and a cream base to prepare ACLE ointments and ACLE oil in water creams. Organoleptic and physiochemical properties of the ACLE formulations were evaluated using standard procedures. In vitro antimicrobial activity of the prepared ointments and creams against selected microorganisms was also carried out using the agar dilution method. All the ointment and cream formulations exhibited good physicochemical properties, in addition, they were found to be physically stable after storage at room temperature for 30 days. Cream formulations containing 5 % ACLE showed strong inhibition against bacteria and fungi organisms.

INTRODUCTION

In Africa and Asian countries, 80 % of the population depend on herbal medicine for their basic health care [1]. Majority of the Nigerian population still rely on herbal medicine as their forefathers did to treat ailments and diseases [2, 3]. Medicinal plants have been reported to possess strong antimicrobial activity in addition to other activities for which they have been exploited [4]. Antimicrobials are the backbone for treatment of infectious diseases which is a leading cause of mortality worldwide but unfortunately, the challenge of increased development of resistance to the available conventional drugs is growing and stirring up concerns among clinicians and scientists worldwide [5]. The increase in multi-drug resistant microbial strains and the development of strains with reduced susceptibility to antibiotic actions has aroused great concerns in the field of medicine [6]. This has prompted scientists to search for novel antimicrobial agents capable of killing or inhibiting the activities of these microorganisms so as to ensure improved health outcomes.

Natural compounds have been used to cure infectious diseases long before the existence of microorganisms [7]. Plants play a major role in medicine as they serve as alternative sources of drugs and in some cases their active constituents have been exploited for production of drugs [8]. Furthermore the practice of herbal medicine has become evolutionary with the advent of techniques that improve the outcome and efficacy of the herbal product [9].

Medicinal plants contain constituents like tannins, flavonoids, saponins that are responsible for their activity against wide range of microorganisms. Superficial infections like those on the skin can be treated with topical preparations like antimicrobial creams, gels, pastes or ointments which act locally at the affected site. Creams are viscous emulsions containing an active ingredient and other

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excipients and are intended for use as cosmetics or for treatment of a particular infection. Ointment is a viscous preparation of oil and/or fat (usually containing an active medicament) used for treatment or as an emollient.

The plant *Alchornea cordifolia* is an evergreen shrub from the family of euphorbiaceae, it is commonly distributed throughout tropical region of Africa and widely used in African traditional medicine and many superstitious attributes have been accorded to it. It is cultivated in many countries for its medicinal value. The plant is commonly called "Christmas bush" because its flower and fruits come out during December and is also known as Dovewood. In Nigeria, it is called "*ububo*" by the Igbo tribe, "*ipaesinyin*" by the Yoruba and "*banbani*" by the Hausa tribe [10]. It is a multipurpose plant used as fodder, food and medicine.

The plant *A. cordifolia* is utilized in African traditional medicine in the treatment of worm infestations, as an anti-inflammatory, antibacterial and antifungal agent. Although all the parts of the plant are useful, the leaves stand out as being the most used probably due to the constituents it contains. The leaves and the leafy stem when taken in large doses are believed to have abortificient, antispasmodic, blood purifier, diuretic and emetic properties, in some cases, they have also been used to treat worms and canker sores [11, 12, 13]. The leaves are taken as infusion or chewed raw as local remedies in the treatment of respiratory problems including sore throat, cough, bronchitis and pneumonia [14, 15]. Other activities related to the leaves include; its use as an antidote for poison, as a sedative and antispasmodic, it is also used for treating anaemia as well as epilepsy. The leaves and fruit juice rubbed onto the skin have been used to treat ringworm [16] while the dried powdered leaves applied externally to wounds have promoted wound healing and treated filariasis [17].

Studies have been carried out to investigate these folklore beliefs [18, 19, 20] and their reports show that the presence of tannins, flavonoids, phenols, steroids and alkaloids in *A. Cordifolia* leaf extracts were responsible for its inhibitory effect on pathogenic fungi. The study by Ebenyi *et al* [10] demonstrated that the aqueous extract of *A. cordifolia* was bactericidal on some organisms (*Klebsiella, Staphylococcus, Pseudomonas* and *Streptococcus*) and bacterio static on *E. coli.* Another study reported the extent of antimicrobial activity of different extracts of the plant leaves (acetone, aqueous, ethanol, methanol extracts) against selected organisms [21]. A separate study revealed that the aqueous and ethanol leaf extract of A. Cordifolia were active against *H. pylori* and four other bacteria implicated in causing diarrhoea [22]. Similarly, Adeshina *et al* [23] and Mohammed *et al* [24] reported substantial activity of the aqueous leaf extract against *S. Aureus* isolated from wound samples and faecal materials respectively. However, the ethyl acetate fraction of *A. Cordifolia* leaf extract was reported to be more active than the aqueous extract against clinical isolates of *E. coli*, *S. aureus*, *P. Aeruginosa* and *Candida albican* [23]. Dermatological cream preparations of the aqueous leaf extract of *A. cordifolia* (5 % w/w) have been shown to facilitate wound healing [25]. *In vitro* antimicrobial studies of the ethanol leaf extract showed marked inhibition of *S. Aureus* compared to ciprofloxacin which was used as the control [26]. In the same study, ointment preparations of the extract containing hydrous lanolin were found to be active against only *P. Aeruginosa* while those containing white ointment and lanolin bases were not active against any of the organisms tested.

