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Studies on the antimicrobial properties of formulated creams and ointments containing *Baphia nitida* heartwood extract

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Abstract

Baphia nitida Lodd (camwood) is widely used medicinal plant in Nigeria for treatment of variety of skin infections. The plant is used in unspecified quantities without standardisation. *B. nitida* extracts were incorporated in some cream and ointment base in order to present the medicinal plant in an elegant and pharmaceutically acceptable dosage form, as one of the major steps in the scientific evaluation and standardisation of the plant product. The formulated products were evaluated for their antimicrobial activities using agar diffusion technique and microbial challenge test. Their performances were compared with those of standard antiseptic creams and ointments. The results of agar diffusion studies on cream and ointment formulations revealed that the topical bases used to disperse the medicaments could significantly affect the antimicrobial effectiveness of the formulation. Formulations prepared with Aqueous cream BP (a more hydrophilic base; 70% aqueous) exhibited greater antimicrobial activity than the formulation with anionic cream base (a less hydrophilic base; 44% aqueous). Antiseptic creams and ointments containing *B. nitida* extract, which were effective against various test organisms used in this study, were successful formulated in this work.

Key words: *Baphia nitida;* Antimicrobial; Antiseptics; Creams; Ointments.

Introduction

Baphia nitida Lodd (camwood) is a shrub or small tree of the leguminous family widely distributed in several parts of evergreen forest, deciduous and secondary forests; and in coastal parts of the West African sub–region (Dalziel, 1958). *Baphia nitida* has been reported to be useful in the treatment of microbial skin infection (Dalziel, 1958). It has also been incorporated in cosmetic preparations to give the skin a smooth and fine appearance (Kafaru, 1994).

The lack of standardization of dosage of many plant drugs is one of the major problems associated with their use in many traditional settings. This problem could lead to wide variations in the content of the active ingredient(s) of traditional medicinal remedies; therapeutic failure, toxicity, emergence of drug resistance and many other deleterious effects on consumers. Another

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problem with the use of traditional medicinal remedies is non-adherence to Good Manufacturing Practice (GMP) during their formulations. Frequently, herbal formulations, due to non-adherence to GMP, are associated with alterations in the physical characteristics, stability and potency of the medicament (Olowosulu, 2001; Olowosulu and Ishaku, 2005). The formulation of phytomedicine into appropriate dosage form is one of the main ways of addressing the problems of standardization of dosage and ensuring adherence to good manufacturing practice in their production. This will, undoubtedly, ensure that herbal medicines are presented in more acceptable pharmaceutically elegant dosage forms, which are convenient for use and are cost-effective. This is also a major step in the scientific evaluation and standardization of phytomedicines.

Skin infections are widely distributed in developing countries especially among children. In most cases, the available remedies are formulated using synthetic antimicrobial agents that have often been reported to elicit allergic and other dermatophytic skin reactions. The antimicrobial property of heartwood extract of *Baphia nitida* has been established (Olowosulu, 2001; Olowosulu and Ishaku, 2005; Olowosulu and Ibrahim, 2005). In these studies, the heartwood extract of *B. nitida* has been shown to be effective on various microorganisms at concentration of 5%-10%w/w. *Baphia nitida* is widely used in Nigerian ethno-medicine in unspecified quantities in relatively crude form, which, in most cases, are pharmaceutically unacceptable. It is therefore the objective of this research to formulate *B. nitida* extract as a topical antimicrobial dosage form for use in the treatment of skin infections. The formulation into topical dosage form (cream & ointment) was informed by their traditional application in the treatment of skin infections.

This paper examines the potential usefulness of *B. nitida* extract cream and ointment formulations in the treatment of skin infections.

