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Effect of Cream Bases on the Antimicrobial Properties of the Essential Oil of *Aframomum meleguata*

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ABSTRACT

The antimicrobial properties of the essential oil of *Aframomum meleguata* (AMO) and the effect of cream bases on these properties were investigated using both Agar dilution and diffusion methods. The oil was found to be most active against gram positive bacteria with a MIC of 0.001% v/v against *S. aureus* and *B. subtilis*. *C. albicans* was inhibited at 0.5% v/v while *Ps. aeruginosa* and *E. coli* were each inhibited by 1.0% v/v. The release rate of the oil from the water soluble cream bases, Cetomacrogol or Aqueous cream was greater than that from the hydrocarbon cream base, oily cream due to the hydrophobicity of the oily cream.

INTRODUCTION

The plant *Aframomum meleguata* (Zingiberaceae) is widely cultivated in West Africa. It has narrow leaves, pink or lilac flower spikes at the base of the stem and bright red fruits which turn brown on drying. Its ovate fruit contains reddish-brown seeds with a pungent aromatic odour. The aromatic odour is attributable to the presence of about 0.5% essential oil while the pungency is associated with a resinous substance, paradol, contained in the seed coat (Trease & Evans, 1978).

Different parts of the plant have been widely used for the preparation of traditional remedies (Breyant *et al.*, 1979). Most of these formulations are used as stimulants in stomachics and tonics. When incorporated into ointments or creams, the extracts from the fruit are used in the treatment of sprains or fractures. The alcohol extracts are used as stomach disinfectants. The fruits have been used in the treatment of throat and skin infections (Sofowora, 1986).

The use of the fruit and seed extracts in traditional remedies against infection suggests the presence of antimicrobial agents in the extracts of this plant. This study was designed to investigate the antimicrobial properties of the essential oil obtained from the fruit of *A. meleguata* against some gram positive and gram negative bacteria and fungi. In addition, the effects of some cream bases on the antimicrobial properties and the release of the oil were investigated.

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MATERIALS AND METHODS

Plant Material

The dried fruits of *A. meleguata* Schum. were purchased locally in Lagos, Nigeria and authenticated by Dr. Z.O. Gbile of the Forestry Research Institute of Nigeria.

The essential oil from the fruits was obtained by steam distillation using a Clevenger still. A stock solution of 4% v/v oil in a 1% aqueous Tween 80 solution was prepared. The relative density of the oil is 0.8327.

Cream Bases

Aqueous, cetomacrogol and oily cream bases were used in this study. Their composition is shown in Table 1.

Microorganisms

The microorganisms used in this study included *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25853), *Staphylococcus aureus* (NCTC 6571), *Bacillus subtilis* (Hospital isolate) and *Candida albicans* (Hospital isolate). They were maintained on blood agar slopes at 4 °C. However, when required for use, each organism was subcultured in blood agar plates, and an overnight culture prepared in nutrient broth.

Microbiological Methods

The antimicrobial property of the oil in 1% Tween 80 was determined using the agar dilution method. In this method, concentrations of between 0.0005% v/v (4.16 µg/ml) and 1% v/v (8.33 mg/ml) of the essential oil were prepared in molten nutrient agar at 42°C. 1 ml of 1% v/v Tween 80 was added to molten nutrient agar to serve as a negative control. Each plate was well mixed, allowed to solidify and then dried in an incubator for 2 hours to allow for absorption of the inoculum when added to it.

0.025 ml of a 1 in 100 dilution of the overnight culture of each organism containing 1 x 10^6 organisms/ml was dropped at the appropriate sector of the labelled plate. The plates were incubated at 37°C, and examined for growth after 24 hours. Each determination was done in triplicate and the mean value calculated. Penicillin G was used as a positive control.

In the determination of the effect of the cream bases on the antimicrobial property of the oil, 10 g of each cream was prepared containing 1-16% w/w concentrations of the oil. 0.5 g of each cream was then mixed with the molten agar and the antimicrobial property determined as above. The different cream bases used are shown in Table 1.

The release of the oil from the cream bases was determined using the agar diffusion method. 1 ml of the overnight culture of *S. aureus* NCTC 6571 containing 1.0 x 10^6 organisms/1 ml was seeded in

Table 1. Composition of the Various Cream Bases.

1. *Aqueous Cream Base*	
Emulsifying Ointment	300 g
Purified water, freshly boiled and cooled	700 g
	1000 g
2. *Cetomacrogol Cream Base*	
Cetomacrogol emulsifying Ointment	300 g
Purified water, freshly boiled and cooled	700 g
	1000 g
3. *Oily Cream Base BP*	
Wool alcohol Ointment	500 g
Purified water	500 g
	1000 g

25 ml of agar at 42°C, mixed and allo... the highest susceptibility to the oil ... agar, and filled with 0.5 g of the ... concentrations of the oil. The zone ... Tween 80 was used to plot the stan... cream base was determined. ... was + 10%. The coefficient of ...

The oil was very active ... mum inhibitory concentration ... Other isolates exhibited ... Tween 80 was sufficient to in... compared favourably with ... *B. subtilis* were very sensitive ... oil in the cream. Other micro...

