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Original Research Article

Evaluation of Antimycotic activity of crude methanolic extract of *Mitrocarpus scaber* on *Candida albican* and *Trichophyton mentagrophytes*

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Abstract: *Mitrocarpus Scaber* is a local herb traditionally used for the treatment of eczema, ringworm and other skin disorders. Dry crude *methanolic* leaf extract was obtained, weighed and dissolved in double distilled deionized water giving a concentration of 20mg/ml solution. Standardized solution of *Nystatin* and *Griseofulvin* were prepared from commercial tablets and used as standard controls. Sterilized Sabauroud's dextrose agar contained in universal bottles containing *chloramphenicol* were divided into groups of 5 in which variable numbers of drops (0.05ml) of the test drug (*Griseofulvin, Nystatin* and *Mitrocarpus scaber)* were made before the bottles were thoroughly mixed and slanted. Purified identified culture of *Candida albicans* and *Trichophyton mentagraphytes* were obtained and used as test cultures. Each of the test agar slants were inoculated with *Trichophyton. mentagraphtes* in one group and *Candida albicans* in the other. The bottles were incubated at 37^0C for three to four days before observing them for growth of the inoculated organisms. No growth in the test bottles was considered as positive inhibition. The experiment was replicated three times and results were recorded. The results indicated that *Mitrocarpus* extract inhibited the growth of both *Trichophyton mentragraphytes* and *Candida albicans* at the agar concentration of 4mg/ml. nystatin was inhibitory to *T. mentagraphytes* at 7mg/ml and *Candida albicans* at 1mg/ml but did not inhibit the growth of *Candida albicans*. It is suggested that further research into the fungistatic effect of *Mitrocarpus scaber* to be carried out to explore its values in developing drugs for treating both human and veterinary *dermatomycosis*
Keywords: *Mitrocarpus Scaber*, Nystatin, *antifungal, Candida albicans*

INTRODUCTION

The history of herbal remedy is closely linked with conventional medicine and is called botanical medicine or phyto medicine. [1] It dates back to thousands of years and refers to the use of plant parts such as seeds, berries, roots, leaves, bark or flower for medicinal and therapeutic purposes [2]. Human beings have been aware of the medicinal use of a wide range of healing plants as far back as the early days of Paleolithic society [3]. Over time, there has been an increase of awareness of the importance of preserving traditional medicinal herbs and effects are being made to integrate orthodox and traditional medicine to remedy various human and animal ailments [4].

In Nigeria, many plants have been known to be rich sources of raw materials for traditional human medicine. Over 92 plants have been identified and used in Ethno-veterinary practices some of which have been shown to possess pesticidal or insect repellants activities [5].

Fungal (*mycotic)* diseases, or mycosis, are categorized as superficial (cutaneous), subcutaneous and systemic, depending on the tissues or organs involved [6]. Such infections can also be of *zoonotic* importance. Several medications have been used to treat fungal infections with varying degrees of success. Traditional medicine often used by people in Nigeria include, kerosene, and hydraulic fluid, which often lead to doubtful results and rates of reoccurrence of the treated infections have more often been high [3].

Mitracarpus scaber is a local herb that is fairly widespread and commonly available in Nigerian farmlands. It is traditionally used for the treatment of eczema, ringworm and other skin disorders in its crude form Gbaguidi [7] evaluated the anti-microbial effect on the crude methanolic extract of a plant on a skin pathogen *Dermatophilus congolensis* and later, Bisignano *et al.*[8] successfully fractioned the methanolic extract and isolated seven separate compounds, that possess antibacterial and *antimycotic* properties. However, since most of the effective proprietary drugs are expensive and difficult to come

by, the reach for cheaper, effective and easily available alternatives are therefore necessary.

The aim of the present study is to evaluate the *anti-mycotic* effect of the crude mehanolic leaf extract of *Mitracarpus scaber* and to determine the in vitro minimum inhibitory concentration (MIC) of the extract on *Trychophyton mentragraophytes* and *Candida albicans.*

MATERIALS AND METHODS

Equipment

Bunsen burner flame, universal sample bottles, droppers, pipette, syringe, porcelain mortar and pestle, conical flasks, incubator.

Agar Base

Four and a half (4.5) grams of sabaroud's Dextrose Agar (SDA) base (45g/L) was weighed on a mettle weighing balance and poured into a 100ml conical flask and water was measured and added. The flask containing the agar was heated over gauze and Bunsen burner flame to dissolve while agitating to prevent charring.

