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Investigation of Antifungal Activity of *Moringa Oleifera* and *Hyphaene Thebaica* Extracts

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ABSTRACT

Background: Plants may provide a promising source of bioactive compounds that could be used to control infectious diseases.

Objective: This study was undertaken to explore antifungal activities of two plant extracts.

Methods: Antifungal activity was determined by the aid of disc diffusion method against three references fungal strains, using different dilution (5, 25, 50, 100, 250 µg/ml).

Results: Inhibition zone of the extracts was compared with different standards (Nystatin and Griseofulvin). Our study illustrated the occurrences of antimicrobial efficacy.

Conclusion: The results of the present study have shown that *Moringa oleifera* and *Hyphaene thebaica* possess a promising antifungal activity. Hence further surveys prerequisite to isolate the effective constituents responsible for this activity.

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Introduction

The emergence and dissemination of multiple drugs represents a great challenge which encourages scientists and researchers towards utilization the products in the nature as a source of new medicine candidates that can be a promising alternate to the already existing medicine. Unfortunately, the antibiotic resistance exerts a detrimental effect on the effectiveness of these agents and consequently affects healthcare outcomes and expenses [1]. Research has been directed to the determine the efficacy of a wide range of plants to be used as antimicrobial agents [2,3].

Moringa oleifera (miracle tree or drumstick tree) is native to India and recently distributed in different parts of the world [4-6]. It consists of nutritive components such as minerals, amino acids proteins, vitamins and β-carotenes [7]. The leaves of *Moringa oleifera* contain a plethora of such alkaloids, saponins, tannins,

steroids, phenolic acids, glucosinolates, flavonoids, and terpenes [8].

Many pharmacological activities have been done to investigate the therapeutic potential of *Moringa oleifera* including cardiovascular activity, anti-inflammatory activity [10], antihypertensive activity, antioxidant activity, anticancer activity, hepatoprotective and nutraceutical activity anti-allergic activity, antimicrobial [9-18].

Hyphaene thebaica (Doum fruit) exists in Western India parts of Africa and Arabian Peninsula [19,20]. It is an origin of fundamental minerals such as calcium, sodium, magnesium, phosphorus and potassium [21]. Moreover, fruits contain nutritive principles including vitamins, carbohydrates, and fiber. Also the fruit contains tannins, saponins, hydroxy cinnamates and Flavonoids [20,22]. The plant possesses a variety of pharmacological properties such as antimicrobial, anticancer, hyperlipidemia, antioxidant, anti-inflammatory, and antidiabetic effects [19,23].

The current study was conducted to confirm the antifungal potential of ethanolic extracts of *Moringa oleifera* leaves and *Hyphaene thebaica* fruits against reference fungi.

Materials and Methods

Collection of Plants Materials

Leaves of *Moringa oleifera* and the fruits of *Hyphaene thebaica* were collected from various areas of Western and Northern districts, Sudan respectively. The identification of the plants was accomplished in the department of Pharmacognosy, Medical Research Centre, and Khartoum, Sudan. Collection based on ethno botanical information survey. The plant materials were labeled by numbers and date.

Preparation of Plants Extract

Preparation and extraction of the *Moringa oleifera* leaves and *Hyphaene thebaica* fruits was conducted as in [24]. The plants were subjected to drying and grinding by a mechanical grinder. (30.0 g) of *Moringa oleifera* and (35.0 g) of *Hyphaene thebaica* powders were defatted by petroleum ether (60-80°C), followed by 900 ml of ethanol by the aid of Soxhlet extractor for 72 hours at a temperature not more than the boiling point of the solvent. The extracts were filtered by Whatman filter paper (No.1) while still hot, concentrated by rotary flask evaporator and dried in a desiccator. The ethanolic extracts yield dark greenish solid residue weighing 8.50 g (24.28% w/w) from *Moringa oleifera* leaves and dark brownish highly viscous semi-liquid residue weighing 7.2 g (24.0% w/w) from *Hyphaene thebaica* fruits. The process was continued to get more extracts. The extracts were then placed into sterile bottles and kept at a refrigerator to be used for biological tests. The crude extracts of ethanol were adjusted to have a concentration expressed as (mg/ml) to be used for antifungal testing.

Test Microorganisms

Three fungal reference strains including *Candida albicans* (MTCC 1345), *Aspergillus niger* (MTCC 2211) and *Aspergillus clavatus* (MTCC 1126), were selected depend on their clinical importance. These strains were provided by Microbiology department, Faculty of Dentistry, Jazan University, Saudi Arabia.

Antifungal Efficacy.

