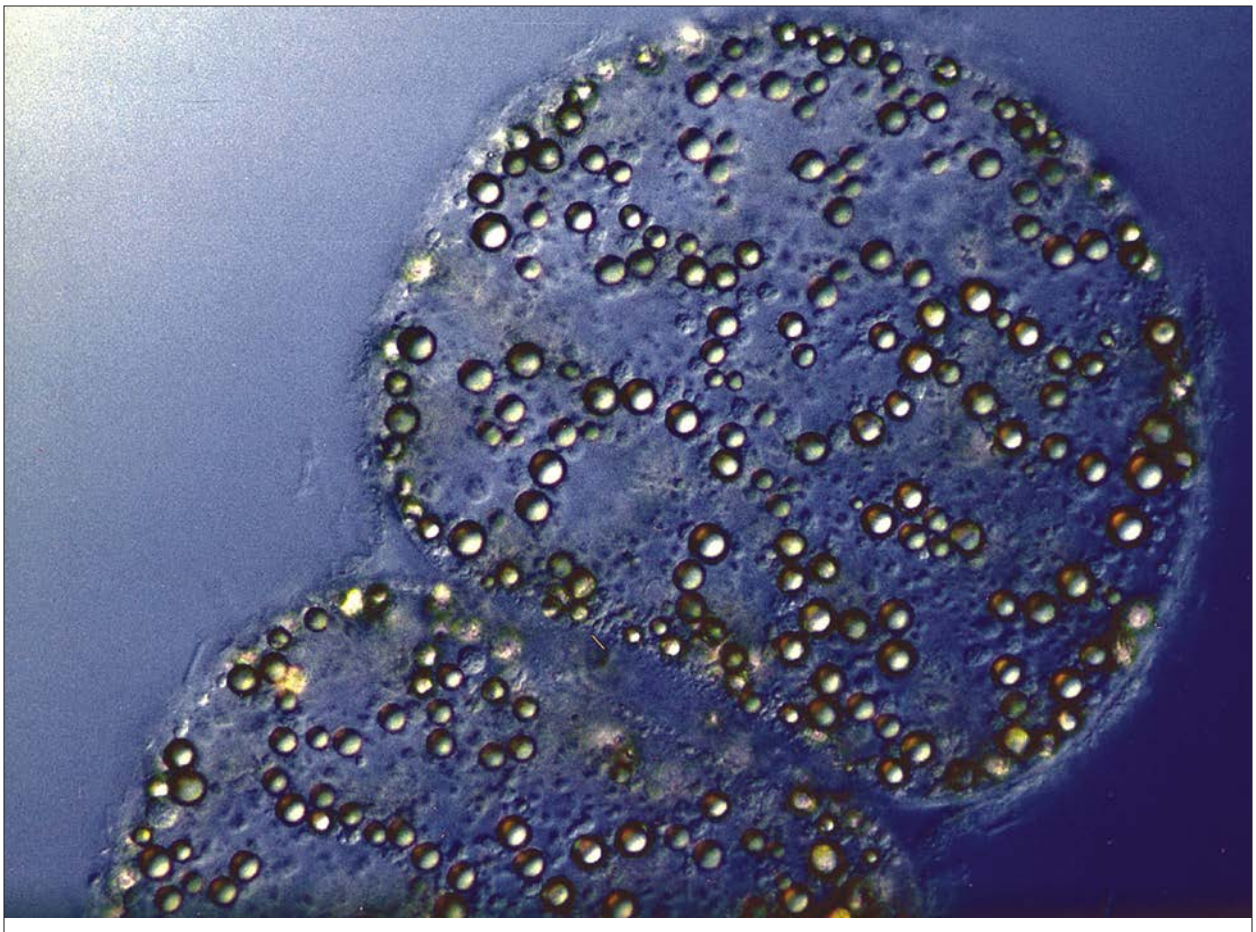

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The *in vitro* Antifungal Activity of the Combinations of *Mitracarpus scaber* and *Occimum gratissimum* Herbal Extracts and Some Non-steroidal Anti-inflammatory Drugs

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ABSTRACT

Mitracarpus scaber and Occimum gratissimum are widely employed in traditional medicine in West Africa. Given the increasing level of consumer acceptance of herbal medicines there is a high possibility of interaction between herbal and orthodox synthetic drugs. This study aims at evaluating the antifungal properties of these plant extracts alone and their combined activities with some non-herbal Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). The *in vitro* antifungal activity of the combination of herbal extracts and different non-steroidal anti-inflammatory drugs were investigated against some clinical fungal isolates using the checker board technique. Findings showed that some combinations of herbal extracts with NSAIDs demonstrated synergistic activity against some clinical isolates of moulds and yeast. These results authenticated the antifungal effects of *M. scaber* and *O. gratissimum* and demonstrated the potential applicability of combinations of aspirin and *O. gratissimum* oil in the treatment of systemic and superficial mycotic infections.