The dissolved agar was sterilized by autoclaving for 15min at 121^0C. After sterilization, it was allowed to cool to 47^0C on the bench and 10ml each was dispensed into 20ml sterile universal bottles. The bottles were grouped into five per *dermatophyte* sample. In group one 0.05ml of *nystatin* was placed in the first bottle. In the remaining four bottles, the number of drops (0.05ml) was increased so that the last bottle in the group had five drops. This was repeated in the subsequent groups (group 2 using *Greseofulvin* and group 3 with *Metracarpus* extract). All the bottles were slanted to solidify before being kept in the refrigerator at 4^0C for 24 hours.

Using a Pasteur loop, a scoop of the *Trichophyton mentagrophytes* isolate was taken to inoculate each test sample. The same procedure was repeated using *Candida albicans*. These were placed in the incubator at 37^0C for 3-4 days before observations for growth were taken by counting the bottles that showed evidence of growth.

Preparation of Nystatin

A commercial sugar quoted tablet 500,000 IU (Mekophar chemical pharmaceutical Joint-Stock Company, Vietnam) was obtained from the Faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria. The tablet was ground in a porcelain mortar and pestle. 1gram of 2% *tragacanth* was added to the ground tablet as a dispenser to obtain a clearly dissolved solution with a final concentration of 50,000 IU *Nystatin* in 25ml of distilled de-ionized water.

Preparation of Griseofulvin

A commercial table of *Griseofulvin* 500ml/tablet (Embassy Pharmaceutical and Chemical Company Ltd. Lagos Nigeria) was similarly treated as *Nystatin* above, in 25ml of double distilled de-ionized water giving a concentration of 20mg/ml.

Preparation of Mitrocarpus Scaber

Mitrocarpus Scaber leaf extract was obtained from the faculty of Pharmaceutical Sciences Ahmadu Bello University, Zaria. One (1) gram of the extract was weighed and placed into a mortar. *Tragancanth* (2% 1gm powder) was added as in the two previous drugs, mixed thoroughly and 50mls of double distilled de-ionized water was added to form a concentration of 20mg/ml.

For each of the five (5) test graded bottles (*Nystatin*, *Griseofulvin* and leaf extract of *Mitracarpus Scaber*), a loopful of previously isolated *Trichophytes mentagrophytes* and *Candida albicans* were inoculated in each of the five paired concentrated graded test bottles. Each of the two *dermatophytes* tested were duly labeled and incubated at 37^0C for 72 to 96 hours. The bottles were observed for inhibition of growth of the two fungal organisms by checking for clarity of SDA surfaces in the test bottles and results were recorded as no growth (-) showing inhibition at the labeled concentration.

RESULTS

Minimum Inhibitory Concentration (MIC)

The results of MIC determination are shown in Table 1 and 2 below. Treatment for the determination of the MICs of *Mitracarpus Scaber* extract, *Griseofulvin*, and *Nystatin* on *Candida albicans* and *Trichophyton mentagraphytes* were determined using solid media impregnation in universal bottles. The experiment was replicated thrice to achieve a stable result, which were recorded accordingly. The results were averaged and tabulated.

The MIC results show that the test organism *Mitracarpus scaber* inhibited the growth of both *Trichophyton* and *candida* at 4mg/ml, while the commercial *nystatin* inhibited Candida *albicans* at 1mg/ml and *Trichophyton* at 8mg/ml. the results showed that *griseofulvin* did not show any observable inhibition to Candida *albicans*, whereas *Trichophyton mentagraphytes* was inhibited at 7mg/ml.

Table-1: Showing the Growth of *Candida Albicans* on various Concentration of the anti-Mycotic Drugs

Concentration Mg/ml	Mitracarpus Scaber	Griseofulvin	Nystatin
10	_	+	_
9	_	+	_
8	_	+	_
7	_	+	_
6	_	+	_
5	_	+	_
4	_	+	_
3	+	+	_
2.5	+	+	_
2	+	+	_
1.5	+	+	_
1	+	+	_
0.5	+	+	+

Keys
+: Growth of Candida as white cotton wool
-: No growth (inhibition of growth by drugs)

Table-2: Showing the Growth of *Trichophyton Mentagrophytes* on Various Concentrations of the *Anti-Mycotic* Drugs

Concentration Mg/ml	Mitracarpus Scaber	Griseofulvin	Nystatin
10	_	_	_
9	_	_	_
8	_	_	_
7	_	+	_
6	_	+	+
5	_	+	+
4	_	+	+
3	+	+	+
2	+	+	+
1	+	+	+

Keys:
+: Growth of Candida as white cotton wool
-: No growth (inhibition of growth by drugs)