Experimental

Preparation of extracts. The plant *Baphia nitida* was collected from the Forestry Reserve of the Ministry of Agriculture, Kabba, Kogi State. The plant was authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria (voucher specimen number 728). The heartwood of the plant was chopped into small pieces and thereafter pulverized in a domestic grinding mill. 100 g of the powdered mass was extracted with 1000 ml of acetone in a Soxhlet apparatus for 12 hours. The extract obtained was concentrated to a semi-solid mass under reduced pressure in a rotary evaporator (Buchi, Germany). This semi–solid extract was subsequently dried in a beaker on a water bath to give a dark red resinous mass (dry extract). The dry extract of the plant *Baphia nitida* has been previously screened and found to possess antimicrobial property against a variety of microorganisms (Olowosulu, 2001; Olowosulu and Ishaku, 2005; Olowosulu and Ibrahim, 2005).

Preparation of medicated cream and ointment Ten (10) gram each of creams or ointments containing different concentrations of *B. nitida* extract (BNE) were prepared using suitable cream and ointment bases which were earlier determined not to adversely affect the antimicrobial activities of the plant extracts (Table 1 and Table 2). The resulting products were subsequently distributed into sterile glass containers. *B. nitida* extract was incorporated into the cream and the ointment bases by trituration method (Collett and Aulton, 1990). The dry extract was incorporated at concentration of 5% and 10% w/w as was previously determined to be effective in exerting antimicrobial activity on

the various test organisms (Olowosulu, 2001; Olowosulu and Ishaku, 2005; Olowosulu and Ibrahim, 2005).

Preparation and standardization of test organism cultures. Cultures of the test organisms were obtained from the collections of Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria. The bacterial test organisms (*Bacillus subtilis* NCTC 8236, *Staphylococcus aureus* NCTC 6571, *Pseudomonas aeruginosa* NCTC 6570 and *Escherichia coli* NCTC 10418) were sub-cultured on Nutrient Agar (NA – Merck, Germany) and incubated at 37°C for 24 hours. The fungal organisms (*Candida albicans* and *Trichophyton mentagrophytes*) were subcultured on Sabouraud Dextrose Agar (SDA, Oxoid, England) and incubated at 30°C for 48 hours. The over night cultures of the test bacterial organisms and 48 hour cultures of *C. albicans* were prepared in the appropriate media and standardized to contain approximately 10^8 CFU ml^{-1} for the test organisms. However, for *T. mentagrophytes* fungal spores were harvested from 21 day old SDA slant cultures by washing with 10 ml sterile normal saline containing 2% w/v Tween 80 with aid of sterile glass beads to help in dispersing the spores (Olonitola *et al.*, 2000). Thereafter, the spore suspension was standardized to 106 spores/ml using sterile normal saline containing 2% Tween 80 and used for agar diffusion studies. All cultures were checked for purity and maintained on Nutrient agar (NA) for bacterial and Sabouraud dextrose agar (SDA) for the fungal test organisms respectively at 4° C (in the refrigerator) until required for use.

(i) Test for microbiostatic activity. The agar-well diffusion method was used in this determination. About 0.2 ml of the standardized microbial suspensions of test organisms were mixed with 20 ml of the appropriate molten agar (40°C) to give a population density of 10^6 CFU ml^{-1}. The seeded agar was aseptically poured into sterile Petri dishes and allowed to solidify. However, for *T. mentagrophytes* 1 ml of spore suspension containing approximately 10^6 spores ml^{-1} was poured on the surface over dried agar. Thereafter, six holes (cups) measuring 8.0 mm in diameter were bored into the over-dried agar using a sterile cork borer. 0.1 g of the test creams or ointments were filled into the cups using sterile spatulas. A pre–incubation diffusion time of 2 hours at room temperature was allowed. Thereafter, the plates were incubated at 37°C for 24 hours and 30°C for 48 hours for bacteria and *C. albicans* respectively and up to 14 days at 300 C for *T. mentagrophytes*. The resulting diameters of zones of inhibition were measured in millimetres after incubation. The test was carried out in triplicates.

