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IN VITRO STUDIES ON THE POTENTIALITY OF BALANITES OIL IN THE TREATMENT OF SOME SUPERFICIAL MYCOSES

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Abbreviations:
AOCS = American Oil Chemist's Society, BKO = Balanites Kernel Oil, FFA= Free Fatty Acids, PNO = Peanut Oil, UM = Unsaponifiable Matter.

ABSTRACT

Fruits of Balanites aegyptiaca are traditionally used in paste form for curing some skin diseases in few areas in Western Sudan.
Our initial results pointed to the fruit kernel containing the active ingredient. Further studies were meant to test, in vitro, the effect of Balanites fruit kernel oil on three isolates which are known to cause superficial mycoses namely: Microsporum audouinii, Trichophyton soudanense and T. mentagrophytes. The three test organisms were subcultured on sabouraud's medium to which different concentrations of Balanites kernel oil were added. In addition to appropriate controls a normal vegetable oil viz. peanut oil, was tested. BKO, at 5% concentration, showed marked (>70%) growth inhibition against the three fungal isolates tested. No antifungal activity was observed in controls, including peanut oil. Two fractions of Balanites kernel oil were prepared namely: the free fatty acids (FFA) and the unsaponifiable matter (UM), and when tested for antifungal activity with the three test organisms, the activity resided exclusively in the free fatty acid fraction. Initial clinical studies on Balanites kernel oil, currently underway, largely confirmed our in vitro work described here.

INTRODUCTION

Balanites aegyptiaca Del. of the family Simaroubaceae is a wild tree widespread in central Sudan and locally known as "hijlij". The partially edible fruits (Lalaob) are abundantly collected and constitute an item of trade in the Sudanese market. The estimated annual production of fruits in Sudan exceeded 400000 tons [(1).] .
Balanites has been hailed as a useful multipurpose tree for the tropics. It is a good source of wood and a fodder for grazing animals [(2)]. The kernels contain a fixed oil [(2, 3)] that is locally edible. The fruit flesh and kernel have long been considered as potential sources of steroidal saponins, important raw-materials needed

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for the manufacture of steroid drugs [4]. We have studied the latter two products in several Sudanese fruit accession as well as their accumulation during fruit development [3].
Some biological activities of Balanites fruits have been reported in the literature. Archibald (1933) [5] reported on the use of these fruits in the control of Schistosomiasis in Sudan. Hypocholesterolemic [6] and hypoglycemic [7] effects of crude fruit pulp extracts have been demonstrated. Aqueous extracts of Balanites had larvicidal activities against Culex mosquitoes [8].
As far as Sudanese folk-medicine is concerned, Balanites fruits are used to cure several diseases, notably bilharzia, diabetes and skin diseases [3]. For the latter purpose the woody outer parts of Balanites fruits are carefully burnt; the kernels, now roasted, are crushed and the resulting paste is applied as a poultice on infected areas of the skin [3]. In an attempt to investigate this claim we have tested crude extracts and partially purified preparations made from Balanites kernels, in vitro, against three human skin disease-causing organisms.
Kernels of the fruit of Balanites aegyptiaca Del. are traditionally used in folk medicine in Sudan to treat skin diseases. The main objective of this study was to verify such claim. This might lead to the development of alternative antifungal or antibacterial drugs readily accessible for the treatment of these diseases especially in rural areas. A wider global scale impact may also be anticipated.

MATERIALS AND METHODS

Preparation of Balanites fruit kernels:
Mature fruits were collected from tagged trees in Umbaroana Forst in Wad Medani area (Central Sudan). Kernels were separated by cracking the woody outer layer mechanically with or without separation of epicarp and mesocarp.

Test organisms:
Three test organisms, known to cause dermatophytoses and pityriasis versicolor were used. These were; Microsporum audouinii, Trichophyton soudanense and T. Mentagrophytes. These test organisms were isolated from infected humans in the White Nile area of Sudan and purified after culturing in a Medical Laboratory [9].
Balanites kernel crude extracts:
Balanites kernel oil and peanut oil were prepared according to AOCS official methods (1993) [10] in a Soxhlet apparatus using hexane as solvent.