Key words: Antifungal, *Mitracarpus scaber*, *Occimum gratissimum*, mycotic infections, interaction

INTRODUCTION

Mitracarpus scaber is widely employed in traditional medicine in West Africa for the treatment of headaches, toothache, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases and leprosy (Bisignano *et al*., 2000). Among the folkloric uses, the juice of the plant is applied topically for the treatment of skin diseases (infectious dermatitis, eczema and scabies) (Dalziel, 1936; Benjamin and Hugbo, 1986). A previous study (Mouhs *et al*., 1992) reported the isolation of pentalogin, from fresh aerial parts of *M. scaber* which demonstrated a potent antifungal activity against *Candida albicans* and *Trichophyton soudanense*. Other investigations (Germano *et al*., 1999) showed that different extracts of *M. scaber* exhibited broad anti-bacterial and anti-fungal activity against standard strains and clinical isolates of *Staphylococcus aureus* and *C. albicans* responsible for common skin infections. On the other hand, *Ocimum gratissimum* is an aromatic medicinal plant belonging to

the Lamiaceae family. It is a small shrub with many branches, commonly found in many gardens around village huts in Nigeria and planted for its medicinal uses. Originating from Central Africa and South Asia (Agnanient *et al*., 2005), it is probably one of the most commonly used of all herbs in cooking and there are many local and foreign recipes that are enhanced by this minty aromatic plant. In Brazil, it is popularly known as alfavacao, alfavaca and alfavaca-cravo (Aguiyi *et al*., 2000). It is believed to have significant health benefit. The leaves of the African varieties are said to contain thymol oil which is regarded as highly antiseptic and it is also used to prevent mosquito bite (Lee *et al*., 2005). In addition to health benefit, it is used widely as a condiment or spice and as a source of flavor in food preparations. Earlier investigation has shown that *O. gratissimum* has a variety of therapeutic indications which includes its use in treating bacterial infections, diarrhea, diabetes (Watt and Breyer-Brandwijk, 1962), respiratory tract infections, pneumonia, fever, cough, headache, wart, worms, kidney function, abdominal pains, sore eyes and ear infections, barrenness, convulsions, tooth gargle, regulation of menstruation and as a cure for prolapse of the rectum (Harjula, 1980; FAO, 1986). Evaluation of the biological activities revealed that extracts of *O. gratissimum* exerted anti-diarrheal effects in experimental animals (Ayisi and Nyadedzor, 2003; Mohammed *et al*., 2007), showed high antiviral effects against HIV-1 and HIV-2 (Atal *et al*., 1986) and very potent antidiabetic properties (Keita *et al*., 2001). Immuno-biological studies revealed that extracts of *O. gratissimum* appeared to improve the phagocytotic function, without affecting the humoral or cell mediated immune system (Nakamura *et al*., 1999). The essential oil of this spices also presented interesting activities such as anti-bacterial (against *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Shigella, Salmonella* and *Proteus*) (El-Said *et al*., 1969), antifungal *(Trichophyton rubrum, T. mentagrophtes Cryptococcus neoformans, Penicillium lilacinum* and *Candida albicans)* (Lemos *et al*., 2005; Lopez *et al*., 2005; Souza *et al*., 2002) and as a relaxant on isolated ileum from guinea pig.

