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Efficacy of Two Commonly Used Antifungal Herbs in Nigeria Against Clinical Isolates of Fungi

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ABSTRACT

Mitracarpus scaber and *Ocimum gratissimum* are used extensively in Nigerian herbal medicinal practice to treat many ailments especially those caused by fungi. In the current study, the antifungal activities of these two herbs against fungal isolates (moulds and yeast) recovered from subjects in the community were evaluated. Twenty species of moulds tested were isolated from 3 clinical samples including skin scrapping (n = 13), scalp (n = 4) and skin/scalp (n = 3) while 18 clinical isolates of *Candida albicans* were isolated from seven clinical samples including high vaginal swab (n = 8), sputum (n = 4), urine (n = 1), endocervical swab (n = 2), groin (n = 1), mouth thrush (n = 1) and palm (n = 1). Studies on the *in vitro* antifungal activity of the ethanol extract of *Mitracarpus scaber* (50 μg mL^{-1}) and *Ocimum gratissimum* oil (50 μg mL^{-1}) showed that the clinical isolates were sensitive to the herbal extracts but more sensitive to *O. gratissimum* oil extract (MIC range of 0.8-1.25 μg mL^{-1}) than to ketoconazole (MIC range of 0.31-5.00 μg mL^{-1}). The MIC ranges for sodium salicylate and aspirin were 0.75-1.60 and 7.81-31.25 μg mL^{-1}, respectively. The MFC results revealed that the *O. gratissimum* oil had greater biocidal effect against most of the tested organisms (MFC range of 0.156-2.5 μg mL^{-1}), whereas the effect of ketoconazole against the tested organisms was biostatic (MFC range of 1.25-5.00 μg mL^{-1}). Biocidal studies showed that the oil started to eliminate the organisms earlier than the ketoconazole. The study has confirmed the *in vitro* activity of these two extracts on the fungal isolates tested.

Key words: Dermatophytes, yeast, Nigeria, herbal extracts, antifungal activities

INTRODUCTION

Dermatophytes are made up of closely related fungi which are classified into *Trichophyton, Epidermophyton* and *Microsporum* (Weitzman and Summerbell, 1995). They are keratinophilic moulds that infect human and animal skin, nails and hair and are typically confined to the superficial keratinized tissue (Dei-Cas and Vernes, 1986) and thus, they can often be treated with topical antifungal medications (Gupta *et al*., 1998). However, some of the topical medications are not very effective as they are unable to penetrate hair or nails. In such cases, systemic therapy is usually administered.

In some countries, *Trichophyton* infection is now considered a major public health problem because of its high prevalence (Fuller *et al*., 2003; Ghannoum *et al*., 2003; Gupta and Summerbell, 1998). In the United States, for instance, dermatophytosis is the most frequently reported skin disease, especially among primary school pupils (Dahl, 1993). It is communicated from person to person through direct or indirect contact with infected skin or hair infected with scales. Available evidence indicates that *Trichophyton* infection promotes the development of asthma and other allergic diseases (Ward *et al*., 1989, 1999). The development of immediate hypersensitivity versus delayed-type-hypersensitivity to *Trichophyton* allergens may be pivotal to the cause and severity of skin infection and also to the development of allergic disease (Ward *et al*., 1989, 1999). Also, dermatophytes account for the majority (90%) of cases of fungal nail infections (onychomycosis) in the United States and Europe (Ellis *et al*., 1997). Some authors have showed that infection increases with age (Elewski and Charif, 1997; Gupta *et al*., 1997; Heikkila and Stubb, 1995; Roberts, 1992; Sais *et al*., 1995; Singh *et al*., 2003). This observation is consistent with the view that changes in the immune response which occur with advancing age, lead to susceptibility to disease. Other authors have argued that this increasing global prevalence and the recent increase in the incidence of dermatophytosis is due to the increase in immunocompromised conditions such as those associated with HIV/AIDS, organ transplantation and diabetes mellitus and the use of immunosuppressants and corticosteroids (Faergemann and Baran, 2003; Ghannoum *et al*., 2003; Mirmirani *et al*., 2001; Woodfolk, 2005). In previous studies, it was demonstrated that dermatophytic infections are highly common and endemic in Nigeria (Nweze, 2001; Nweze and Okafor, 2005). The cost of health care associated with the management of dermatophytoses is very high in many parts of the world (Drake *et al*., 1996; Smith *et al*., 1998). The situation in Nigeria and other developing countries is even worse due to poverty, poor health facilities and poorly trained diagnostic personnel. There are several antifungals available, some of these are very toxic to the host and must be used with caution. Concerns have also been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the newer or modern antifungals such as terbinafine (Mukherjee *et al*., 2003). The problem posed by the high cost and adulteration of these synthetic drugs (Cohen, 1992) cannot be overemphasized. Also, in the case of severely immunosuppressed patients, the rising incidence of failures in the treatment of mycoses found especially in the developing countries cannot be overestimated (Shariff, 2001). Thus, there is a need for an alternative therapy that is safer, cheaper and easily available to those who need it in countries facing peculiar difficulties. This study investigated the aetiology of dermatophyte and other mould infections in a local Nigerian primary school and tested the susceptibility of dermatophyte isolates to local herbs used in the area for the treatment of these infections to ascertain their efficacy. The study also tested various clinical isolates of *Candida* species isolated from the Medical Laboratory Department of the Federal Medical Center (FMC), Owerri, Imo State, Nigeria against these herbal extracts.