Balanites kernel cake remaining after hexane extraction was re-extracted with ethanol in a Soxhlet apparatus for 5 hours. Preparation of Balanites oil crude fractions:
Two crude fractions were prepared from extracted Balanites oil namely, the free fatty acids (FFA) fraction and the unsaponifiable matter (UM) fraction. Oil saponification was carried out using KOH according to AOCS methods (1973) [11]. First the unsaponifiable matter was separated using chloroform and the remaining aqueous layer was acidified with HCL to a pH value of 5.5, the free fatty acids recovered by n-hexane.
Sensitivity test:

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The three test organisms were subcultured on Sabouraud's medium. Initial test were carried out by flooding petri-plates contiaing Sabouraud's solid medium with the suspension of the test organism separatly. The activity of crude extracts was carried as follows:
Three microfiber glass-discs saturated with crude ethanol extracts of defatted Balanites kernels were transferred to each of the prepared flooded petri-plates as described above. Controls were made by using microfiber glass-discs saturated with pure ethanol.
Three microfiber glass-discs saturated with either Balanites kernel oil (BKO) or peanut oil (PNO) were transferred to each of the flooded petri-plates. Controls were made by transferring micorfiber glass-discs without oils.
Another test was done by inoculating cork-borer-cut discs from peripheries of colonies of the three test organisms on petri-plates with Sabouraud's solid medium containing 5% Balanites kernel oil. The medium without oil was used as control. Balanites kernel oil (BKO) fractions were tested by saturating the microfiber glass-discs with free fatty acid (FFA) fraction or unsaponifiable matter (UM) fraction which were placed in the centre of petri-plates containing Sabouraud's agar, separately. The petri-plates were then flooded with the suspension of the three test organisms separately. Controls contained untreated discs.

RESULTS

Initial tests were carried out by flooding 1 ml of the suspension of each of the three test organisms on petri-plates containing Sabouraud's solid media. Microfibre discs saturated with solvent-free hexane or ethanol extracts of Balanites kernels were placed on the petri-plates. Control discs were treated with only the pure solvent which was subsequently completely removed before bioassay.

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Fig.1(a,b,c) shows clear fungal growth inhibition around discs treated with the hexane extracts (oil) of Balanites kernels, for the three test organisms.On the other hand, kernel ethanolic extracts showed no antifungal activity against the three fungi tested. Only the result observed with T. mentagrophytes is shown (Fig. 2).

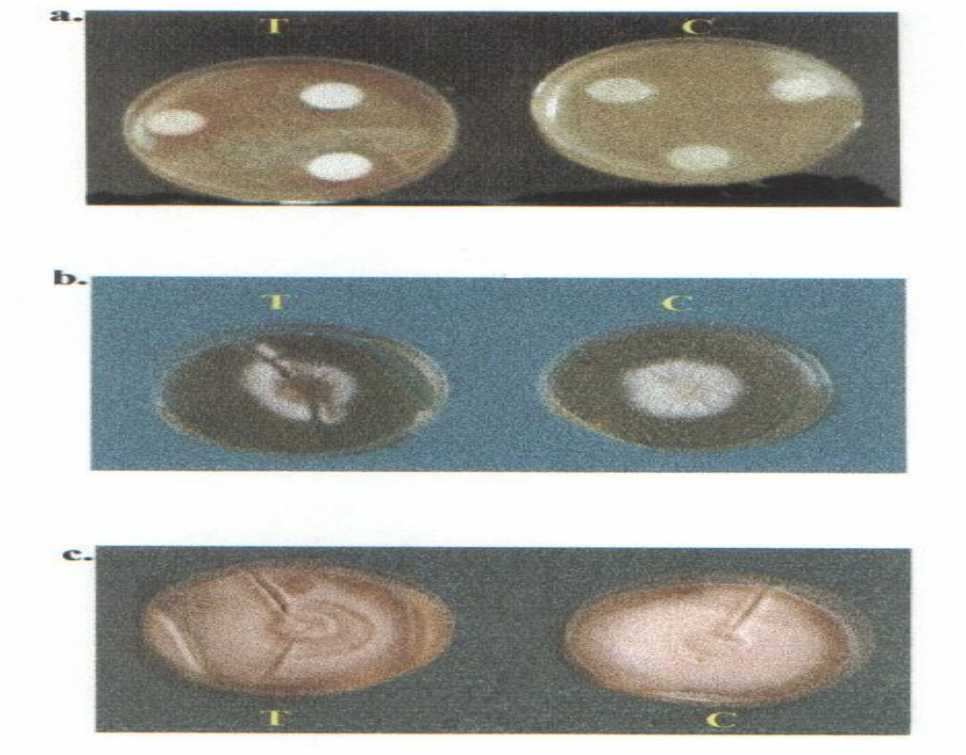

Fig. 1. Antifungal activity kernel oil againsts M. audouinii (a), T. soudanense (b) and T. mentagrophytes (c).