The purpose of this study is to prepare cream and ointment formulations of the hydro-ethanol extract of the leaves of *A. cordifolia*, to evaluate the physicochemical properties and antimicrobial activity of the prepared formulations against selected organisms (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Trichophyton rubrum*, *Candida albicans*, *Staphylococcus parathyphi*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*).

MATERIALS AND METHODS

Materials

Triethanolamine (Central Drug House Corp, New Delhi, India), Benzyl alcohol (Kermel), Stearic acid (Fissons Lab, England), Glycerine (Guangdang Couanghua Chemical Factory co. LTD, China), Cetyl alcohol (Sigma, Germany), Liquid paraffin, Emulsifying wax (Fisher Chemicals, USA), White soft paraffin (Fisher Chemicals, USA), Mueller Hinton agar (Titan Biotech LTD, India). Nutrient agar, Nutrient broth (Sigma-Aldrich, St. Louis, USA), Tryptic Soy Agar, Tryptic Soy Broth, Potato Dextrose Agar, Potato Dextrose Broth (Sigma-Aldrich, St. Louis, USA).

Microorganisms

Staphylococcus aureus ATCC 25923, *Pseudomonas. aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 10798), *Salmonella paratyphi* ATCC 9150, *Trichophyton rubrum* ATCC 28188 and clinical isolates of *Bacillus subtilis*, *Candida albicans*, *Streptococcus pyogenes, Klebsiella pneumonia* from the Diagnostic Laboratory of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria.

Collection, Identification and Preparation of the Crude Leaves

The leaves of the plant *Alchornea cordifolia* were obtained from the botanical garden of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The plant was authenticated in the institute's herbarium by Mallam Muazam and assigned a voucher specimen number (NIPRD/H/7002). The leaves were air-dried at room temperature, pulverised using the mortar and pestle then passed through a sieve (250 µm mesh size); this was packaged in an air-tight container and stored in the desiccator until further use.

Preparation of the Hydro-Alcohol Leaf Extract

Three hundred grams (300 g) of the powdered leaf was placed in a container and macerated with 50 % v/v hydro-ethanol (1:8) for 72 h with intermittent stirring. The filtrate was removed from the marc and concentrated over the water bath (Karl Kobb, Dreieich, West Germany) at 40 ˚C; the yield (%) of the dried extract (ACLE) was calculated, then the extract was packaged appropriately and stored in a desiccator until required.

Preparation of Ointment Formulation

Emulsifying ointment (BP) was used as the base for preparing these ointments. Ointments containing the base and ACLE were prepared according to the formula in Table 1. Appropriate amount of the base was placed on a tile, and then weighed amounts of ACLE was incorporated into the base in geometric portions until all the extract was exhausted and a homogenous mixture was obtained. The prepared ointment was packaged into an ointment jar and stored until further use. This procedure was used for all the ointment formulations as presented in Table 1.

Preparation of Cream Formulation

Oil in water (o/w) cream formulation was prepared using the stated formula in Table 1. The oily portion was prepared by mixing appropriate quantities of stearic acid, cetyl alcohol and liquid paraffin in a beaker by stirring continuously over the water bath at 70 °C. The aqueous phase was prepared by dissolving the water soluble ingredients (glycerol, triethanolamine, benzyl alcohol and ACLE) in water and heating the mixture to 75 °C. The oily phase was incorporated into the aqueous phase while continuously stirring until a smooth, homogenous mixture was obtained. The prepared creams were packaged in appropriate containers and stored until required.

Table 1: Composition of *Alchornea cordifolia* leaf extract (ACLE) ointments and creams

Ingredients/Batch	O1	O2	O3	C1	C2	C3
ACLE (g)	-	1.5	3.0	-	1.5	3.0
Stearic acid (g)	-	-	-	3	3	3
Cetyl alcohol (g)	-	-	-	1.5	1.5	1.5
Liquid paraffin (mL)	-	-	-	2.4	2.4	2.4
Glycerin (mL)	-	-	-	1.5	1.5	1.5
Triethanolamine (mL)	-	-	-	0.6	0.6	0.6
Benzyl alcohol (mL)	-	-	-	0.6	0.6	0.6
Emulsifying ointment (g)	30	28.5	27	-	-	-
Water (mL)	-	-	-	20.4	18.9	17.4

Key: O1, O2 and O3 = ointment preparations containing 0, 5 and 10 % w/w ACLE respectively.
C1, C2 and C3 = cream preparations containing 0, 5 and 10 % w/w ACLE respectively.