MATERIALS AND METHODS

Sample collection, isolation and identification of dermatophytes: Samples were collected from 20 primary school children with suggestive lesions at Central school, Nsude, Enugu State, Nigeria and processed as previously described by one of us (Nweze, 2001). Identification was based primarily on the criteria elaborated by Rebell and Taplin (1974).

Isolation and identification of clinical isolates of *Candida albicans*: Samples were collected from different clinical samples-high vaginal swab, sputum, endocervical swab, urine, groin, mouth thrush and palm-of patients manifesting symptoms of candidiasis at Federal Medical Center, Owerri, Imo State, Nigeria using established methods (Nweze and Ogbonnaya, 2011).

Plant materials: *Mitracarpus scaber* and *Ocimum gratissimum* were collected from Nsukka, Enugu State, Nigeria in March, 2007 and authenticated in the Department of Botany, University of Nigeria, Nsukka, Enugu State, Nigeria. Voucher specimens were deposited accordingly.

Extraction of plants: Sun-dried powdered plant material of *Mitracarpus scaber* was extracted with 2000 mL of ethanol using the cold maceration method previously described by Esimone and Adikwu (1999). This filtrate was exposed to air until the solvent evaporated to dryness. The residue recovered after drying (which is the extract from the plant) was collected, weighed and kept in a container for further use.

Solvent extraction of *Ocimum gratissimum*: The ground dried sample of 500 g was put in a 50x wet extractor thimbles and then set up. The different solvents, chloroform, ethanol and n-hexane, respectively at a batch were introduced and the extraction was allowed for 12 h. The extracts were concentrated with a rotary evaporator at 50°C and the residues were collected.

Volatile oil extraction: Fresh leaf samples were subjected to steam distillation in a modified Clevenger-type apparatus (Sunbim, India) for a minimum of 3 h. The oil was obtained in a yield of 0.3% per 100 g, stored in a sealed glass vial and kept in a refrigerator at 4°C until required.

Maintenance and standardization of stock culture: Stock cultures of each clinical isolates of moulds and *C. albicans* were stored in Sabouraud Dextrose Agar (SDA) slants at 4°C prior to use, the cultures were activated by subculturing onto SDA plates and incubated at 28°C for 24 h to two to seven days depending on the isolate. Suspensions of the organisms were made before inoculation. The cultures were standardized. For *C. albicans*, overnight (18 h) subcultures in Sabouraud dextrose broth were adjusted to 90% transmittance at 530 nm using distilled water. For moulds, a 40 mm diameter of the mycelia growth was inoculated into 2.0 mL SDA plate and processed as previously described (Leven *et al*., 1979).

Antimicrobial screening test

Preliminary sensitivity testing of the isolates: The sensitivity of selected fungi to the herbal extracts (ethanolic extract of *M. scaber* and *O. gratissimum* oil), sodium salicylate and standard antifungal drugs (nystatin, ketoconazole and fluconazole) were evaluated using a modified cup-plate agar diffusion method (Okore, 2005).

Evaluation of MIC of *O. gratissimum* oil alone and ketoconazole alone against fungal isolates using the agar dilution method: The MFC is the least concentration with no growth after this extended incubation period.

The MIC of the two herbal extracts in addition to ketoconazole, sodium salicylate and aspirin were prepared separately in distilled water or dimethyl sulfoxide (DMSO). Each of these solutions was diluted two-fold serially with dilute DMSO up to eight dilutions. Thereafter, 1 mL from each

dilution was seeded into 19 mL of molten sterile SDA and allowed to solidify. The plates were divided into eight segments and eight representative isolates (four isolates each of moulds and *C. albicans* were selected based on their sources) were streaked in triplicates in each segment. The plates were then incubated for 48 h (*C. albicans*) and two to five days (moulds). Signs of growth were checked.

Evaluation of MFC of *O. gratissimum* oil alone and ketoconazole alone against fungal isolates: The MIC was the least concentration that showed no growth. Based on the outstanding antifungal properties exhibited by *O. gratissimum* oil extract, it was further selected for further testing in comparison with ketoconazole as the positive control. For the determination of MFC, the plates were further incubated for twice the incubation period (four days for *C. albicans* and four to ten days for moulds).

Evaluation of the killing rate of *O. gratissimum* oil alone and the killing rate of ketoconazole alone against fungal isolates: Stock solution (10 times the MFC) of the oil and ketoconazole that had a minimum fungicidal concentration was prepared in Sabouraud dextrose broth. One microliter of this stock was added into 8 mL of Sabouraud dextrose broth and 1 mL of representative tested organisms was added immediately. At various time intervals of 0, 20, 40, 60, 90, 120, 180 min, 6 h and 24 h, 0.1 mL were withdrawn from the reaction mixture and diluted 100-fold in sterile normal saline. These various dilutions were plated in sterile SDA plates and colonies were counted. The total viable count was determined using the formula:

$$\text{TVC} = \frac{\text{Mean count/drop} \times \text{dilution factor}}{\text{Vol./drop}}$$

Statistical analysis: All statistical analyses were performed using StatView (version 4.5; Abacus Concepts Inc., CA, USA). A two-tailed Student's t-test was used for analysis of two groups. Values with a p-value of 0.05 were considered statistically significant.