C = control disc(s), T = BKO-treated disc(s).

Note that three replicate discs (a) or one disc (b & c) were used.

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Fig. 2. Effect of ethanolic extracts of Balanites kernel on growth of the fungus T. mentagrophytes (on Sabouraud's medium). T = disc saturated with ethanolic extract (solvent free), C = discs without Balanites kernel extracts.

Balanites kernel hexane extract (oil) thus seemed to exclusively contain the antifungal constituent(s). When an ordianry oil, Peanut oil (PNO), was tested for antifungal acitivty against the three test organisms, using the disc method, no inhibition of fungal growth was observed with T. mentagrophytes (Fig. 3).

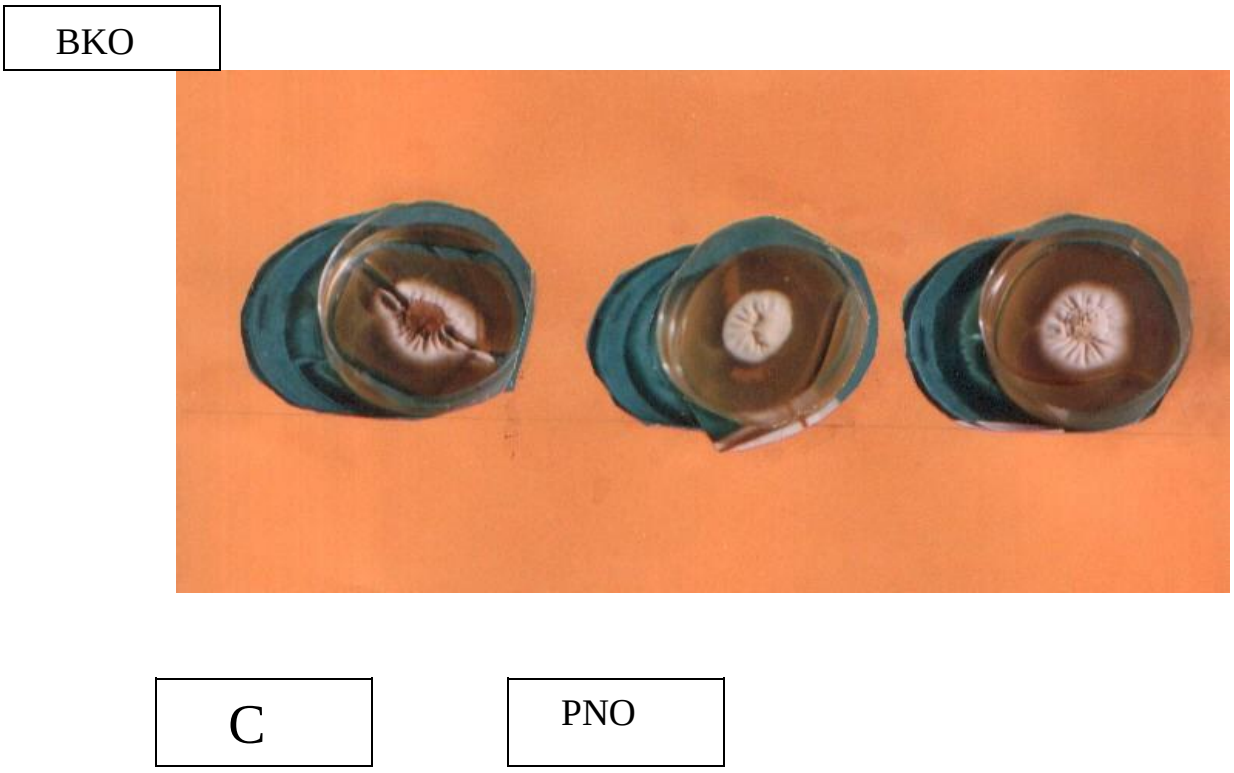

Fig. 3. Effect of PNO on the growth of T. soudanense
Left = BKO, right = Peanut oil, C= control