(ii) Challenge test. Ten (10) g each of the creams or ointment containing 10%w/w BNE was inoculated with 0.1 ml of overnight cultures of the test bacterial suspensions of *S. aureus*, *Ps. aeruginosa* standardized to contain approximately 10^8 c.f.u. ml^{-1} and 0.1 ml of spore suspension of *T. mentagrophytes* containing approximately 10^8 spores ml^{-1}. The creams or ointments were properly mixed with test organisms using sterile stirring rod to ensure even distribution of test organisms. Thereafter, 0.1 ml of the microbial suspensions was added to 10 ml of sterile normal saline to serve as a control count of number of organisms added to the creams or ointments. Approximately, 0.1 g quantities of the creams or ointments were removed at different time intervals (0 min-120 min). Each withdrawn sample was carefully dispersed and then serially diluted with sterile normal saline containing 2% Tween 80. About 0.2 ml aliquots of the final dilution were spread on triplicate plates. The colony forming units on each plate was counted and from this, the total number of viable organism per gram in the original cream was computed. Plots of

survival of the organisms against time in the formulated creams were then made.

Results and Discussion

The diameters of zones of inhibition obtained for the different cream and ointment formulations using the agar-well diffusion method is shown in Tables 3 and 4. The release of *B. nitida* extracts from the topical bases is indicated as zones of inhibition. The sizes of the zones of inhibition are indicative of the level of the antimicrobial activities of the extracts. The diameters of the zones of inhibition were generally larger for the Gram-positive test bacteria compared with values obtained for the Gram-negative bacteria and fungal test organisms. This indicates that the Gram-positive organisms (*B. subtilis and S. aureus*) were more susceptible to the effect of the extracts in the formulations. This may be due to the inherent resistance of Gram-negative bacteria to a large number of chemical antimicrobial agents and antibiotics that are otherwise effective against Gram-positive organism (Hugo and Russell, 1996). This resistance of Gram- negative bacteria has been attributed to lipopolysaccharide layer on their cell wall (Hugo and Russell, 1996). The antimicrobial activity of BNE may be attributed to the flavonoid content of the extract detected from earlier phytochemical studies on the plant (Olowosulu, 2001; Arnone *et al.*, 1981; Banerjee and Mukherjee, 1981). A large number of flavonoids have been reported to possess antimicrobial property (Cowan, 1999; Tsuchiya *et al.*, 1996).

The result in Table 3 also showed that the cream base used to disperse BNE could affect the antimicrobial activity of the incorporated extracts. For example BNE cream formulated with an aqueous cream base (containing 70% aqueous component) exerted greater activity than that formulated with an anionic cream base (containing 44% aqueous component). Hydrophilic cream bases were used for the formulation of antiseptic creams because such bases are known to increase or enhance the release of sparingly soluble medicaments like BNE into a more hydrophobic matrix (Odusote and Mendie, 1992). The release of sparingly soluble medicament from hydrophilic bases may be due to the low affinity of the extract for the cream base thereby enhancing their release for non polar (hydrophobic) matrix (Odusote and Mendie, 1992).

Table 1: Formulation of creams containing *Baphia nitida* extract (BNE).

Ingredients	Weight of ingredients in formulation (% $^{w}/_{w}$)						
	A	B	C	D	E	F	G
Baphia nitida extract	5	5	10	10	-	-	-
Cetrimide*	-	-	-	-	0.5	-	-
Aqueous cream base (Formulations I)	95	-	90	-	95.5	100	-
Anionic cream base (Formulations II)	-	95	-	90	-	-	100

* Cetrimide cream is used as standard antiseptic cream.

