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The formulation of an effective topical antibacterial product containing *Ocimum gratissimum* leaf essential oil

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Abstract

The antibacterial potential of *Ocimum gratissimum* essential oil was explored. Liquid and semisolid formulations of the oil were designed in a variety of bases for topical antiseptic medication. The products were evaluated by agar diffusion assay against type strains and clinical isolates from boil, wound and pimples. Remarkable antibacterial effects, higher than those of commercial antiseptic products, were demonstrated at 2% *Ocimum* oil concentration in some bases. The properties of base into which the oil was incorporated affected its activity. It was more effective in hydrophilic bases than in lipophilic bases. Solubilization and microemulsification grossly reduced its activity. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: *Ocimum* oil; Bases in formulation; Antibacterial activity; Clinical isolates

1. Introduction

Antimicrobial activities of the essential oil and preparations derived from the aerial parts of the plant *Ocimum gratissimum* Linn., family Lamiaceae, have been reported. The *Ocimum* oil from various countries is active against several species of bacteria and fungi (El-Said et al., 1969; Jedlickova et al., 1992; Begum et al., 1993), though some microbial strains are resistant (Ndounga and Ouamba, 1997). Aqueous decoctions of the leaves are taken orally in Nigeria for diarrhoeal treatment (El-Said et al., 1969; Njoku et al., 1997) and in India for bronchitis (Atal et al., 1986). The *Ocimum* oil has been reported to have considerable inhibitory activity against strains of *Escherichia coli* (Orafidiya et al., 2000), that are implicated in the aetiology of persistent, infantile and travellers' diarrhoea (Scotland et al., 1993; Fang et al., 1995). The antidiarrhoeal property of *Ocimum* oil and aqueous leaf extract has also been demonstrated in laboratory animals (Offia and Chukwendu, 1999; Orafidiya et al., 2000).

The objective of the present study was to develop an effective topical antibacterial formulation of *Ocimum* oil that could serve as an antiseptic agent for the treatment of minor wounds, boils and pimples.

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2. Materials and methods

2.1. Plant material and preparation of Ocimum oil

The leaves of *O. gratissimum* Linn. (Lamiaceae) were collected at Ile-Ife, Nigeria, in September 1999. Dr H.C. Illoh of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria identified and authenticated the plant. It was identical with the voucher specimens (Ife 1930 and Ife 1819) previously deposited at the Department of Botany herbarium. *Ocimum* oil was extracted from the fresh leaves using the volatile oil extractor (II) (British Pharmacopoeia, 1980). The oil was then stored in fully filled glass containers in a refrigerator until needed.

2.2. Preparation of test samples

Solutions of *Ocimum* oil, 0.25–5% v/v, were prepared in 50% aqueous methanol (MeOH). Stable dispersions of 10 and 50% v/v concentrations of the oil were only obtainable in 80% aqueous MeOH and were so prepared. Solutions of the oil (2, 10 and 50% v/v) were prepared in liquid paraffin. The following formulation bases were used: a sodium laurate monostearin cream base (glyceryl monostearate 5%, sodium lauryl sulphate 3%, cetostearyl alcohol 2%, liquid paraffin 25%, water to 100%); a macrogol cream base (cetomacrogol emulsifying wax BP 9%, liquid paraffin 6%, white petrolatum 15%, water 70%); a macrogol blend ointment base (macrogol 4000 20%, and macrogol 600 80%); simple ointment (The Pharmaceutical Codex, 1979: cetostearyl alcohol 5%, hard paraffin 5%, wool fat 5%, white petrolatum 85%); and white petrolatum alone. Formulations containing 0.25–50% v/w *Ocimum* oil were prepared in these bases. Dispersions containing 0.5, 1.0 and 2.0% v/v *Ocimum* oil were prepared by mixing the requisite amounts of oil separately with 1.0, 3.0, or 5.0% v/v Tween 80 solutions. The following commercial products were used as positive controls: BP™ benzoyl peroxide 10% lotion (Thames Pharmacal Co., NY), OXY $10^{(SB)}$ benzoyl peroxide 10% lotion (SmithKline Beecham, UK), and Cetavlex® (0.5% w/w cetrimide) cream (Zeneca, Cheshire, UK). The cream and ointment bases were also used as negative controls.

