In vitro Antibacterial Activity of the Ethanolic Extract of C*hrysophyllum albidum* Cotyledon and its Formulation into a Topical Cream for the Treatment of Dermatological Infections

S.F. USIFOH, F.E. EICHIE AND J.O. AGBONKONKON

[1]*Department of Clinical Pharmacy and Pharmacy Practice, University of Benin, Benin City, Nigeria*
[2]*Department of Pharmaceutics and Pharmaceutical Technology, University of Benin, Benin City, Nigeria*

Minimum inhibitory concentrations (MIC) and antibacterial activities of the ethanolic extract of *Chrysophyllum albidum* cotyledon and its cream on dermatological infection causing agents was investigated using agar diffusion method with gentamicin as the positive control. Zones of inhibition diameters were: 11.8 ± 0.2 mm (*Pseudomonas* a*eruginosa)*, 11.3 ± 0.5 mm (*Staphylococcus aureus*) and 11.3±0.5mm *(Escherichia coli)*. The cream formulation of the extract displayed related MIC ≥ 11.7 ± 0.3 mm at higher concentrations. Gentamicin (80µg/ml) displayed 11.5 ± 0.5 mm for *P. aeruginosa* and at 40 µg/ml a higher value 16.3 ± 0.5 mm for *S. aureus* and *E. coli* 26.3 ± 3.3 mm. Formulated cream of extract had antibacterial activity in the at 150 - 250 mg/ml range with zone of inhibition diameter values for *E. coli* (11.7 ± 0.9 mm), *S. aureus* (11.8 ± 0.3 mm) and *P. aeruginosa* (17.5 ± 0.5 mm). Gentamicin cream manifested antibacterial activity at concentrations ≥ 100µg/ml. The extract possessed bactericidal effects comparable with gentamicin. Formulation of extract into cream decreased antibacterial activity, suggestive of the need to increase its concentration in the cream for treatment and management of dermatological conditions.

Keywords: *Chrysophyllum abidum,* anti-bacterial activities, gentamicin, topical cream, minimum inhibitory concentration, zone of inhibition.

INTRODUCTION

Skin diseases are caused by viruses, fungi or bacteria, such as *Staphylococcus aureus* (*Staph.aureus*), group A β-hemolytic streptococci and coryneforms. These pathogens or their toxins can enter into systemic circulation through broken skin thereby causing infections. Dermatophytic fungi have a strong affinity for keratin and invade keratinized tissue of the nails, hair, and skin [1]. The search for antimicrobial drugs that may be effective against such organisms could be obtained from plant sources.

Herbal medicine or phytomedicine and herbalism have a long tradition of use outside conventional medicine. It is becoming more main stream as improvements in analysis and quality control, along with advances in clinical research; show the value of herbal medicine in treating and preventing disease [2]. The awareness and general acceptability of the use of herbal drugs in today's medical practice is increasing. Herbal medicine has led to the discovery of several useful new drugs, and non-drug substances [3].

Chrysophyllum albidum plant (popularly called African star apple and Otien in the local Edo language) is a small to medium buttressed tree species, which can grow up to 25-37 m in height and is distributed throughout the southern part of Nigeria [4, 5]. The seeds are about 1-1.5 x 2 cm, beanlike, dark brown in colour, shiny when ripe, compressed with one sharp edge and with a star-shaped arrangement in the fruit. The seed coats are hard, bony, shiny, dark brown, and when broken reveal white-coloured cotyledons [6, 7].

The roots, barks, and leaves of *C. albidum* have been employed in folk medicine. The bark is used for the treatment of yellow fever and malaria, while the leaf is used for the treatment of skin eruptions, stomach ache and diarrhea [8]. Studies have shown a diminished risk of chronic diseases in populations consuming diets high in

Author to whom correspondence may be addressed. Email: sfusifoh@uniben.edu

fruits and vegetables and it has been suggested that antioxidants found in large quantities in fruits and vegetables may be responsible for this protective effect [8, 9]. Previous study on the antimicrobial constituents of *C. albidum* seed cotyledons extract reported the isolation and characterisation of eleagnine components and the minimum inhibitory activities against various microorganisms [4]. However, there are no further literature reports on the antimicrobial activity of the extracted components from *C. albidum* formulated into a topical preparation. Hence the objective of this study to formulate an ethanolic extract of *C. albidum* into a topical preparation and to determine its antimicrobial activity.