Evaluation of ACLE Ointment and Cream Preparations

Organoleptic Properties

The organoleptic characteristics such as colour and odour of the ointment and cream preparations were evaluated. Other physical parameters like ease of application and removal, texture, skin irritancy and homogeneity were also evaluated.

Determination of pH

The pH of the preparations (1 % w/v) was measured using the digital pH meter (Denver pH meter), three determinations were recorded and the average calculated.

Determination of Spreadability

The preparations (1 g) were placed in between two glass slides and compressed to uniform thickness by placing a known weight (5 g) on the slides. The time taken to separate the upper slide from the lower slide was recorded and spreadability (S) of the preparations was calculated as;

$$S = M \, X \, {}^{L}/_{T} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where,
M = weight placed unto the upper slide,
L = Length of the glass slide,
T = Time taken to separate the two slides

Determination of Extrudability

This was carried by placing a known weight of cream/ointment formulation (a) in an empty syringe which was extruded at a rate of 1 press/sec. The weight of cream/ointment formulation (b) extruded from the syringe was noted and extrudability was expressed in percentage.

Physical Stability Test

The presence or absence of physical incompatibility between the ingredients in the preparations was assessed visually and the observations were recorded. The preparations were also assessed for the presence or absence of microbial growth after being storage at room temperature for 30 days.

Antimicrobial assay of ACLE ointment and cream preparations

The inoculums were prepared by culturing bacteria isolates on Mueller Hinton agar (MHA) plates at 37 °C for 24 h while fungi isolates were cultured on Sabouraud dextrose agar (SDA) plate at 37 °C for 48 h. The agar dilution method as described by Babayi *et al* [27] was modified and adopted for screening the antimicrobial activity of the formulations. The ointments and creams (1 g) were dissolved in hot Muller Hinton agar and Sabouraud Dextrose agar for bacteria and fungi respectively, swirled to effect adequate mixing, poured into sterile petri dishes and allowed to cool. Sterile filter paper discs were placed equidistantly on the surface of the agar and 10 µL of the standardized culture of test organisms was dispensed on the discs and incubated at 37°C for 24 h (bacteria) and 25 – 30 °C for 48 h (fungi) after which the plates were assessed for growth. Control plates comprising inoculums without extract were made. Terbinafine HCl and chloramphenicol were used as standard drugs.

RESULTS AND DISCUSSION

The percentage yield of the *Alchornea cordifolia* hydro-ethanol leaf extract (ACLE) was calculated as 21.07 % which is significantly higher than 7 % and 8 % previously reported for ethanol and aqueous leaf extract respectively [28] but slightly lower than 28.75 % reported for ethanol leaf extract [26]. Variations could be due to differences in geographical location and time of collection, in addition, the method of extraction and solvent used for extraction can be factors responsible for the differences observed [29].

The organoleptic and physical properties of ointments and creams prepared with ACLE are presented in Table 2. The colour of the prepared ointments (O2 and O3) was found to be white to grey while the creams (C2 and C3) were brown to dark brown. The progressive increase in colour intensity of the preparations is attributed to the increasing concentration of the extract in the preparations. Formulations O1 and C1 which contained no extracts were white due to the colour of the base used in the preparation. The preparations; O1, O2 and C1 were odourless while O3, C2 and C3 were fruity to leafy due to the presence of extract in them.

Table 2: Properties of *Alchornea cordifolia* leaf extract (ACLE) ointments and creams

Properties	O1	O2	O3	C1	C2	C3
Colour	white	Ash	Grey	white	brown	dark brown
Odour	odourless	Odourless	Fruity	odourless	leafy	Leafy
Texture	smooth	Gritty	Gritty	smooth	smooth	Smooth
Homogeneity	+++	+++	+++	+++	+++	
Ease of application	+++	+++	+++	+++	+++	+++
Washability	+	+	+	+++	+++	+++
Irritancy	-	-	-	-	-	-
Presence of growth	-	-	-	-	-	-
Physical incompatibility	-	-	-	-	-	-

Key: O1, O2 and O3 = ointment preparations containing 0, 5 and 10 %w/w ACLE respectively, C1, C2 and C3 = cream preparations containing 0, 5 and 10 %w/w ACLE respectively. +++ =easily applied, washable and homogenous, + = not easily washable, - = non-irritant, no visible growth, no physical incompatibility.

All the prepared creams and ointments had smooth texture and were homogenous, this shows that there was uniform distribution of ingredients and good consistency which could be attributed to the method and expertise employed during the preparation. This implies that there would be easy spreadability, penetration and accurate dose would be dispensed when the ointments are applied.