RESULTS

Several mould and yeast isolates were recovered from patients within the localities investigated. Twenty species of moulds tested were isolated from three clinical samples including skin scrapping (n = 13), scalp (n = 4) and skin/scalp (n = 3) while 18 clinical isolates of *C. albicans* were isolated from 7 clinical samples including high vaginal swab (n = 8), sputum (n = 4), urine (n = 1), endocervical swab (n = 2), groin (n = 1), mouth thrush (n = 1) and palm (n = 1), as demonstrated in Table 1 and 2, respectively. Table 1 shows two things: (1) different mould species are recoverable from the locality investigated and (2) the spectrum of mould species causing skin infections is wide in this locality. *Candida* still remains an important aetiological agent in Southeast Nigeria with this species implicated in several kinds of infections, as shown in Table 2. The inhibitory effects of antifungal extracts (ethanol extracts of *M. scaber* and *O. gratissimum* oil) and standard antifungal drugs against isolates of moulds and *C. albicans* are presented in Table 3, which is composed of ten representative isolates of *Candida albicans* and one isolate each of *T. soudanense* (TS1), *T. mentagrophytes* (TM1), *P. linacinum* (PL1), *Fusarium* spp. (FS1), *Cladosporium* spp. (CS1), *Curvularia* spp. (CSS1) and *A. niger* (AN1). This preliminary test illustrated in Table 3 reveals that both antifungal extracts and drugs have antifungal activity against the fungal isolates tested. The activity of the antifungal extracts was very comparable with that of the conventional drugs. For

Table 1: Different species of moulds isolated from the pupils in central school, Nsude

Sex (CS1)	*Year of study	Age	Source(s)	Isolates (codes)
M	2	9	Skin/scale	*T. soudanense* (TS1) and *Cladosporium* sp.
M	2	7		*Trichophyton soudanense* (TS2)
	1	6		*Cladosporium* sp. (CS2)
	5	12		*Penicillium* sp. (PS1)
	6	11		*Curvularia* sp. (CSS1)
	6	11	Scalp	*Aspergillus niger* (AN1)
	6	12	Skin/scalp	*Fusarium* sp. (FS1)
	5	8		*Trichophyton soudanense* (TS3)
	5	11	Skin	*Aspergillus niger* (AN2) and *A. flavus* (AF1)
	1	4	Skin	Mixed growth of *Cladosporium* (CS3) and *Trichophyton soudanense* (TS4)
F	1	4	Skin scrapping	*T. soudanense* (TS5)
F	3	11		*Aspergillus niger* (AN3)
	2	4		*Fusarium* sp. (FS2)
F	4	9		*Fusarium* sp. (FS3)
	3	14		*Cladosporium* sp. (CS4)
M	3	9	Scalp	*Aspergillus flavus* mixed with growth of *Trichophyton mentagrophytes* (TM1)
M	3	9	Skin/scalp	*Cladosporium* sp. (CS5)
M	3	10	Scalp	*Penicillium lilacinum* (PL1)
F	4	10	Scalp	*Cladosporium* sp. (CS6)
F	6	11	Skin	*Fusarium solani* (FSS1)

M: Male, F: Female, *Year of study by the pupil in the 6 year school, Parenthesis values are isolate numbers

Table 2: Isolates of *C. albicans* recovered from the study and their sources

Strain No.	Source of isolate
UNFPCAP1	Palm
UNFPCAH1	High vaginal swab
UNFPCAH2	High vaginal swab
UNFPCAH3	High vaginal swab
UNFPCAH4	High vaginal swab
UNFPCAH5	High vaginal swab
UNFPCAH6	High vaginal swab
UNFPCAH7	High vaginal swab
UNFPCAH8	High vaginal swab
UNFPCAU1	Urine
UNFPCAE1	Endocervical swab
UNFPCAE2	Endocervical swab
UNFPCAM1	Mouth thrush from AIDS patient
UNFPCAS1	Sputum
UNFPCAS2	Sputum
UNFPCAS3	Sputum
UNFPCAS4	Sputum
UNFPCAG1	Groin

instance, the inhibition zone diameters of extract A (*O. gratissimum*) against the isolates UNFPCASI, UNFPCAM1, UNFPCAE1 of *C. albicans* were 65, 64 and 70 mm, respectively and the corresponding values for all the standard antibiotics tested were correspondingly lower, thus indicating the potential in this extract. This trend is also evident among some of the mould species tested i.e., *P. linacinum* (PL1). Table 4 shows the MIC results of *M. scaber, O. gratissimum* oil

Table 3: Preliminary test on selected strains of *Candida albicans* (S/nos 1-10) and moulds (S/nos 11-17) recovered from the study

	Zone of inhibition (mm)								
Strain No./isolate	Nyst	Keto	Fluco	A	B	C	D	E	F
UNFPCAS1	13	20	22	65	14	+	+	+	+
UNFPCAM1	15	20	20	44	15	+	+	+	+
UNFPCAE1	17	14	16	64	15	+	+	+	+
UNFPCAHI	17	-	-	70	15	+	+	+	+
UNFPCAS3	17	30	25	54	-	+	+	+	+
UNFPCAG1	15	25	25	60	14	+	+	+	+
UNFPCAP1	22	20	27	60	13	+	+	+	+
UNFPCAH3	15	-	-	55	10	+	+	+	+
UNFPCAS5	19	33	37	53	-	+	+	+	+
UNFPCAU1	16	18	15	64	10	+	+	+	+
T. soudanense (TS1)	13	35	30	54	15	+	+	+	+
T. mentagrophytes (TM1)	30	35	27	55	15	+	+	+	+
P. linacinum (PL1)	22	-	-	66	13	+	+	+	+
Fusarium sp. (FS1)	15	17	15	40	13	+	+	+	+
Cladosporium sp. (CS1)	15	20	17	50	14	+	+	+	+
Curvularia sp. (CSS1)	14	18	22	40	15	+	+	+	+
A. niger (AN1)	15	18	15	61	15	+	+	+	+