Table 2: Formulation of ointments containing *Baphia nitida* extract (BNE)

Ingredients	Weight of ingredients in formulation (% $^{w}/_{w}$)			
	H	I	J	K
BNE	5	10	-	-
Benzoic acid*	-	-	6	-
Emulsifying Ointment BP	95	90	94	100

*Benzoic acid ointment is used as a standard antiseptic ointment

Table3: Antimicrobial susceptibility profiles of BNE in formulated creams against the test microorganisms

Formulated cream	Inhibition zone diameters in mm					
	S. aureus	*B. subtilis*	*Ps. aeruginosa*	*E. coli*	*C. albicans*	*T. mentagrophytes*
5 % $^{w}/_{w}$ BNE aqueous	15.1 ± 1.6	19.0 ±1.0	12.0 ±1.0	10.0 ± 0	10.0 ± 0	8.0 ± 0
5 % $^{w}/_{w}$ BNE anionic	12.3 ± 0.7	14.3 ±0.3	8.0 ± 0	8.0 ± 0	10.0 ± 0.5	8.0 ± 0
10 % $^{w}/_{w}$ BNE aqueous	16.1 ± 2.5	20.7 ± 0.6	14.2 ± 1.6	13.2 ±1.6	14.1 ± 1.1	11.0 ±1.0
10 % $^{w}/_{w}$ BNE anionic	14.0 ±1.0	15.5 ± 0.3	14.0 ± 0.5	11.0 ± 1.0	11.0 ±1.0	10.0 ±0
0.5 % $^{w}/_{w}$ Cetrimide BP	20.5 ±1.0	25.2 ± 0.5	15.0 ±1.0	17.7 ± 1.7	15.3 ± 1.5	13.3 ±0.6

Aqueous cream: a more hydrophilic base, 70% aqueous.; Anionic cream: a less hydrophilic base, 44% aqueous.
Cork borer diameter 8.0mm ; Control: Aqueous cream base and anionic cream base showed no zones of inhibition

Table 4: Antimicrobial susceptibility profiles of formulated ointments against the test microorganisms.

Formulated ointment	Inhibition zone diameters in mm					
	S. aureus	*B. subtilis*	*Ps. aeruginosa*	*E. coli*	*C. albicans*	*T. mentagrophytes*
5% BNE ointment	17.7 ± 1.1	17.7 ± 0.6	17.3 ± 0.6	15.3 ± 0.6	10.0 ±1.0	10.0 ± 1.0
10% BNE ointment	18.3 ±1.6	19.0 ±1.0	17.1 ± 0.5	16.0 ±1.0	12.0 ±1.0	10.0 ± 0
Benzoic acid ointment BP	24.0 ±2.6	25.5 ±1.7	15.1 ±1.1	22.7 ± 0.6	13.0 ±1.0	11.0 ±1.0

Cork borer diameter: 8.0mm; Control: Ointment base: Emulsifying Ointment BPC showed no zones of inhibition.