2.3. Test organisms

Three patients reporting for wound dressing at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria, and two healthy individuals with a boil and pimples, respectively, provided clinical specimens for the bacterial isolates used in this investigation. Type cultures (*Staphylococcus aureus* NCTC 6571 and *Pseudomonas aeruginosa* ATCC 10145) were also used. Specimens from the infected wound sites were taken using sterile cotton-tipped applicators (Sterilin, England) and transferred to the microbiology laboratory. Specimens from the boil and from pimples were taken following surface treatment of the skin with a cleansing lotion. The specimen samples were immediately applied to freshly prepared blood agar as well as MacConkey agar plates (Oxoid, England) and streaked to obtain isolated colonies, which were later Gram stained. The Gram-positive isolates from the blood agar showing positive reaction to catalase test were streaked on mannitol salt agar (Oxoid) for presumptive identification of *S. aureus*. This was subsequently confirmed by coagulase test according to Jorgensen and Kloos (1987). Gram-negative non-lactose-fermenting organisms on MacConkey plates were differentiated using standard microbiological methods including oxidase test and oxidation of sugars, for identification of *P. aeruginosa* (Sewell, 1987), and urease, MRVP and D-mannitol fermentation tests for the identification of *Proteus* species (Farmer et al., 1987).

2.4. Tests for antibacterial activity

A 0.2 ml volume of overnight nutrient broth culture (Oxoid) of the respective microorganisms was seeded into 20 ml of molten and cooled (45 °C) nutrient agar. Three equidistant cups were bored into the seeded plates with sterile 9-mm diameter cork borers. Plates challenged with high concentrations of *Ocimum* oil contained one centrally bored cup only. Following the re-

moval of the agar plugs and prior to testing *Ocimum* oil solutions, one Pasteur pipette drop of molten agar (45 °C) was placed into the cups to seal the bottom and prevent seeping of test solutions beneath the agar. Into the cups were placed 0.1 ml of the various test liquids or ≈ 0.2 g of the semisolid test preparations in duplicate experiments. The plates were allowed to stand for 1 h at room temperature, and then incubated at 37 °C. The diameter of zones of inhibition was measured, excluding cup size, after 24-h incubation.

2.5. Determination of physical parameters of Ocimum oil dispersions in Tween 80 solutions

The kinematic viscosities of the *Ocimum* oil dispersions, *Ocimum* oil, Tween 80 solutions, and MeOH at 28 °C were determined with an Ostwald U-tube viscometer (Size C). A Size D viscometer was used for liquid paraffin. The mean droplet sizes of the emulsions were determined with a microscope fitted with a calibrated eyepiece. At least 600 droplets were measured for each sample. The dispersions were observed for creaming.

3. Results

Solutions of *Ocimum* oil in aqueous MeOH exhibited antibacterial activity. The 50% v/v solution, in some cases, showed markedly higher activity than the undiluted oil. Control 50% aqueous MeOH was inactive, while 80 and 100% MeOH showed only slight activity (Table 1). The methanolic solutions of the oil exhibited higher antibacterial effects than corresponding liquid paraffin solutions. Comparing the various 2% *Ocimum* oil preparations investigated, the methanolic solution exhibited the highest antibacterial activity, followed by the ointment in macrogol blend base, the cream in sodium laurate monostearin base, and then the emulsion. The activities of these preparations were higher than those of the positive controls used in this study. Ointment formulations of *Ocimum* oil in white petrolatum base exhibited weak activity, while 0.25–2.0% formulations in macrogol cream base and in simple ointment base were completely inactive (Table 2). Increasing the *Ocimum* oil content of the inactive formulations up to 50% did not improve their activity appreciably.

The antibacterial effect of the dispersions in Tween 80 increased with increase in *Ocimum* oil content and decrease in concentration of the surfactant (Table 3). Only dispersions that appeared white exhibited antibacterial activity. The transparent or translucent dispersions, whose droplet sizes were submicroscopic, exhibited no apparent antibacterial activity (Tables 3 and 4). All the dispersions remained stable under storage for 6 months.