Table 2. Inhibitory Activity of A.M.O.

Percentage of A.M.O. in 1% v/v Tween 80	*Staph. aureus*
1%	–
0.5	
0.25	
0.125	
0.0625	
0.03125	–
0.016	–
0.008	–
0.004	–
0.002	
0.001	
0.005	
1% v/v Tween 80 in distilled water	++++
100% A.M.O.	
Penicillin G 160 mg/ml	–

Key: – = No Growth
++ = Little Growth
+++ = Moderate Growth
++++ = Profuse Growth
A.M.O. = Essential oil from A...

25 ml of agar at 42°C, mixed and allowed to set. *S. aureus* NCTC 6571 was used because it exhibited the highest susceptibility to the oil in Tween 80. Holes 7 mm in diameter were bored in the solidified agar, and filled with 0.3 g of the various preparations of the cream bases containing the different concentrations of the oil. The zone of inhibition of the different concentrations of oil (alone) in 1% Tween 80 was used to plot the standard curve from which the concentration of oil released from the cream base was determined. Each determination was done in quadruplicate. Assay reproducibility was ± 10%. The coefficient of variation of each point in the graph ranged from 0.001 - 0.09.

RESULTS AND DISCUSSION

The oil was very active against *S. aureus* and *B. subtilis* giving a very low minimum inhibitory concentration of 0.001% v/v (8.33 µg/ml) for each organism. Other isolates exhibited varied susceptibility. However, 1% v/v of the oil in Tween 80 was sufficient to inhibit all the species of microorganisms tested and compared favourably with penicillin G (Table 2). In aqueous cream *S. aureus* and *B. subtilis* were very sensitive, being inhibited by 1% w/w concentration of the oil in the cream. Other microorganisms were inhibited by 4% w/w of the oil in

Table 2. Inhibitory Activity of A.M.O. at Different Concentrations Against Five Organisms.

Percentage of A.M.O. in 1% v/v Tween 80	*Staph aureus*	*Ps. aeruginosa*	*E. coli*	*B. subtilis*	*Candida albicans*
1%	–	–	–	–	–
0.5	–	++	++	–	–
0.25	–	++	++	–	+++
0.125	–	+++	+++	–	++
0.0625	–	++	+++	–	+++
0.03125	–	+++	+++	–	+++
0.016	–	+++	+++	–	+++
0.008	–	+++	+++	–	++++
0.004	–	++++	++++	–	++++
0.002	–	++++	++++	–	++++
0.001	–	++++	++++	–	++++
0.005	++	++++	++++	++	++++
1% v/v Tween 80 in distilled water	++++	++++	++++	++++	++++
100% A.M.O.	–	–	–	–	–
Penicillin G 160 mg/ml	–	–	–	–	–

Key: – = No Growth
++ = Little Growth
+++ = Medium Growth
++++ = Profuse Growth
A.M.O. = Essential oil from *A. meleguata* fruits

the aqueous cream. The activity of the oil in Cetomacrogol cream was very similar to that of aqueous cream bases.

The antimicrobial activity of the oil in the oily cream BP was observed to be very low. *S. aureus* and *B. subtilis* were inhibited at 4% w/w of the oil in oily cream, while other organisms were inhibited at 8% w/w. The very low activity of the oil in oily cream formulation may be due to the hydrophobic nature of the oily cream which may have retarded the diffusion of the oil out of the cream base (Onawunmi & Ogunlana, 1986). Comparatively, the high antimicrobial activity of the oil in the water soluble cream bases may be attributed to the less hydrophobic nature of these cream bases and, therefore, better diffusion of the oil from the base to exert its activity on the microorganisms. Generally, the lower activity of the oil in the presence of the cream bases compared to the oil in Tween 80 suggests that the hydrophobic portion of the base inhibits the partitioning of the oil to the aqueous surrounding.

Figure 1 shows the release of the oil from the various cream bases when 8% w/w of the oil was incorporated in the cream bases. The water soluble cream bases released the highest amount of the oil from their formulations. In contrast,

Table 3. Effect of Cream Bases on the Antimicrobial Activity of the Essential Oil of *A. meleguata* (AMO).

Cream Base	Conc. of the Oil	*B. subtilis*	*S. aureus*	*Ps. aeruginosa*	*E. coli*	*Candida albicans*
Aqueous Cream	0%	+++	++++	++++	++++	++++
	1%	–	–	+++	+++	++
	2%	–	–	++	++	+
	4%	–	–	–	–	–
	8%	–	–	–	–	–
	16%	–	–	–	–	–
	* All notations as in Table 2.					
Cetomacrogol Cream	0	++++	++++	++++	++++	++++
	1	–	–	++	++	++
	2	–	–	+	+	–
	4	–	–	–	–	–
	8	–	–	–	–	–
	16	–	–	–	–	–
Oily Cream BP	0	++++	++++	++++	++++	++++
	1	+++	+++	+++	+++	+++
	2	++	++	+++	+++	+++
	4	–	–	+	+	+
	8	–	–	–	–	–
	16	–	–	–	–	–