Each antifungal drug conc. used: 50 μg mL^{-1}, A: *O. gratissimum*, B: *Mitracarpus scaber*, C, D, E and F: Chloroform, water, ethanol, n-hexane extracts of *O. gratissimum* oil, -: No growth, +: Growth, Nyst: Nystatin, Keto: Ketoconazole, Fluco: Fluconazole

Table 4: MIC of extracts in comparison to ketoconazole and the NSAIDs (sodium salicyclate and aspirin) against selected isolates of mould and *C. albicans* recovered in the study

		MIC (μg mL^{-1})			
Test organisms (isolate No.)	*M. scaber*	Oil	Keto	Sod. Sal	Aspirin
T. soudanense (TS1)	40.00	0.625	1.25	31.25	0.95
T. mentagrophytes (TM1)	20.00	0.625	5.00	31.25	0.85
A. niger (AN1)	20.00	1.250	2.50	31.25	1.35
T. soudanense (TS2)	40.00	-	+	15.23	1.25
C. albicans (UNFPCAS3)	40.00	0.160	5.00	15.23	0.85
C. albicans (UNFPCAM1)	40.00	0.310	2.50	15.63	0.75
C. albicans (UNFPCAH1)	40.00	0.160	2.50	15.63	1.60
C. albicans (UNFPCAE1)	20.00	0.800	0.30	7.81	1.10

+: Growth, -: No growth, Sod. Sal: Sodium salicyclate, Oil: *O. gratissimum* oil

extracts, ketoconazole and NSAIDs (sodium salicyclate and aspirin) against selected fungal isolates. The result, presented in Table 4, revealed that the MIC of *O. gratissimum* oil was much lower than ketoconazole for all tested isolates. While ketoconazole had no activity against a particular isolate of *T. soudanense* (TS2), *O. gratissimum* oil completely killed it. In this study, the MIC value of less than 1 μg mL^{-1} indicates good antifungal activity while MIC of 5 μg mL^{-1} is a suggestive of a fairly good antifungal activity especially for crude plant extracts. As can be seen in Table 4, the MIC values for *Candida* isolates were consistently lower (0.16-5 μg mL^{-1}) against *O. gratissimum* compared to ketoconazole (1.25-5 μg mL^{-1}).

Based on the impressive MIC data exhibited by *O. gratissimum* oil, the MFC was further determined with ketoconazole used as the positive control for comparative purposes. The MFC

Table 5: MFC of ketoconazole and oil against selected isolates of moulds and *C. albicans*

Test organisms	MFC ($\mu g\ mL^{-1}$) Ketoconazole	Oil of *O. gratissimum*
T. soudanense (TS1)	2.50	0.625
T. mentagrophytes (TM1)	5.00	2.500
A. niger (AN1)	2.50	1.250
T. soudanense (TS2)	2.00	0.156
C. albicans (UNFPCAS3)	+	0.156
C. albicans (UNFPCAM1)	2.50	2.500
C. albicans (UNFPCAH1)	5.00	0.625
C. albicans (UNFPCAE1)	1.25	0.625

+: Growth

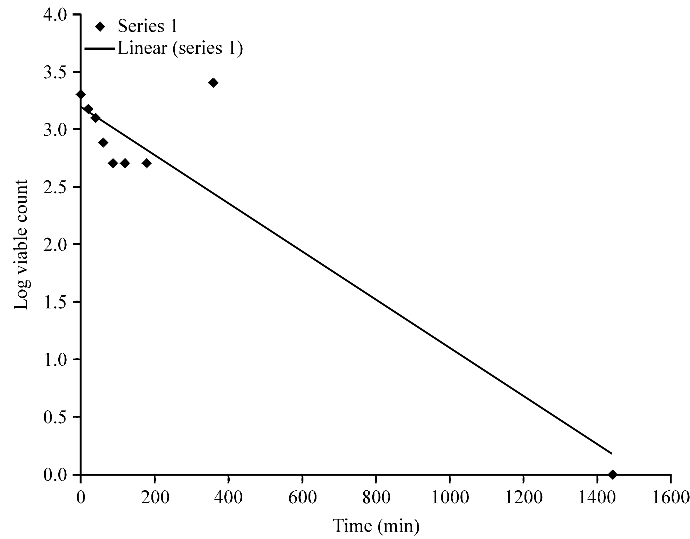

Fig. 1: Killing rate results of ketoconazole against *A. niger* (AN1)

results of ketoconazole and *O. gratissimum* oil against isolate of moulds and *C. albicans* are shown in Table 5. The data shows that in contrast to ketoconazole, *O. gratissimum* oil had a greater biocidal effect against most of the test organisms. However, the MFC of ketoconazole against the tested organisms indicated antagonism. It appears that the effect of ketoconazole on the organisms is biostatic rather than biocidal; while that of *O. gratissimum* oil is more of a biocidal. The results of killing rates of ketoconazole and *O. gratissimum* oil against moulds and *C. albicans* are presented in Fig. 1-8. These results are further illustrated in Table 6-9.

In Table 6, the total viable counts were reducing with increase in time though; there was no growth at 1440 min (24 h). This indicates that ketoconazole is effective on moulds (AN1 and TS1) and eliminated both organisms within 24 h.

In Table 7, the total viable counts were also reducing as the time increased and were totally eliminated at 24 h. This indicates that ketoconazole has activity on *C. albicans* isolates (UNFPCAS3 and UNFPCAM1) but has a better activity against moulds.