MATERIALS AND METHODS

The ripe fruits of *C. albidum* fruits were obtained from a local market in Benin City, Edo State, Nigeria. The Department of Pharmaceutical Microbiology, University of Benin, Benin City provided the bacteria species, *Staphylococcus aureus*, *Pseudomonas aeruginosa (P. aeruginosa)* and *Escherichia coli* (*E. coli*) used for the study. Nutrient agar (Muller Hinton) was obtained from Tulip Diagnostics Ltd, India, while cetomacrogol emulsifying wax, white soft paraffin and liquid paraffin were obtained from Halewood Chemicals Ltd, England. Ethanol (98 %) was obtained from Sigma-Aldrich, Germany. All other chemicals employed in the study were of reagent grade.

Extract preparation from *Chrysophyllum albidum* seed cotyledons

The seeds of *C. albidum* were air-dried for 48 hr after which the hard shells were removed to obtain the cotyledons. The cotyledons were then air-dried for two weeks and blended into powder. About 740g of the powdered cotyledon was dispersed in 1.5 L ethanol. The mixture was macerated, allowed to soak for 48 hr and filtered using Whatman filter paper. The filtrate was concentrated by evaporation at room temperature for 4 days and was then stored in an airtight container in a refrigerator until further investigation.

Preparation of *Chrysophyllum albidum* extracts cream

Varying concentrations (150-250 mg/ml) of *C. albidum* extract were individually incorporated into cetomacrogol ointment BP base with appropriate volume of water to form an oil–in-water creams as described by BP 2004 [10]. In a similar manner, varying concentrations of gentamicin (100– 250 µg/ml) were incorporated into cetomacrogol ointment base BP.

Determination of MIC and assessment of antibacterial activity of *C. albidum* extract, the formulated cream and gentamicin.

The agar diffusion method was used to assess the antibacterial activities of the *C.albidum* extract and the formulated cream using gentamicin as the standard. Four Petri dishes were each filled with 20 ml molten sterile nutrient agar and allowed to set before each was then inoculated with the test organisms, by streaking the surface of the set agar plates with sterile cotton swab sticks of 0.5 McFarland standards of the test organisms.

Several wells were bored, with the aid of a sterile cork-borer, into the surface of the nutrient agar plates. The bottom of each well was sealed with a drop of molten agar. A sterile syringe was used to introduce 0.2 ml each of the different concentrations (120, 130, 140, 150, 160 and 180 mg/ml) of the *C. albidum* extract and the cream formulations of the extract into the respective wells. The same volume of 5 % Tween-80 that served as negative control and gentamicin cream (150-250 µg/ml) the positive control were similarly introduced into their respective wells. The samples agar plates were left for about 30 min to allow for diffusion of the test and control samples' solutes into the agar. The plates were then incubated for 24 hr at 37 °C.

After incubation, the diameter of the zones of inhibition on each plate were determined, recorded and used as a measure of antibacterial activity. This procedure was carried out in triplicate for each concentration and the mean results and standard deviations were reported.

RESULTS

Organoleptic properties of the *Chrysophyllum albidum* extract

A total yield of 14 % w/w of the ethanolic extract was obtained. The extract was a reddish-brown, powdered dried substance with a characteristic unique pleasant odour that was readily soluble in water and ethanol.

Minimum Inhibitory Concentration of the extract

Results of the MIC of varying concentrations of the extract and the formulated cream extract against organisms under investigation are shown in Table 1. Generally, the MIC values of the extract for all organisms investigated was ≥ 130mg/ml while that of the formulated creams was ≥160 mg/ml as measured by the zones of inhibition diameters. The zones of inhibition displayed at varying MIC values were: *E. coli* (130 mg/ml) 11.5±0.5 mm, *S. aureus* (140 mg/ml) 11.3±0.5 and *P. aeruginosa* (150 mg/ml) 11.8±0.2, respectively. It was observed that the zones of inhibition increased with increase in the concentrations of the extract. However, the zone of inhibition was highest for *P. aeruginosa* and least for *E. coli*.