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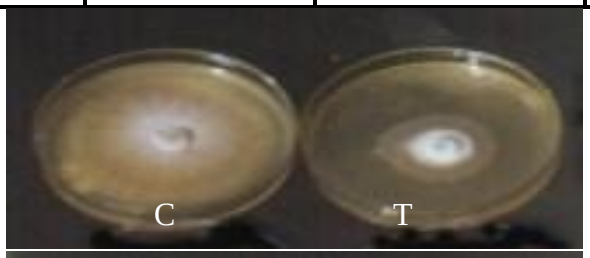
When the three test organisims were subcultured on media containing 5% Balanites kernel oil (BKO) there was more than 70% decrease in colony diameter compared with media containing no BKO (i.e. control), (Fig. 4 and Table 1). The growth of M. audouinii colony diameter decreased by 73.3%, T. soudanense by 75.6% while the colony diameter of T. mentagrophytes decreased by as much as 82%.

Table 1: Decrease % in colony diameter of three fungal isolates grown on Sabouraud's Agar containing 5% BKO shown in Fig. 4.

Test organisms	Colony diameter in mm.		% decrease
	Treated	Control	
M audouinii	1.30 mm.	4.50 mm.	73.30
T. soudanense	1.10 "	4.50 "	75.60
T. mentagrophytes	0.90 "	5.00 ''	82.00

(a)



(b)

(c)

Fig. 4. Decrease in colong diameter of M. audouinii (a), T. soudanense (b)

And T. mentagrophytes (c) grown on Sabrouraud's agar contianing 5% Balanites kernel oil. C = control, T = BKO treatment

Balanites kernel oil was separated into two fractions, by saponification and solvent partitioning, namely, the unsaponifiable matter (UM) and the free fatty acid (FFA) fractions. When suspensions of fungal propagules of the three test organisms were inoculated separatly, on discs saturated with either of the two separated BKO fractions, the three test organisms behaved differently (Fig.5 a, b, c).

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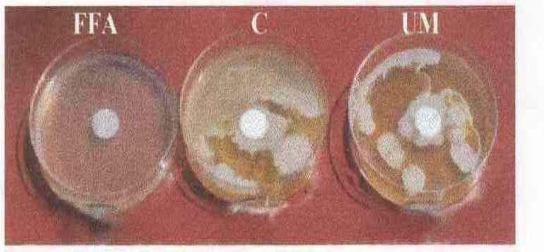
The growth of T. soudanense was completely inhibited by the FFA fraction treatment, the control and the UM fraction treatment showing no inhibition. On the other hand, T. mentagrophytes seems to be least affected by either oil fraction (Fig. 5).

(a)

(b)



(c)

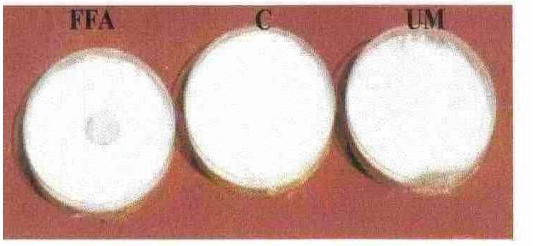


Fig. 5. Effect of the free fatty acid (FFA) and unsaponifiable matter (UM) fractions prepared from BKO on growth of the three test fungi and applied on microfibre discs.
C= control treatment. The three fungal isolates were M. audouinii (a), T. soudanense (b) and T. mentagrophytes (c)

DISCUSSION

Crude BKO (hexane extracts) had considerable antifungal activity, decreasing fungal growth by more than 70% when tested against three human skin diseasecausing fungi, namely, Microsporum audouinii, Trichophyton soudanense and T. mentagrophytes. Treatment of the three fungi with Peanut oil (PNO), common oil (Fig. 3), did not significantly reduce growth, showing that antifungal activity was not due to some physical properties of the oils, but rather to a chemical component in the Balanites kernel extractable by hexane. Thus intact triacylglycerols, normally comprising more than 90% of vegetable oils, would be excluded as the active antifungal ingredient(s).Antifungal activity of Balanites kernel resided exclusively in the hexane extract (the oil fraction); the ethanolic extract of the kernel showing little activity. Ethanol is a good general purpose solvent that is expected to extract, at least partially, most organic plant constituents, and particularly polar compounds. In our hands Balanites kernels were exhaustively extracted with ethanol by the Soxhlet method for several hours. Within the kernel oil extract antifungal activity was exclusively associated with the FFA fraction with very little or no activity detected in the UM fraction. The latter fraction would contain non-glyceride, hexane-soluble compounds such as