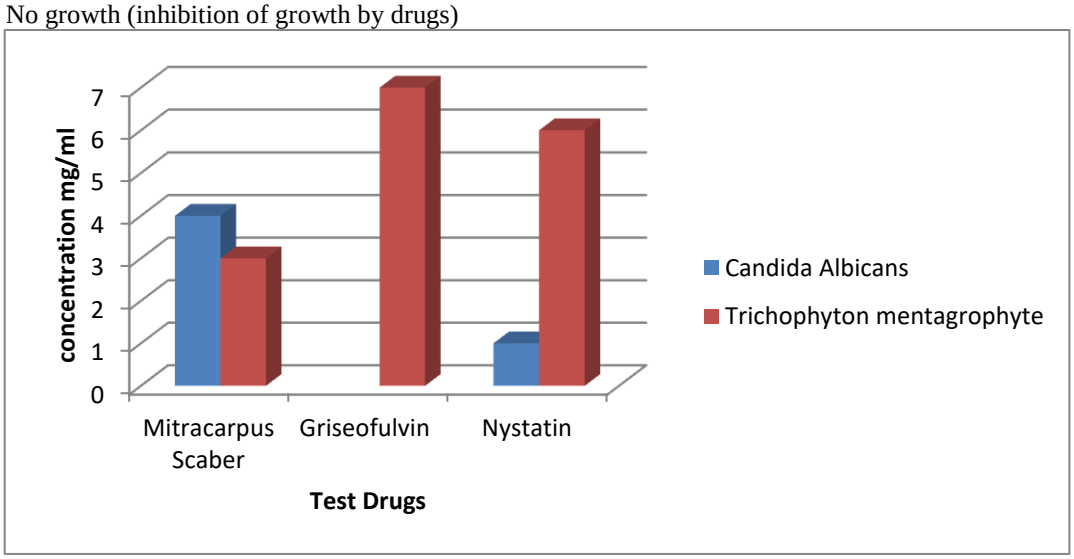

Fig-1: Minimum inhibitory concentration of Mitracarpus Scaber, Griseofulvin, Nystatin on Cadida Albicans and Trichophyton mentagrophytes

DISCUSSION

From the result obtained using the crude methanolic extract, it was observed that the *MIC* for *Mictracarpus scaber* against *Candida albicans* and *Trichophyton mentagrophytes*, was 4mg/ml. *Nystatin* however was observed to inhibit the growth of *Candida albicans* at 1mg/ml and *Trichophyton mentagrophytes* at 7mg/ml. *griseofulvin* was not observed to have any inhibitory effect on the growth of Candida albicans but inhibited the growth of *T. mentagrophytes* a 8mg/ml. in conclusion, it is therefore reasonably to state that *Mitracarpus scaber* should be regarded as a fungistatic agents but it must be borne in mind that in vitro experimental result must be interpreted with caution because in vivo experimental results most often influenced by many factors that may include tissue absorption, detoxification by body *microsomal* enzymes and availability of the test drug at the site of infection with the micro-organism [9]. In view of the fungistatic nature of *Mitracarpus scaber*, it is therefore recommended, that further in vitro studies be carried out on this plant extract.

From the above results *Mitracarpus scaber* plant extracts should be regarded as an effective fungicidal agent when compared to commercial drugs such as *nystatin* and *griseofulvin*. The plant has been observed to contain compounds like *Methoxyacetophenone 3,4,5-trimethoxyacetophenone* that have fungicidal properties [10]. In an earlier study, Benjamin and Hugho, [11] isolated a coumerin-like compound reported to be responsible for an anti-fungal activity. In another report by Moulis *et al*., [12], *pentagonin* a *naphaquiniod* pigment of the plant isolated from the aerial part of the plant, demonstrated some fungistatic activities.

Nystatin that is primary used in the topical therapy of superficial *candidiasis* [13] was observed to be very effective at 1mg/ml on *Candida albicans* but its activity was quite slow on *Trichophyton* indicating that despite its in vitro activity on Candida in vivo topical therapy on *dermatophytes* infections is ineffective [13].

Griseofulvin is a conventional drug used in the treatment of superficial *dermatomycosis*, the activity of which is attributed to its ability to inhibit the synthesis of *hyphal cell* wall by the destruction of the *cytoplasmic* microtubules [14]. Systemic administration of drugs may for several reasons be efficacious even when topical application is not. This is because when *griseofulvin* is administered orally metabolites with more potent pharmacological activity are produced and deposited on various body parts before exacting their activity. This may partly explain why *griseofulvin* showed no effect on Candida with only a slow activity on *Trichophyton.*

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