4. Discussion

Ocimum oil contains about 75% thymol, which is mainly responsible for its antimicrobial activity (Hammer et al., 1999). The sensitivities of *S. aureus* isolates from a boil, wound and pimple to the *Ocimum* oil preparations were similar and comparable to that of the type strain (Table 1). Hence, only the activity of *S. aureus* (wound) was illustrated subsequently. However, the clinically isolated *P. aeruginosa* (wound) appeared more resistant than the type strain.

The nature of base in which a drug is formulated has considerable effect on its efficacy (Omotosho et al., 1986; Florence and Attwood, 1990). Factors that affect the release of an active principle from a base include its affinity for the base and the viscosity of the preparation (Florence and Attwood, 1990; Oyedele et al., 2000). The markedly higher activity of *Ocimum* oil solutions in aqueous MeOH than in liquid paraffin (Table 1) can be attributed to the lipophilic affinity of the oil for liquid paraffin, which impairs the release of its active constituents into the more hydrophilic agar medium. Liquid paraffin is much more viscous than *Ocimum* oil and MeOH (Table 4) and this would further retard diffusion of the active principle from the oil into agar. Preliminary experiments showed no difference in the antibacterial activity of 2% cetrimide in water, in 50, 80, and 100% MeOH. Therefore, aqueous MeOH as solvent was probably not synergistic to the an-

Table 1
Antibacterial activity of *Ocimum* oil solutions

Test samples	Inhibition zone diameter (mm)						
	S. aureus boil isolate	*S. aureus* pimples isolate	*S. aureus* wound isolate	*P. aeruginosa* wound isolate	*Proteus* spp. wound isolate	*S. aureus* (NCTC 6571)	*P. aeruginosa* (ATCC 10145)
100, 80 and 50% aqueous MeOH	2, 0, 0	1, 0, 0	2, 2, 0	1, 1, 0	0, 0, 0	2, 2, 0	1, 0, 0
Ocimum oil							
0.25% in 50% MeOH	2	3	4	1	3	1	3
0.5% in 50% MeOH	3	4	4	2	6	3	5
1.0% in 50% MeOH	6	8	8	2	7	7	6
2% in 50% MeOH	12	11	11	2	7	7	6
10% in 80% MeOH	18	16	20	2	19	16	7
50% in 80% MeOH	22	20	30	2	22	30	18
2% in liquid paraffin	1	3	1	0	0	1	0
10% in liquid paraffin	7	9	9	0	7	9	3
50% in liquid paraffin	10	13	14	0	10	15	12
100% undiluted oil	16	20	22	1	11	28	16

tibacterial activity of the oil, but rather enhanced diffusibility of its active constituents into agar, being less viscous than the pure oil.

Ocimum oil in lipophilic semisolid bases (petrolatum, simple ointment) exhibited much lower or no activity compared to its formulation in the more hydrophilic macrogol blend ointment base. The higher antibacterial activity of the macrogol blend ointment preparation may, however, be linked to the inherent activity of the bland macrogol blend base (Table 2). The preparation of *Ocimum* oil in sodium laurate monostearin

Table 2
Antibacterial activity of 2% *Ocimum* oil formulations and controls

Test samples	Inhibition zone diameter (mm)				
	S. aureus wound isolate	*P. aeruginosa* wound isolate	*Proteus* spp. wound isolate	*S. aureus* (NCTC 6571)	*P. aeruginosa* (ATCC 10145)
2% Ocimum oil in					
Macrogol cream base	0	0	0	0	0
Simple ointment base	0	0	0	0	0
White petrolatum	1	2	5	2	3
1% Tween 80	5	5	3	6	4
Sodium laurate monostearin cream base	5	5	5	6	6
Macrogol blend ointment base	9	4	6	7	4
50% MeOH	11	2	7	7	6
Positive controls					
BP™ benzoyl peroxide lotion	3	2	0	2	0
OXY $10^{(SB)}$ benzoyl peroxide lotion	4	4	1	3	0
Cetavlex® cream	2	4	5	4	7
Bases showing activity					
Sodium laurate monostearin cream base	4	0	4	4	0
Macrogol blend ointment base	5	4	3	3	4
3% Sodium lauryl sulphate solution	10	0	6	13	1