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phytosterols and carotenoids, but not the steroidal saponins. Recently Zhang et al. (2005) [12] reported that steroidal saponins of Tribulus terrestris L. demonstrated anti-candida activities. Takchi and Tanaka (1991) [13] claimed antifungal acitivty for the synthetic steroidal saponin, diosgenyl monoglyceride. Steroidal saponins are present in considerable amounts in Balanites kernels, amounting to 5% or more of the kernel weight [3]. These compounds were not extracted by hexane as infra-red spectroscopy of BKO revealed (Fig. 6). We have good reason not to attribute any antifungal activity to Balanites kernel saponins, as ethanolic extracts of Balanites kernel which would contain largely steroidal saponins, had no antifungal activity (Fig. 2). It is known that all fatty acids in the free or salt form have antifungal activity [14]. This would partially explain why antifungal acivity was associatd with the free fatty acid fraction. However, explanation of the remarkable antifungal activity observed with Balanites kernel oil remains to be established. The amount of the free fatty acids inherantly present in the kernel oil was typically low and usually at the level 1% or less [3]. The question arises whether BKO is more prone to hydrolysis (by fungal lipases) under the antifungal test conditions, releasing free fatty acids. Our gas-chromatographic and infra-red spectroscopic analyses of BKO [3] did not reveal the presence of 'unusual' fatty acids, except perhaps for traces of epoxy fatty acids.

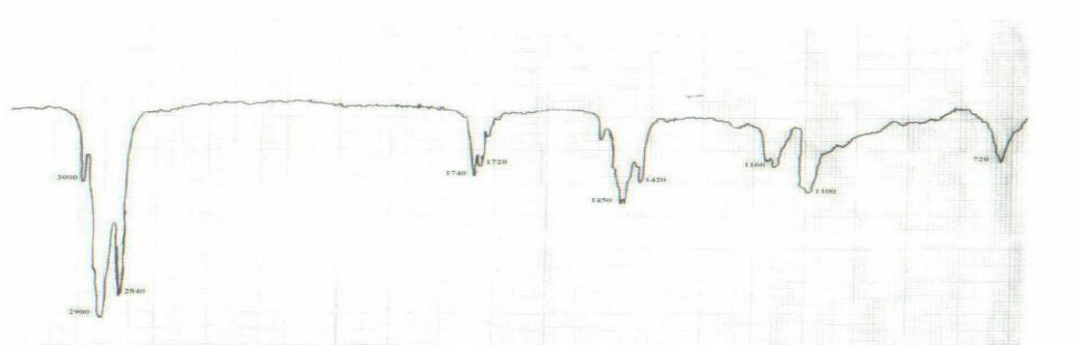

Fig. 6: I.R. absorption spectrum of the whole oil of Balanites kernel. Note the lack of spirostan absorption (expected at 980, 960, 940, 915 and/or 900cm^{-1}).

The anomalous behaviour of T. mentagrophytes, viz, its extreme sensitivity to crude BKO (Fig. 1) and lack of senitivity to the FFA fraction (Fig. 5) may support the finding of Gershon and Shanks (1978) [14] . These authors studied the antifungal activity of several homologues and derivatives of fatty acids against Aspergillus niger, Trichoderma viride, Myrothecium verrucaria and Trichophyton mentagrophytes. They found that the first three mentioned organisms were markedly less affected by the fatty acid methyl ester than by the corresponding free acid. With T. mentagrophytes, the ester form was quite fungitoxic, equal in toxicity to the free acid form. Gershon and Shanks (1978) [14] attributed this to differences in the membranes of T. mentagrophytes, compared to the other organisms they tested, allowing for pentetration of the more lipid soluble esters. These authors used monocarboxylic acid esters while in our case we were dealing with triacylglycerol esters.
Further work is underway to clarify the nature of the antifungal active ingredient present in BKO and to proceed to clinical trials.

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