All the cream preparations (C1, C2 and C3) had smooth appearance while ointments containing ACLE were gritty however; they were all easily applied unto the skin and also very easy to wash off the skin because the base used in their formulation is aqueous-based. The ointments (O1, O2 and O3) on the other hand were greasy and more difficult to wash off the skin under running water without addition of friction.

The ointment and cream preparations did not produce any visible sign of irritation like redness, soreness or allergic sensitization when applied unto the skin which indicates they are safe for topical application. In addition, none of the formulations showed signs of physical incompatibility or growth upon storage at room temperature (29 °C) for 4 weeks.

The efficiency of any semi-solid dermatological preparation is based upon its ability to be spread unto the skin and penetrate through the dermis of the skin. This spreadability portrays the extent of dispersion of the formulation on the skin, accuracy of delivering the appropriate dose to the affected area and ultimately the bioavailability of the medication [30]. All the prepared ointments and creams were found to be easily spread showing the ease with which the formulations can be applied with or without friction and the consequent rapid release of the active ingredient from the formulation.

Extrudability test shows the ease with which a formulation can be released from its final container upon application of force. The formulation is required to be pressed-out easily while not being expelled too rapidly to prevent loss and wastage. This factor indicates how assessable the formulation is to the user which is also a pointer towards improving compliance. As presented in Table 3, the prepared ointments and creams were found to have % extrudability between 80.3 and 94.2, showing that all the formulations were easily expelled from the tube.

The pH of the preparations, are presented in Table 4. Ointment formulations O2 and O3 were observed to have pH of 7.26 and 6.07 respectively while the creams; C2 and C3 had near similar pH between 7.53 and 7.05 respectively. Preparations with no extract (O1 and C1) were observed to have neutral pH, as a result the observed pH for the cream (C2 and C3) and ointment formulations (O2 and O3) can be attributed to the presence of the extract. The values were all near neutral which shows that the formulations are compatible with the skin and corroborates the irritability test results already presented.

Table 3: Extrudability and spreadability of ointments and creams

Batch	Extrudability (%)	Spreadability
O1	89.1	1.08
O2	87.4	1.71
O3	86.7	1.11
C1	94.2	3.81
C2	85.3	4.59
C3	80.3	6.35

Table 4: pH of the prepared ointments and creams

Preparations	pH
O1	7.66 ± 0.00
O2	7.26 ± 0.01
O3	6.07 ± 0.01
C1	7.53 ± 0.04
C2	7.53 ± 0.02
C3	7.05 ± 0.02

Microbiological Assessment of ACLE Ointment and Cream Preparations against Selected Microorganisms

The results show that the antibacterial efficacy of ACLE was best demonstrated in the cream formulations than the ointment formulations (Table 5) with the later showing greater inhibition on the tested organisms than the ointment formulations. The inability of the bioactive extract to diffuse out of the ointment formulations may be attributed to the oily base used which prevented the release of the extract from the formulation. Oleaginous bases as used in this study is known to be occlusive thereby leading to possible low/poor drug release while oil in water cream formulations allow for easier drug release [31]. Another study has also demonstrated the negative effect of oily formulation bases on extract release as observed in this study [26].

Table 5: Antimicrobial activity of the formulated ointments and creams on selected microorganisms

Organisms/ Batches	O1	O2	O3	C1	C2	C3	Control
S. aureus	-	±	±	-	+	±	-
E. coli	-	+	+	-	±	-	-
P. aeruginosa	-	+	-	-	+	±	-
S. paratyphi	-	-	-	-	+	+	-
K. pneumonia	-	+	+	-	+	+	-
B. subtilis	-	-	-	-	±	±	-
T. rubrum	-	-	-	-	+	-	-
C. albicans	-	-	-	-	+	-	-

Key: - = inactive; + = strongly active; ± = weakly active

However, ointment formulations (O2 and O3) showed strong active against *E. Coli* and *K. pneumonia* while being weakly active against *S. aureus*. The cream formulations (C3) was observed to be active against only the bacteria microorganism whereas, formulation C2 showed strong inhibitory activity against the eight organisms commonly responsible for human infections. Interestingly, C2 showed strong inhibition of fungi growth portraying its propensity to be used in fungal infections. These results however show that the cream formulations at lower concentration (5 %; C2) showed stronger overall inhibition over the higher concentration (10 %; C3). This could be due to poor solubility of the higher concentration in the medium thus limiting the extent of diffusion out of the medium.

CONCLUSION

The hydro-alcohol extract of the leaves of *Alchornea cordifolia* (ACLE) has been successfully incorporated into ointment and cream formulations. The cream formulation containing 5 % w/w ACLE has demonstrated good *in vitro* anti-bacterial and anti-fungi effect against some microorganisms implicated in dermatological infections.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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