In Table 8, the total viable counts reduced significantly with increase in time. In the cases of two of *C. albicans* isolates (UNFPCAH1 and UNFPCAE1), respectively, there was no growth at

Table 6: Killing rate (total viable counts) results of ketoconazole against *A. niger* (AN1) and *T. soudanense* (TS1) at different time intervals

	Time (min)								
Organisms	0	20	40	60	90	120	180	360	1440
A. niger	8	6	5	3	2	2	2	1	-
T. soudanense	7	6	4	2	1	1	1	1	-

Table 7: Killing rate (total viable counts) results of ketoconazole against *C. albicans* (UNFPCAS3 and UNFPCAM1) at different time intervals

	Time (min)								
Organisms	0	20	40	60	90	120	180	360	1440
C. albicans	69	39	34	23	16	14	12	12	-
C. albicans	50	46	31	30	28	24	22	20	-

Table 8: Killing rate (total viable counts) result of *O. gratissimum* oil against *C. albicans* (UNFPCAH1 and UNFPCAE1) at different time intervals

	Time (min)								
Organisms	0	20	40	60	90	120	180	360	1440
C. albicans	44	19	12	6	4	2	-	-	-
C. albicans	33	13	-	-	-	-	-	-	-

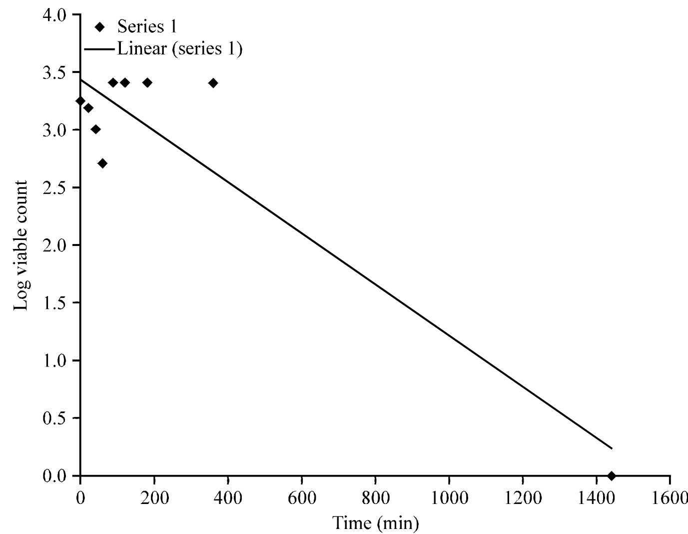

Fig. 2: Killing rate result of ketoconazole against *T. soudanense* (TS1)

180 and 40 min. This indicates that *O. gratissimum* oil is highly effective against *C. albicans* and eliminated them faster than ketoconazole. It is also an indication that the oil will be good for the treatment of *C. albicans*.

In Table 9, the total viable counts were reducing with increase in time. More so, notwithstanding the deviation where the total no of viable counts at 90 and 120 min are higher than others, there is still evidence that the oil has a strong activity against moulds (AN2 and TS2).

Table 9: Killing rate (total viable counts) results of *O. gratissimum* oil against *A. niger* (AN2) and *T. soudanense* (TS2) at different time intervals

	Time (min)								
Organisms	0	20	40	60	90	120	180	360	1440
A. niger	80	34	28	15	38	42	27	3	-
T. soudanense	80	62	48	44	53	73	47	30	-

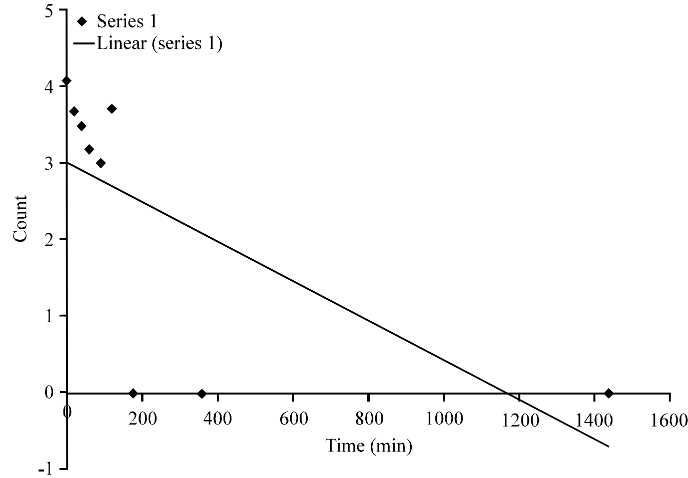

Fig. 3: Killing rate result of ketoconazole against *C. albicans* (UNFPCAS3)

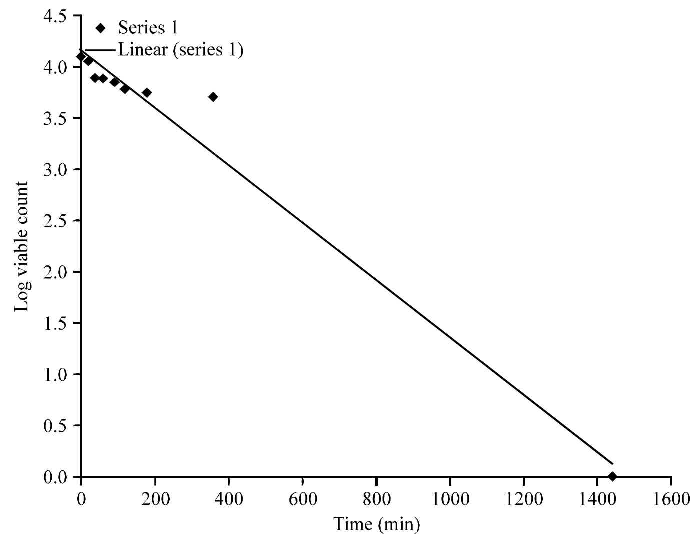

Fig. 4: Killing rate result of ketoconazole against *C. albicans* (UNFPCAM1)

The apparent rate constant (Kapp) is presented in Table 10. In summary, the results indicate that *O. gratissimum* oil has a very good activity against the isolates and started to eliminate the organisms earlier than ketoconazole.