The MIC of varying concentrations of the formulated cream of the extract was markedly affected by the formulation. Generally, there was a remarkable increase in the MIC values of the formulated creams with regards to all organisms and the values ranged as follows: *E. coli* (160 mg/ml), *S. aureus* (200 mg/ml) and *P. aeruginosa* (250 mg/ml), respectively. The zones of inhibition of the formulated creams were also concentration dependent. The corresponding zones of inhibition of the cream against the organisms at an equivalent MIC value of ≥ 250 mg/ml was more marked with *P. aeruginosa* (17.5 ± 0.3 mm), followed by *S. aureus* (15.0±3.0 mm) and *E. coli* (13.7± 1.3 mm) the least.

Antibacterial activity of the of the extract, gentamicin and their formulated creams

The MIC of gentamicin against the organisms under investigation is shown in Tables 1 and 2. *S. aureus* and *E. coli* were highly susceptible to gentamicin at very low concentrations of 40 µg/ml while *P.aeruginosa* was susceptible at a two-fold higher concentration of the other organisms. The resulting zones of inhibitions was least at MIC value 80 µg/ml for *P. aeruginosa* (11.5±0.5 mm) and highest for *E. coli* which had a zone of inhibition of 26.3±3.3 mm at 40 µg/ml.

Results of antibacterial activities of the formulated creams of the extract and gentamicin are presented in Tables 1 and 2. The results showed decreasing zones of inhibition as the concentration of the extract in the cream decreased. The extract had the lowest MIC of 130 mg/ml on *E. coli* with a zone of inhibition of 11.3 ± 0.5 mm and the highest minimum inhibitory concentration of 150 mg/ml on *P. aeruginosa* with a zone of inhibition of 11.8 ± 0.2 mm. The MIC for *S. aureus* was 140 mg/ml with zones of inhibition of 11.3 ± 0.5 mm. The highest MIC for the formulated cream of the extract was 250 mg/ml for *P. aeruginosa* (17.5±0.5 mm) while the least MIC was 150 mg/ml for *E. coli* (11.7±0.9 mm). Gentamicin cream also showed decreasing zones of inhibition as the concentration of gentamicin in the cream decreased. Generally, gentamicin had higher antibacterial activity compared with the extract cream. A comparative analysis of the antibacterial activities of the extract with the standard gentamicin (Table 3) showed very high susceptibility of the organism to gentamicin at very low potent doses, whereas higher doses would be required from the extract to achieve a similar antibacterial effect against the test organisms.

Table 1: Antimicrobial activity of varying concentrations of ethanolic extract and the formulated cream extract against organisms under investigation

Test Organism	Conc. of pure extract/formulated cream (mg/ml)	Zone of inhibition (Mean ± SD mm)	
		Pure extract	Formulated cream
Pseudomonas aeruginosa	150	11.8±0.2	-
	160	12.7±0.5	-
	180	13±0.8	-
	200	13.7±1.5	-
	250	16.3±0.5	17.5 ± 0.3
Staphylococcus aureus	140	11.3 ±0.5	-
	150	11.7±0.5	-
	160	12.3±0.5	-
	180	12.3±0.5	-
	200	13±0.8	11.8±0.3
	250	17±0.8	15.0±3.0
Escherichia coli	130	11.3±0.5	-
	140	12.3±0.5	-
	150	11.7 ± 0.8	-
	160	11.7 ± 0.5	11.3 ± 0.5
	180	13.3 ± 0.5	13.3 ± 0.5
	200	13.0 ± 0.8	13.0 ± 0.8
	250	13.7± 1.3	13.7± 1.3

Key: - No inhibition of bacterial growth.