Recently, herbal medicinal products are becoming increasingly popular (Brevoort, 1998; Fisher and Ward, 1994; Eisenberg *et al*., 1998). At the same time, the safety issues related to herbal drugs continue to be ignored by the public, neglected by manufacturers and legislative bodies as well as under-researched by the medical professions (Ernst, 2000a-d). With the increasing level of consumer acceptance of herbal medicines for diverse reasons, there is a high possibility of interaction between herbal and synthetic drugs. This is because users of herbal medicines tend to believe that these botanicals are inherently safe and are thus likely to concomitantly use self-prescribed synthetic drugs. A survey conducted in South Australia showed that almost half of 3004 respondents had used at least one type of complementary (herbal) remedy in the previous 12 months and that one-fifth had consulted a complementary medicine practitioner (Maclennan *et al*., 1996). Two other important surveys carried out in the U.S in 1991 and 1997/98, involving over 1500 and 2000 individuals, respectively revealed some useful information on trends in complementary medicine use (Eisenberg *et al*., 1998, 1993). Use of at least one form of complementary therapy in the 12 months preceding the survey increased significantly from 34% in 1990 to 42% in 1997. Herbal medicine was one of the therapies showing the most increase over this time period. There was a statistically significant increase in safe-medication with herbal medicines from 2.5% of the samples in 1990 to 12.5% in 1997 (Eisenberg *et al*., 1998). Disclosure rates to physicians of complementary medicines use were below 40% in both surveys. Furthermore, 18.4% of prescription medicine users took prescription medicines concurrently with herbal remedies. These aspects of user behaviour clearly have application for safety (Ernst, 2000d). All herbal

medicines are complex mixtures of more than one active ingredient. This multitude of active ingredients increases the possibilities of interactions between herbal medicines and conventional (synthetic) drugs (Ernst, 2000a, c).

The implications of herb-drug interactions are multi-dimensional (Ernst, 2000a-c). On the one hand, since there are evidences that a clinically relevant interaction exist (Ernst, 2000a-c), health care professionals should no longer ignore their patients' use of herbal medications. Physicians should realize that a large proportion of their patients regularly use herbal remedies in addition to prescribed medicines and that without informing their doctor. Physicians therefore, ought to be aware about possible herb-drug interactions and regularly ask their patients about the use of herbal remedies. Patients should also be adequately informed about the possibility of herb-drug interactions. On the other hand, manufacturers of herbal products should be obliged to take responsibility on informing the public about herb-drug interactions involving their product. Also, regulatory bodies should reconsider the stand that herbal remedies can be classified as food supplements.

The aim of this study was therefore, to evaluate the combined effects of these herbal extracts with some Non-Steroidal Anti-Inflammatory Drugs (NSAIDs).

MATERIALS AND METHODS

Drugs: Sodium salicylate (Poole, England), acetyl salicylic acid, diclofenac, ibuprofen, cycloheximide (Merk, Germany), Nystatin, Ketoconazole Fluconazole (Janssen-Cilag, Nigeria), Chloramphenicol (India), Aspirin, Ibuprufen, Diclofenac (India), Clotrimazole Cream (FIDSON, Nigeria) and Miconazole Ointment (FIDSON, Nigeria) were used.

Extraction of plants: Sun-dried powdered plant material of *Mitracarpus scaber* (500 g) was extracted with 2000 mL of ethanol using the cold maceration method previously described by Bisignano *et al.* (2000). This filtrate was exposed to air until the solvent evaporated to dryness. The residue seen after drying (which is the extract from the plant) was collected, weighed and kept in a container for further use.

Solvent extraction of *Occimum gratissimum*: About 500 g of the ground dried sample was put in a 50µ wet extractors thimble 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 and stored in a sealed glass vial and kept in a refrigerator at 4°C until required.

Evaluation of combined activity of herbal extracts and NSAIDs against fungal isolates using the checkerboard-technique: Stock solutions of extract and NSAID were prepared in sterile distilled water and these solutions were combined in different ratios (1:4, 4:1, 5:0, 0:5 and 5:5), following slight modification of a previously reported method (Okore, 1990; Scott, 1989; Esimone *et al.*, 1998). Each of these combinations was diluted two-fold serially up to five dilutions in sterile test tubes. Replicates of each dilution were also prepared in order to obtain a reproducible result. At the end, 2.5 mL were collected from each tube and mixed with 7.5 mL of molten double

strength sabouraud dextrose agar. These were allowed to solidify and the fungal inoculum (0.1 mL) streaked on designated segment of sterile SDA plates. The plates were incubated at 28°C for 24 h (for *C. albicans*) and 4-7 days (for moulds and dermatophytes) and observed for presence or absence of growth. The MICs were deduced as the minimum concentration of drug or extract inhibiting visible growth following a reported method (Esimone and Adikwu, 1999; Esimone *et al.*, 2003). Interaction was assessed algebraically by determining the Fractional Inhibitory Concentration (FIC) indices according to the relationship.