Key: ++++ = Profuse Growth
+++ = Medium Growth
++ = Little Growth
+ = Very Little Growth
– = No Growth

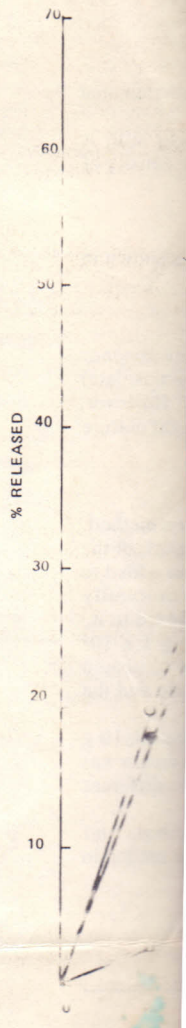

Fig. 1. The release of [illegible]
Key: ● Aqueous [illegible]

the oily cream gave a [illegible]
oil from the water [illegible]
water soluble [illegible]
matrix of the agar gel
diffuse well into the [illegible]

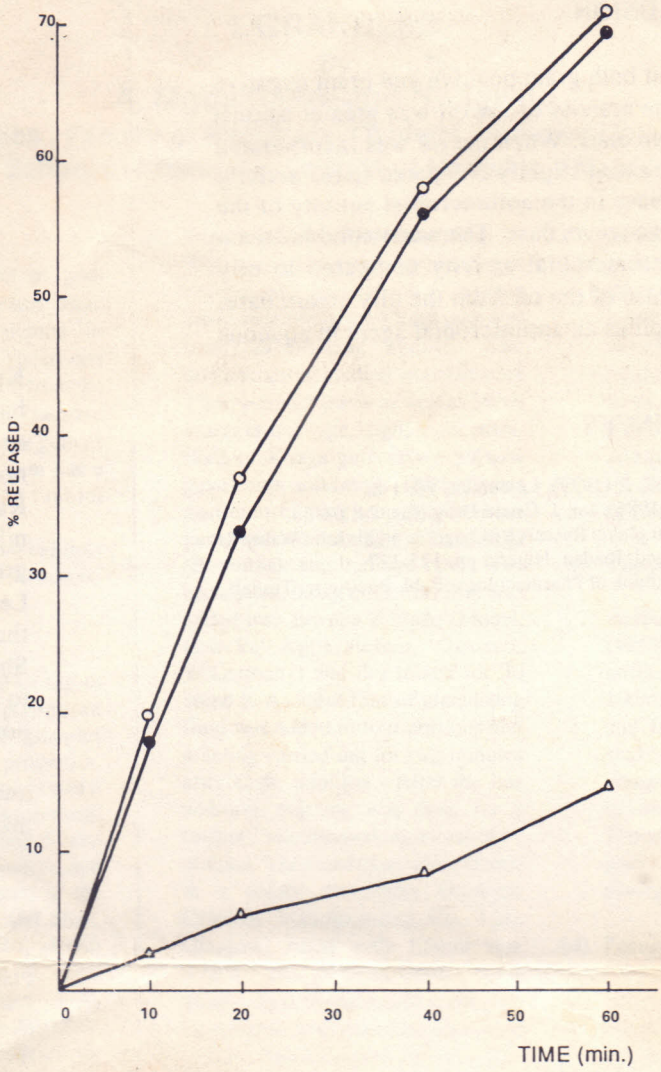

Fig. 1. The release of 8% w/w essential oil of A.M.O. from three cream bases.
Key: ● Aqueous cream; O Cetomacrogol cream, and Δ oily cream bases.

the oily cream gave a very low release rate of the oil. The greater release of the oil from the water soluble cream bases may be due to the fact that the bases are water soluble and able to diffuse with the incorporated oil into the aqueous matrix of the agar gel. The oily cream base is not water soluble and may not diffuse well into the aqueous matrix.

CONCLUSION

The oil shows appreciable activity against both gram positive and gram negative bacteria as well as *Candida albicans*. The activity of the oil was greater against gram positive bacteria than gram negative ones. When the oil was incorporated into various cream bases, its activity decreased slightly compared to the activity of the oil in 1% v/v Tween 80. The decrease in the antimicrobial activity of the oil may be associated with the nature of the cream base. The water soluble cream bases containing the oil gave higher antimicrobial activity compared to oily cream base, possibly due to the slow release of the oil from the oily cream base. The results suggest a possible use of the oil as an antimicrobial agent in aqueous and Cetomacrogol cream bases.

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Screening for

Department of Biological S

Kano is a region of Niger
has the sudan
to semi-arid conditions
nomedicine is
Karatela, 1989) and
microbial
growing on hosts
Lewis, 1979; Oliver,
thought it might be
Shamma and Mitscher,
to screen these
nomic value.

In this paper
antimicrobial
alkaloids.

Plant Material

Plant materials were
identified in the
specimens in the
and Gbile, 1980).

Extraction Procedure

Dried and powdered
solvent

Test
The test organisms
terium diphteriae, *Escherichia*
Proteus vulgaris, *Pseudomo*
Streptobacillus sp.
Health Clinic, except
Streptobacillus sp.
Walp.).