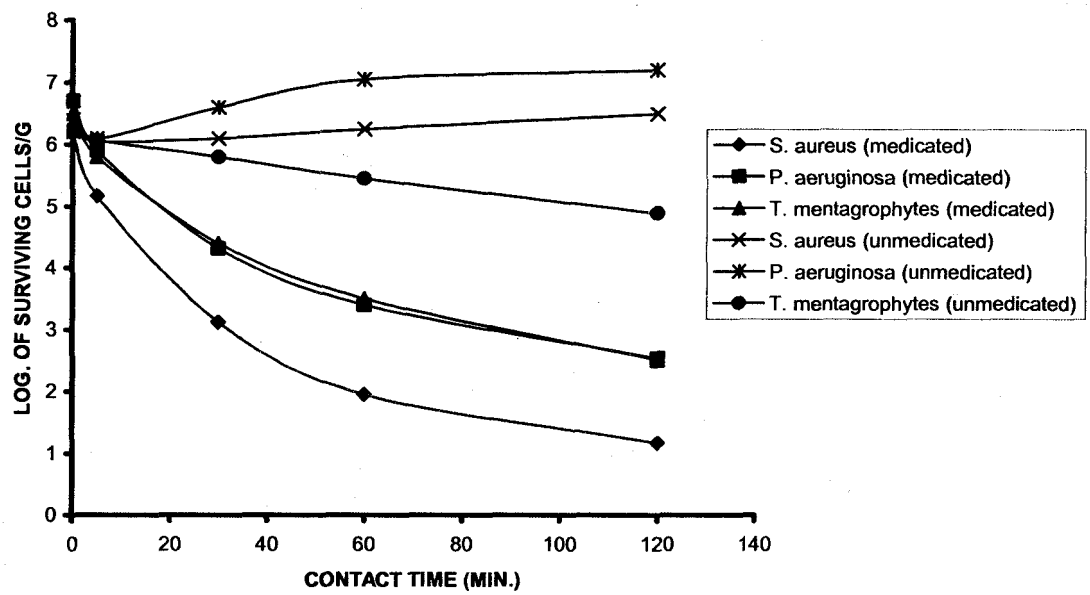

Fig 1: Reduction in the viability of test microorganisms in the cream base and Baphia cream

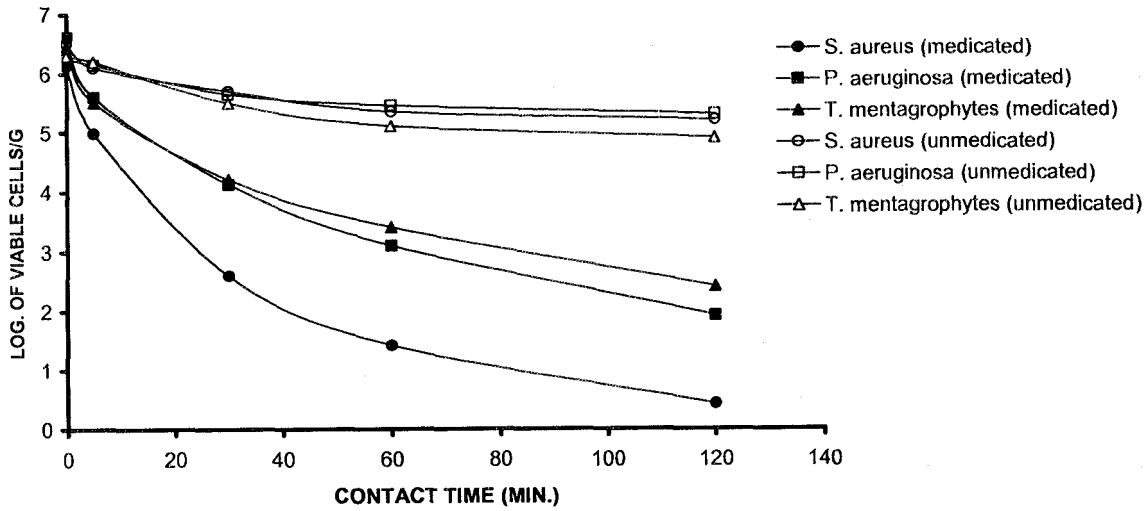

Fig 2: Reduction in the viability of test microorganisms in ointment base and 10% $^{w}/_{w}$ Baphia ointment

Generally, cream and ointment bases used in formulation of topical medicaments could cause reduction in antimicrobial efficacy of the incorporated medicaments. This could be attributed to the reduction in the amount of freely available medicaments; some proportion of incorporated drugs had probably complexed with or bound with the different phases of the cream or solubilised in the oily environment of the ointment. It should be noted that it is only freely available unbound drug portions that will diffuse and exert antimicrobial action. The reduction in the viability of the test organisms as the contact time increases in the unmedicated cream and ointment bases as well as in the medicated preparations is shown in (Fig 1 and 2). The results obtained showed that the formulated creams and ointments containing *Baphia nitida* extract exerted significant microbiocidal activities on the test organisms and a marked reduction in their viability when compared with the unmedicated preparations. This may be due to the antimicrobial property exhibited by the incorporated BNE.

In conclusion, the incorporated BNE was sufficiently released from the topical bases to exert significant antimicrobial activity against the various test organisms used in this study. This indicates that *B. nitida* heartwood extract can be successfully formulated as antiseptic creams and ointments in the treatment of microbial skin infections. This is a first step in the scientific evaluation and standardization of the plant as skin antiseptics.

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