$$FIC_{index} = FIC_{extract} + FIC_{NSAID}$$

$$FICNSAID = \text{Fractional inhibitory covcentration of non-steroidal anti-inflammatory durg} = \frac{\text{MIC of NSAID in Combination with extract}}{\text{MIC of NSAID alone}}$$

$$FIC_E = \text{Fractional inhibitory concentration of extract} = \frac{\text{MIC of extract in combination with NSAID}}{\text{MIC of extract alone}}$$

RESULTS

The MIC result of ketoconazole, extracts and NSAIDs against selected fungal isolates: The results revealed that the MIC of ketoconazole, *M. scaber* extract and sodium salicylate showed a decrease in activity indicating antagonisms against the isolates of both moulds and *C. albicans* correspondingly (Table 1). There were exceptions however on one particular isolate of *C. albicans* (isolate 4) where ketoconazole had activity and another mould isolate where it had no activity at all. MIC value of less than 1 μg mL^{-1} indicates good antifungal activity while MIC of 5 μg mL^{-1} is suggestive of a fairly good antifungal activity.

Antifungal herbal extracts and non-steroidal anti-inflammatory drug (NSAIDs) combinations against fungal isolates: The combinations of *M. scaber* ethanol extract, *O. gratissimum* oil and NSAIDs (sodium salicylate, aspirin, ibuprofen and diclofenac) against representative fungal isolates were synergistic with some variations (Table 2-4). On the whole, some combinations especially 5:5 produced synergistic effect, some indifferent effect, while some exhibited additive effect and few produced antagonistic effect.

Table 1: MIC of Extracts, Ketoconazole and NSAIDs against isolate of moulds and *C. albicans*

		MIC (μg mL^{-1})			
Test organisms	*M. scaber*	Oil	Keto	Sod. sal.	Aspirin
T. soudanense	40	625.0	1.25	31.25	0.95
T. mentagrophytes	20	0.625	5	31.25	0.85
A. niger	20	1.25	2.5	31.25	1.35
T. soudanense	40	-	+	15.23	1.25
C. albicans 1	40	0.16	5	15.23	0.85
C. albicans 2	40	0.31	2.5	15.63	0.75
C. albicans 3	40	0.16	2.5	15.63	1.60
C. albicans 4	20	0.8	0.31	7.81	1.10

+: Growth, -: No growth

Table 2: Combination of *O. gratissimum* oil and ibuprofen against isolates of *C. albicans*

Drug, Comb.	Org. code	MIC_O	MIC_I	FIC_O index	FIC_I	FIC	Inference
5:0	01	1.30	0	1	0	1	Additive
	02	1.40	0	1	0	1	,
1:4	01	0.50	1.55	0.38	0.78	1.16	Indifference
	02	0.35	1.50	0.18	0.25	1.35	,,
4:1	01	0.10	1.00	0.85	0.50	1.35	Indifference
	02	0.50	0.50	0.26	0.42	0.68	,,
5:5	01	1.30	1.58	1	0.79	1.79	Indifference
	02	0.75	1.00	0.40	0.83	1.23	,,
0:5	01	0	2.00	0	1	1	Additive
	02	0	1.20	0	1	1	,,

O. gratissimum: Ibuprofen combinations. O = *O. gratissimum*. I = Ibuprofen

Table 3: Combination of *O. gratissirmum* oil and ibuprofen against *Trichophyton* and *Aspergillus niger* isolates