The biocidal study indicated also that the oil has very good activity against the isolates and kills them faster than ketoconazole especially against *C. albicans* (Table 8, Fig. 5, 6). This is an indication that the oil will be good for the treatment of *C. albicans*. More so, not with

Table 10: Killing rates of ketoconazole and *O. gratissimum* oil against isolates of moulds and *C. albicans*

Drug	Killing rate (min^{-1}) *A. niger* (AN1) and *T. soudanense* (TS1)	*C. albicans* (UNFPCAS3)
Ketoconazole	0.0048	0.0064
	0.0051	0.0064
Oil	0.0067	0.0060
	-0.0062	0.0025

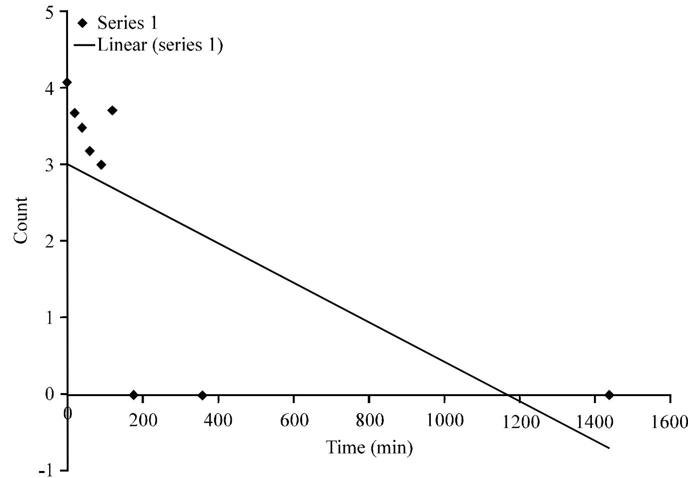

Fig. 5: Killing rate of oil against *C. albicans* (UNFPCAH1)

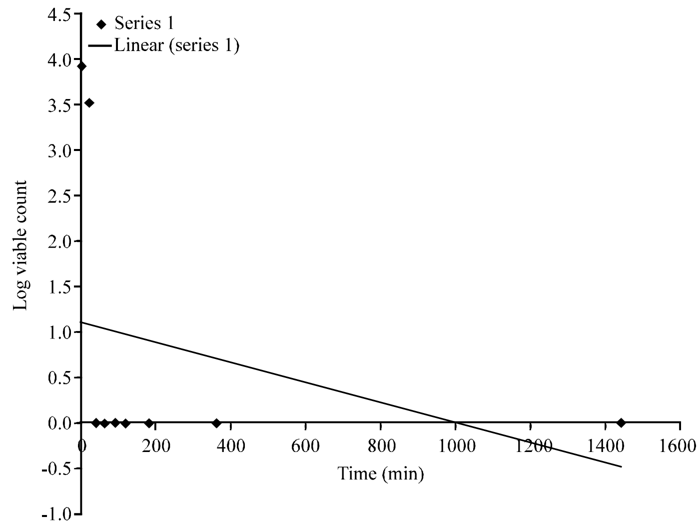

Fig. 6: Killing rate of oil against *C. albicans* (UNFPCAE1)

standing the deviation of the total viable count in Table 9, (where the total no of viable counts at 90 and 120 min) are higher than others, there is still evidence that the oil has a strong activity against moulds (Fig. 7, 8).

The regression equations and their corresponding correlation coefficients reveal that the killing kinetics of ketoconazole and oil against moulds and *C. albicans* are generally linear when the data was treated according to the classical second-order kinetic model. This implies that the

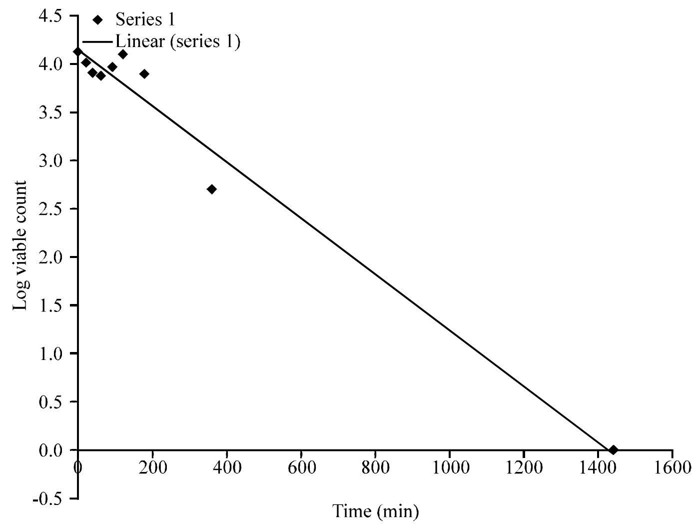

Fig. 7: Killing rate of oil against *A. niger* (AN2)