Table 2: MIC of varying concentrations of the extract and gentamicin against the test organisms

Microorganism	Zone of inhibition of extract (mm) (Mean ± SD)					
Concentration(mg/ml)	**180**	**160**	**150**	**140**	**130**	**120**
P. aeruginosa	13±0.8	12.7±0.5	11.8±0.2	-	-	-
S. aureus	12.3±0.5	12.3±0.5	11.7±0.5	11.3±0.5	-	-
E. coli	13.3±0.5	11.7±0.5	12±0.8	12.3±0.5	11.3±0.5	-
	Zone of inhibition of gentamicin (mm) (Mean ± SD)					
Concentration (µg/ml)	**80**	**70**	**60**	**50**	**40**	
P. aeruginosa	11.5±0.5	-	-	-	-	
S. aureus	19.7±1.3	19.7±0.5	17.7±0.5	20±3.6	16.3±0.5	
E. coli	30.7±2.6	28.7±3.3	26.3±1.9	29.3±1.7	26.3±3.3	

Key:- No inhibition of bacterial growth.

Table 3: Comparison of the antibacterial activity of the formulated creams of the extract and gentamicin cream

Concentration	Extract cream zone of inhibition (mm) (Mean ± SD)			Gentamicin cream zone of inhibition (mm) (Mean ± SD)			
	250 mg/ml	**200 mg/ml**	**150[a] mg/ml**	**250 µg/ml**	**200 µg/ml**	**150 µg/ml**	**100 µg/ml**
P. aeruginosa	17.5 ± 0.5	-	-	12.8± 0.2	12.5 ±0.5	-	-
Staph. aureus	15 ± 3	11.8 ± 0.3	-	14 ± 1	-	-	-
E. coli	13.7 ± 1.3	13 ±0.8	11.7 ±0.9	20.7 ± 3.1	20.8 ±0.3	18 ±2.9	16.7 ± 0.9

Key:- No inhibition of bacterial growth, [a] - No inhibition at 100 mg/ml of extract cream.

DISCUSSION

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that is required to inhibit the growth of a microorganism. Cotyledons from the seeds of *C. Albidum* G. Don-Holl (Sapotaceae), have been used in ointments in the treatment of vaginal and dermatological infections in Western Nigeria [4]. The zones of inhibition obtained in this study indicated sensitivity of the microorganisms to the ethanolic extract of the cotyledons from the seeds of *C. albidum* which is comparable to earlier work done by Idowu *et al,* 2003 [4]. The sensitivity of the microorganisms to the *C. albidum* extract implies that the crude extract can be used for skin infections normally caused by the tested organisms. A previous report had been made on the use of the leaves of *Cassia alata* in the treatment of *Tinea imbricate* in Western Pacific [11].

The formulated cream of the extract had antimicrobial activity as indicated by the zones of inhibition recorded. It had the highest effect on *E. coli* with a zone of inhibition of 11.7 ± 0.9 mm at 150 mg/ml concentration. It had the lowest effect on *P. aeruginosa* with a zone of inhibition of 17.5 ± 0.5 mm at 250 mg/ml. concentration. This observed antimicrobial effect of the extract may not be unrelated to the presence of fatty acid constituents, with known antimicrobial activity, in the plant [12].

Higher concentrations of the extract were required in the cream to have antibacterial activity against the organisms due to further dilution of the extract by formulation into cream which is probably due to the reduction in the concentration of the active ingredient. Formulation of the extract into cream may have affected the antimicrobial activity of the extract. The positive control (standard antibiotic) gentamicin had a much lower MIC than the crude extract and its cream had antimicrobial activity even at very low concentrations. This means that the cream containing the crude extract of *C. albidum* cotyledons is less effective as an antimicrobial cream than the gentamicin cream.

CONCLUSION

Chrysophyllum albidum cotyledons ethanolic extract was found to possess antimicrobial activity against *P. aeruginosa, S. aureus* and *E. coli*. Topical formulation of ethanolic extract into cream for use in the management of possible dermatological conditions may require an increase in the concentration of the extract to achieve a favourable therapeutic and comparable antibacterial activity with the standard gentamicin.

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