Drug, Comb.	Org. code	MIC_O	MIC_I	FIC_O	FIC_I index	FIC	Inference
5:0	01	0.50	0	1	0	1	Additive
	02	1.40	0	1	0	1	,,
	03	0.15	0	1	0	1	,,
	04	0.60	0	1	0	1	,,
	05	0.50	0	1	0	1	,,
	06	0.65	0	1	0	1	,,
1:4	01	0.30	0.25	0.60	0.28	0.88	Synergism
	02	0.50	1.80	0.36	0.92	1.28	Indifference
	03	0.10	1.35	0.67	1.67	2.34	Antagonism
	04	0.05	1.40	0.08	1.27	1.35	Indifference
	05	0.05	1.15	0.10	0.92	1.02	,,
	06	0.40	1.25	0.62	0.66	1.28	,,
4:1	01	0.20	0.25	0.40	0.28	0.68	Synergism
	02	1.00	0.85	0.71	0.44	1.15	Indifference
	03	0.40	0.40	2.67	0.50	3.17	Antagonism
	04	0.25	0.25	0.42	0.23	0.65	Synergism
	05	0.65	0.75	1.30	0.60	1.90	Indifference
	06	0.60	1.60	0.92	0.84	1.76	,,
5:5	01	0.20	0.90	0.40	1.00	1.40	Indifference
	02	0.35	1.40	0.25	0.72	0.97	Synergism
	03	0.10	0.80	0.67	1	1.67	Indifference
	04	0.15	0.75	0.25	0.68	0.93	Synergism
	05	0.15	0.60	0.30	0.48	0.78	,,
	06	0.40	1.12	0.62	0.59	1.21	Indifference
0:5	01	0	0.90	1	0	1	Additive
	02	0	1.95	1	0	1	,,
	03	0	0.80	1	0	1	,,
	04	0	1.10	1	0	1	,,
	05	0	1.25	1	0	1	,,
	06	0	1.90	1	0	1	,,

Organism 01-04 = *Trichophyton* species; 05-06 = *Aspergillus niger*; *O. gratissimum*: Ibuprofen combinations; O = *O. gratissimum;* I = Ibuprofen

Combined effect of *O. gratissimum* oil and diclofenac against isolates of moulds and *C. albicans*: The results shown in Table 4 indicated that with the exception of the combination

Table 4: Combination of *O. gratissimum* oil and diclofenac against isolates of dermatophytes and *C. albicans*

Drug, Comb.	Org. code	MIC_O	MIC_D	FIC_O	FIC_D index	FIC	Inference
5:0	01	1.00	0	1	0	1	Additive
	02	1.35	0	1	0	1	,,
	03	1.25	0	1	0	1	,,
	04	1.25	0	1	0	1	,,
	05	1.35	0	1	0	1	,,
	06	1.35	0	1	0	1	,,
1:4	01	0.45	1.95	0.45	1.19	1.64	Indifference
	02	0.75	1.25	0.56	0.71	1.27	,,
	03	0.35	1	0.28	0.57	0.85	Synergism
	04	0.75	1	0.60	0.61	1.21	Indifference
	05	0.65	1.15	0.48	0.67	1.15	,,
	06	0.50	0.50	0.74	0.28	1.02	,,
4:1	01	1.00	1.25	1.00	1.56	2.56	Antagonism
	02	1.25	1.75	0.93	1	1.93	Indifference
	03	1.12	1.70	0.90	0.97	1.87	,,
	04	1.35	1.75	1.08	1.06	2.14	Antagonism
	05	1.25	1.75	0.93	1.06	1.99	Indifference
	06	1.20	1.75	0.89	0.97	1.86	Indifference
5:5	01	1.00	1.50	1.00	1.88	2.88	Antagonism
	02	1.00	1.30	0.74	0.74	1.48	Indifference
	03	0.90	1.30	0.72	0.74	1.46	”
	04	1.05	1.55	0.84	0.94	1.78	”
	05	0.80	1.30	0.60	0.79	1.39	”
	06	1.00	1.52	0.37	0.84	1.21	”
0:5	01	0.00	0.80	0.00	1.00	1.00	Additive
	02	0	1.75	0	1	1	”
	03	0	1.75	0	1	1	”
	04	0	1.65	0	1	1	”
	05	0	1.65	0	1	1	”
	06	0	1.80	0	1	1	”

Key: 01-08 = *Trichophyton* species; 04-06 = *C. albicans*. *O. gratissimum*: Ibuprofen combinations; O = *O. gratissimum*; I = Ibuprofen

ratio 1:4 which exhibited synergism against isolate of mould (isolate 03), all the other combinations exhibited either antagonism, additive and indifference against the test organisms (moulds and *C. albicans*). However, both drug and oil (alone) exhibited some levels of activity on the test organisms, respectively.