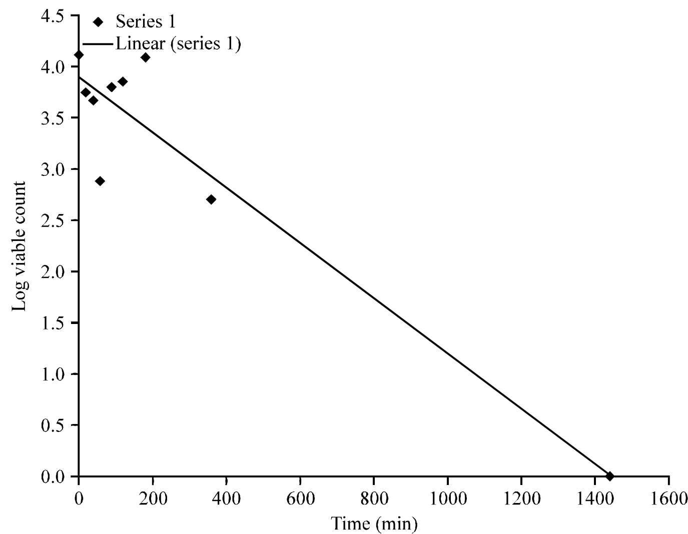

Fig. 8: Killing rate of oil against *T. soudanense* (TS2)

apparent rate constants (Kapp) obtained from the slope of the second-order kinetic equation (Log Nt = -kt/2.303+Log No) could be used to describe or explain the effect of the interaction on the antimicrobial kinetics of these antifungal agents against moulds and *C. albicans*. The apparent rate constant (Kapp) is presented in Table 10. The results reveal that the *O. gratissimum* oil has a very good activity against the isolates and started to eliminate the organisms earlier than ketoconazole.

In Fig. 1, the regression equation and their corresponding correlation coefficient reveal that the killing kinetics of ketoconazole against AN1 is linear when the data was treated according to the classical second-order kinetic model. The apparent rate constant (Kapp) obtained from the slope of the second-order kinetic equation (Log Nt = -kt/2.303+Log No) is 0.0048 min^{-1}. This result reveals that ketoconazole has activity against this isolate (Fig. 1).

In Fig. 2, the regression equation and their corresponding correlation coefficient reveal that the killing kinetics of ketoconazole against TS1 is linear when the data was treated according to the

classical second-order kinetic model. The apparent rate constant (Kapp) obtained from the slope of the second-order kinetic equation (Log Nt = -kt/2.303+Log No) is 0.0051 min^{-1}. This result reveals that ketoconazole has activity against this isolate.

In Fig. 3, the regression equation and their corresponding correlation coefficient reveal that the killing kinetics of ketoconazole against *C. albicans* isolate UNFPCAS3 is linear when the data was treated according to the classical second-order kinetic model. The apparent rate constant (Kapp) obtained from the slope of the second-order kinetic equation (Log Nt = -kt/2.303+Log No) is 0.0064 min^{-1}. This result reveals that ketoconazole has activity against this isolate but has a better activity against moulds (Fig. 3).

In Fig. 4, the regression equation and their corresponding correlation coefficient reveal that the killing kinetics of ketoconazole against *C. albicans* isolate UNFPCAM1 is linear when the data was treated according to the classical second-order kinetic model. The apparent rate constant (Kapp) obtained from the slope of the second-order kinetic equation (Log Nt = -kt/2.303+Log No) is 0.0064 min^{-1}. This result reveals that ketoconazole has activity against this isolate but has a better activity against moulds. Figure 5 reveals that the killing kinetics of oil against *C. albicans* isolate UNFPCAH1 is linear when the data was treated according to the classical second- order kinetic model. The apparent rate constant (Kapp) obtained from the slope of the 2nd-order kinetic equation (Log Nt = -kt/2.303+Log No) is 0.0025 min^{-1}. This result reveals that *O. gratissimum* oil has a very good activity against this isolate and started to eliminate the organisms faster than ketoconazole even at a lower concentration. Figure 6 shows that the killing kinetics of oil against *C. albicans* isolate UNFPCAE1 is linear when the data was treated according to the classical second-order kinetic model. The apparent rate constant (Kapp) obtained from the slope of the second-order kinetic equation (Log Nt = -kt/2.303+Log No) is 0.0060 min^{-1}. This result reveals that *O. gratissimum* oil has a very good activity against this isolate and started to eliminate the organisms faster than ketoconazole even at a lower concentration.

In Fig. 7, the regression equation and their corresponding correlation coefficient reveal that the killing kinetics of oil against AN2 is linear when the data was treated according to the classical second-order kinetic model. The apparent rate constant (Kapp) obtained from the slope of the second-order kinetic equation (Log Nt = -kt/2.303+Log No) is 0.0067 min^{-1}. This result reveals that *O. gratissimum* oil has a good activity against the isolate. Figure 8 reveals that the killing kinetics of oil against TS2 is linear when the data was treated according to the classical second-order kinetic model. The apparent rate constant (Kapp) obtained from the slope of the second-order kinetic equation (Log Nt = -kt/2.303+Log No) is -0.0062 min^{-1}. This reveals that *O. gratissimum* oil has a good activity against the isolate notwithstanding that a lower concentration is used.

DISCUSSION

Pathogenic fungi such as *Candida albicans* cause both superficial and serious systemic infections and are now widely recognized as important agents of hospital-acquired infection (Douglas, 2003). Medicinal plants have been used for several purposes including antimicrobial effects and have exhibited inhibition of growth to fungi. After a downturn in medicinal plants research in recent decades (Alper, 1998), the pace is again gathering momentum as scientists realized that the effective life span of any antibiotic is limited. World spending on finding new anti-infective agents (including vaccines) is expected to increase by 60% from the spending levels in the early nineties (Alper, 1998). New sources, especially plant sources, are also being investigated. This is the major motivation for our study.