Table 3
Antibacterial activity of *Ocimum* oil dispersions in Tween 80 solutions

Test sample	Inhibition zone diameter (mm)				
	S. aureus wound isolate	*P. aeruginosa* wound isolate	*Proteus* spp. wound isolate	*S. aureus* (NCTC 6571)	*P. aeruginosa* (ATCC 10145)
0.5% oil in 5, 3, or 1% Tween 80	0	0	0	0	0
1% oil in 5 or 3% Tween 80	0	0	0	0	0
1% oil in 1% Tween 80	2	0	2	4	3
2% oil in 5% Tween 80	1	2	0	3	3
2% oil in 3% Tween 80	3	3	2	5	4
2% oil in 1% Tween 80	5	5	3	6	4

Table 4
Physical properties of *Ocimum* oil dispersions in Tween 80 solutions and viscosity of some liquids

Test sample	Kinematic viscosity (cSt)	Mean droplet diameter (μm)	Appearance of dispersions
Ocimum oil dispersions in Tween 80 solution			
0.5% oil in 5% Tween	1.48	NO	Transparent
0.5% oil in 3% Tween	1.31	NO	Transparent
0.5% oil in 1% Tween	1.09	NO	Translucent
1% oil in 5% Tween	1.45	NO	Transparent
1% oil in 3% Tween	1.29	NO	Translucent
1% oil in 1% Tween	1.09	4.38	White
2% oil in 5% Tween	1.36	NO	White
2% oil in 3% Tween	1.21	1.72	White
2% oil in 1% Tween	1.07	6.28	White
5% Tween solution	1.43	–	–
3% Tween solution	1.21	–	–
1% Tween solution	1.07	–	–
Methanol	1.03	–	
Liquid paraffin	46.76	–	–
Ocimum oil	3.46	–	–

(NO) Not observable; (–) not applicable.

cream base exhibited considerably higher activity compared to its formulation in macrogol cream base that showed no activity at 2% oil concentration (Table 2). The presence of petrolatum in the macrogol cream base possibly inhibited release of the active principle from the oil. Intrinsic antibacterial property of sodium lauryl sulphate may have contributed to the remarkable activity of the monostearin cream preparation of the oil (Table 2).

The dispersion of oil in a surfactant solution may result in its solubilization (producing a transparent solution), in microemulsion (forming transparent or translucent dispersion), or in macroemulsification (giving a white emulsion) depending, among other factors, on the relative concentrations of the two materials (Porter, 1994). Solubilization and emulsification result in entrapment of the dispersed droplets within micellar structure, which greatly hinders their release (Rees and Collett, 1975; Mitchell and Kazmi, 1977; Florence and Attwood, 1990). Thus solubilized and microemulsified *Ocimum* oil exhibited little or no antibacterial activity compared to the macroemulsified products (Tables 3 and 4). The Tweens are non-ionic surfactants that are able to bind phenolic antimicrobial agents (such as the thymol content of *Ocimum* oil), thereby reducing their antimicrobial activity (Lund, 1994). The dispersion of *Ocimum* oil as very fine droplets increases the surface area of its thymol content that would be available for binding to the Tween 80, which will further impair its antimicrobial activity. These observed effects of a surfactant on the antibacterial activity of *Ocimum* oil calls for caution in the use of the modified agar dilution method for determination of antimicrobial activity of essential oils (Hammer et al., 1999), where Tween 20 (0.5% v/v) is included in the agar to enhance oil dissolution. Observed slight increases in viscosity with decrease in oil concentration in the emulsions containing identical surfactant concentrations (Table 4) may be alluded to the formation of finer droplets, which caused an increase in the resistance to flow (Florence and Attwood, 1990).

Cetavlex® cream is antiseptic; and OXY $10^{(SB)}$ and BP™ lotions are used for the treatment of acne pimples. The 2% *Ocimum* oil preparations in MeOH and in macrogol blend ointment base demonstrated considerably higher antibacterial activity than the positive controls (Table 2). The methanolic solution would offer a cooling and soothing effect on skin, but may require cotton

swab for its application. The macrogol blend ointment formulation is, however, considered the best for *Ocimum* oil, being semisolid and greaseless.

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