DISCUSSION

Pathogenic fungi cause both superficial and serious systemic diseases and are now widely recognized as important agents of hospital-acquired infection (Douglas, 2003). Medicinal plants have been used for several purposes and are known to inhibit the growth of several microorganisms including fungi. After a downturn in medicinal plants in recent decades (Alper, 1998), the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. World spending on finding new anti-infective agents is expected to increase by 60% from the spending levels in 1993 (Alper, 1998). New sources, especially plant sources, are also being investigated. The MIC of *O. gratissimum* oil and aspirin against the various isolates tested showed that both have greatly pronounced activity against the test organisms. *O. gratissimum* oil exhibited a lower MIC

against *C. albicans* than against moulds showing a high degree of potentiation. *O. gratissimum* oil completely inhibited the growth of one mould isolate tested. Essential oils as well as compounds derived from them possess wide range of activities but the antimicrobial properties are the most studied (Hammer *et al.*, 2003). In 1977, it was reported that 60% of essential oil derivatives examined to date were inhibitory to fungi while 30% inhibited bacteria (Chaurasia and Vyas, 1977). One of the potent antimicrobial compounds isolated from essential oils is lipophilic monoterpens extensively applied as preservatives (Pauli, 2001). It is thus obvious from our data that *O. gratissimum* oil has much greater activity on the test organisms than other test agents. Also, *O. gratissimum* oil was more effective against *C. albicans*. This finding is consistent with data from previous studies (Hammer *et al.*, 2003; Chaurasia and Vyas, 1977; Pauli, 2001).

Many studies have found instances of improved efficacy of certain antimicrobials when they are combined with antimicrobials of other classes (Nakata *et al.*, 1992). Since there is a clinical interest in the use of combinations of antimicrobial agents to improve the spectrum of drug activity, studies with *M. scaber* ethanol extract and *O. gratissimum* oil combined with NSAIDs were performed to determine if synergism, antagonism, indifference or additivity would be the predominant response when the combinations were tested against fungal isolates. These combinations were to determine the possibility of combating fungal isolates through herbal extracts and NSAIDs in some ratios combinations. The paradoxical effect of synergism, antagonism, indifference and additive outcome in drug combinations appears to be dependent on the ratio of drug in combination. In all the antifungal herbal extracts and drugs combinations, ratios 5:0 and 0:5 exhibited additivity against all the isolates tested. In interactions, the presence of one drug alters the pharmacological effect or modifies the pharmacokinetics of another drug. The fungus when exposed to such combinations of these antifungal agents may therefore, be attacked on two fronts. Cases abound where a drug enhances the effectiveness of an antimicrobial agent (Georgopapadakuo and Tkaez, 1995; Esimone *et al.*, 1999). Nikkomycin Z inhibits the formation of new wall material via its competitive inhibition of chitin synthase, while econazole inhibits the biosynthesis of membrane lipids (Esimone *et al.*, 1999). In a previous study by Esimone *et al.* (1999). EDTA was shown to enhance the antimicrobial properties of cetrimide against the fungi by chelating the metal ions essential for the growth of fungi which contributes in inducing fungal stasis. Improvements in the efficacy of antifungal drug therapy and reductions in toxicity may be achieved by using combinations of antifungal agents. The synergism observed between these herbal extracts and NSAIDs may be due to the complementary modes of action of these two compounds. Although, the modes of action of the antifungal activity of these herbal extracts (*M. scaber* ethanol extract, *O. gratissimum* oil) and NSAIDs are still unknown. Sanyal *et al.* (1993) reported that the non-steroidal anti-inflammatory drug ibuprofen exhibits weak antifungal activity against *C. albicans in vitro*. In the current investigation, combining *M. scaber* ethanol extract and *O. gratissimum* with NSAIDs produced some antifungal effect which was significantly synergistic. However, combining these antifungal extracts with NSAIDs may not only result in increased antifungal efficacy, but the recognized anti-inflammatory properties of the drugs could also prove to be clinically useful in the topical treatment of candidiasis and other fungal skin infections because it would help to relieve the pain caused by inflammation (Tariq *et al.*, 1995).

CONCLUSION

This study has demonstrated the differences in the efficacy of antifungal combinations against clinical isolates of moulds and *C. albicans*. The results obtained suggest that the antifungal extracts

(*M. scaber* extract and *O. gratissimum* oil) and some non-steroidal anti-inflammatory drugs (Aspirin and Sodium salicylate) combinations are of potential therapeutic interest and show that the test applied is of potential use in studies on antifungal synergy involving fungi (moulds and *C. albicans*).

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