From the MIC data, it can be observed that *O. gratissimum* oil, ketoconazole and aspirin showed MIC values (greater activity) against all tested isolates. *O. gratissimum* oil exhibited a lower MIC against *C. albicans* than against moulds and completely inhibited one isolate of *T. soudanense* (TS2). This extract therefore holds a great promise for use in treating a wider spectrum of fungal pathogens. This finding is in agreement with reports from previous investigators (Hammer *et al.*, 2003; Chaurasia and Vyas, 1977; Pauli, 2001). It has been previously shown that essential oils as well as compounds derived from *O. gratissimum* possess wide range of activities with the antimicrobial properties being the most studied (Hammer *et al.*, 2003). In 1977, it was reported that 60% of essential oil derivatives examined to that time were inhibitory to fungi while 30% inhibited bacteria (Chaurasia and Vyas, 1977). One of the most anti-microbial compounds isolated from essential oils is lipophilic monoterpenes, such as thymol, carvacrol, linalool, citral, geraniol and 1-8-cineole. Their applications as preservatives have also been widely studied (Pauli, 2001).

Moreover, the inhibitory effects of antifungal extracts (ethanol extracts of *M. scaber* and *O. gratissimum* oil) and standard antifungal drugs (nystatin, ketoconazole and fluconazole) against isolates of moulds and *C. albicans* shown in Table 3 are also consistent with the findings of other workers. The findings of Bisignano *et al.* (2000) revealed that the phenolic compound isolated from *M. scaber* extracts are used for the treatment of skin infections caused by *Staphylococcus aureus* and *C. albicans*. Adebolu and Oladimeji (2005) also showed that different extracts of *M. scaber* exhibited broad antibacterial and antifungal activity against standard strains and clinical isolates of *S. aureus* and *C. albicans* responsible for common skin infections. Among the compounds isolated, psoralen is used for the management of mycosis fungoides (Anderson and Voorhees, 1980).

Several workers (Hammer *et al.*, 2003; Chaurasia and Vyas, 1977; Pauli, 2001) have extensively investigated the antimicrobial activities including antifungal activity of *O. gratissimum* oil. Their findings reveal that the oil has activity against both moulds and *C. albicans*. Our study also shows that *O. gratissimum* oil has the highest level of activity against all the test fungal isolates.

The activity of the antifungal extracts compares well with that of the standard antifungal drugs. The MFC results of ketoconazole and *O. gratissimum* oil against isolates of moulds and *C. albicans* indicated that, in contrast to ketoconazole, *O. gratissimum* oil had a greater biocidal effect against most of the test organisms (Table 4). This suggests that the effect of ketoconazole on the organisms is biostatic rather than biocidal; while that of *O. gratissimum* oil is more of a biocidal.

The results of biocidal study of ketoconazole and oil against moulds and *C. albicans* (Fig. 1-8) reveal that *O. gratissimum* oil has a very good activity against the isolates and started to eliminate the organisms earlier than ketoconazole. Kinetic studies have been consistently used to quantify antimicrobial activity (Garrett, 1996) or interactions (Seydel *et al.*, 1972). In many ways, they appear to be more superior when compared to other conventional methods of assessing antimicrobial interactions based on broth dilutions, agar diffusions or experimental animal infections (Seydel *et al.*, 1972). For instance, diffusion of drug in agar plates may not be independent resulting in a false presumption of synergy or antagonism of antimicrobial action. Whole animal experiments-while perhaps best related to the clinical effects of the combinations may show synergy or antagonism as a result of pharmacological interactions rather than antimicrobial effects (Seydel *et al.*, 1972). Also, in the checkerboard titration method, interpretation of fractional inhibitory concentration indices leaves little scope for the interpretation of true additive interactions (Tariq *et al.*, 1995). Mackay *et al.* (2000) used the same checkerboard on three separate days to test

reproducibility and found no statistically significant differences. However, they did conclude that all tested drug combinations that showed synergism according to the FIC index after 24 h also showed synergism by the time kill assays after 24 h (but the correlation between synergy at two or 5 h according to the FIC index and by the time-kill assay was poor). Certain problems have been associated with the checkerboard micro dilution method itself, such as variability in the inocula as a potential source of error as well as the solution in appropriate antibiotic concentrations (Eliopoulos and Eliopoulos, 1998). Checkerboard assays are laborious and time consuming. The robotic instrument used in the experiment described allows large number of strains and drug combinations to be tested. However, in our experiment, the results for each herbal extracts was successfully obtained upon re-testing. In addition, the end-point (the complete inhibition of growth is a qualitative measure, rather than a quantitative parameter that can be predicted on a dose-response basis (Tariq *et al.*, 1995).

CONCLUSION

This present study has therefore demonstrated that the ethanolic extracts of *M. scaber* and *O. gratissimum* oil have antifungal activity against moulds and *Candida albicans*. These findings justify their local use in Nigeria and other countries. Generally, the activity of *O. gratissimum* oil was better than *M. scaber* extract and the tested antifungal drugs as shown by the *in vitro* susceptibility test data of the fungal isolates to the antifungal herbal extracts. The killing rate study indicated also that the oil has very good activity against the isolates. The killing kinetics showed that the extracts started killing the tested isolates completely from 2 h and upwards. However, further studies involving animal studies are warranted to confirm, among other things, the safety profile of these extracts.

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