Determination of Zone Inhibition

The antifungal efficacy was investigated by using agar disk diffusion method [25]. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. The dilution of (5, 25, 50, 100, and 250 µg/ml) of each extract and reference drugs were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator strains (108 cfu) and allowed to stay at 37°C for 3 hours. Control tests were performed under similar conditions by using Nystatin and Griseofulvin as authentic samples. The extracts were then examined for their antimicrobial activities versus *Candida albicans*, *Aspergillus niger* and *Aspergillus clavatus*. The inhibitory zones were recorded after incubation at 28°C for 48 to 96 hours

Determination of Minimum Fungal Concentrations (MFCs)

The minimum fungal concentrations were examined by serial sub-cultivation of a 2 µl into micro titer plates containing 100 µl of broth per well and further incubation 72 hours at 28 °C. The lowest concentration with no visible growth was defined as MFC indicating 99.5% killing of the original inoculum. A commercial standard, Fluconazole (Sigma), was used as positive controls (1–3000 µg/ml) for fungi. All experiments were performed in duplicate and repeated three times.

Results

The antimicrobial efficacy of the *Moringa oleifera* leaves and *Hyphaene thebaica* fruits extracts were examined using various concentrations (5, 25, 50, 100, and 250 µg/ml) versus *Aspergillus niger*, *Aspergillus clavatus* and *Candida albicans*.

The antifungal efficiency was estimated in terms of zone inhibition. The antimicrobial efficacy raised with increase in the concentration of extracts (µg/ml). Compared with standard our finding revealed that *Candida albicans* showed good results compared with *Aspergillus niger* and *Aspergillus clavatus*. Inhibition zones measured ranged from 10 to 30 mm for all the sensitive fungi. The extracts of *Moringa oleifera* leaves and *Hyphaene thebaica* fruits found to be more efficient versus all examined microbes. [Figures 1-3, Table 1].

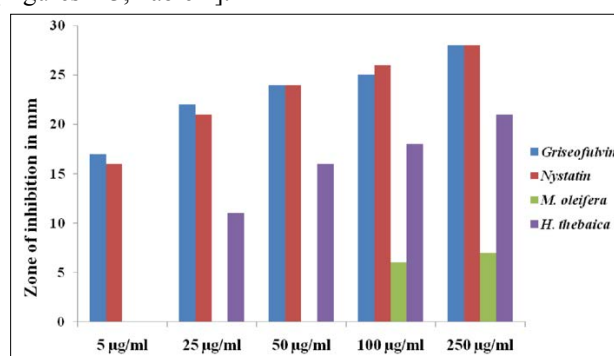


Figure 1: Antifungal Activity of *Moringa Oleifera* and *Hyphaene Thebaica* Extracts Against *Candida Albicans* (MTCC 1345) Compared to Standard Drugs

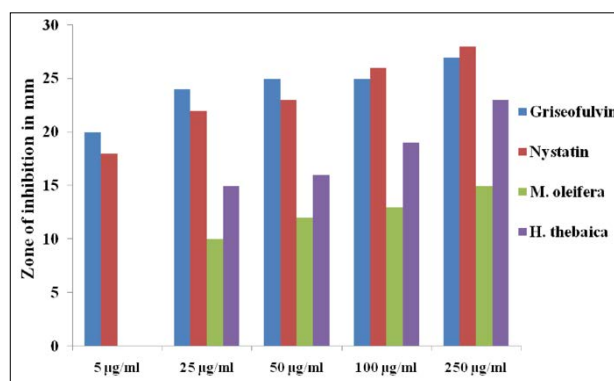


Figure 2: Antifungal Activity *Moringa Oleifera* and *Hyphaene Thebaica* Extracts Against *Aspergillus Niger* (MTCC 2211) Compared to Standard Drugs

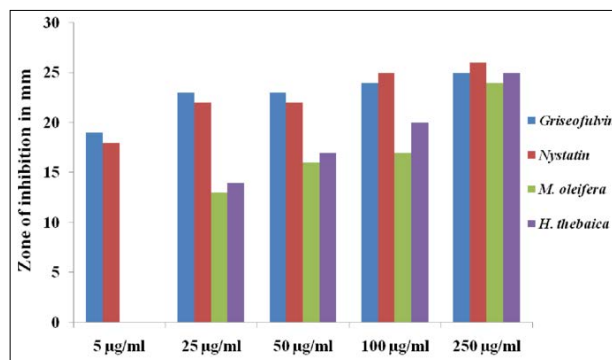


Figure 3: Antifungal Activity of *Moringa Oleifera* and *Hyphaene Thebaica* Extracts Against *Aspergillus Clavatus* (MTCC 1126) Compared to Standard Drugs

Table 1: Minimum Inhibition and Fungal Concentrations (MFC) of *Moringa Oleifera* and *Hyphaene Thebaica* Extracts on *Aspergillus Niger* and *Aspergillus Clavatus*

Plant tested	<i>A. niger</i>		<i>A. clavatus</i>	
	MIC	(µg/ml) MFC	MIC	MFC
<i>Moringa oleifera</i>	44.2	56.0	28.6	42.2
<i>Hyphaene thebaica</i>	32.4	38.8	33.3	46.3

Discussion

The study aimed to explore the prominence utilization of the medicinal plant. The use of medicinal plants as antimicrobial agents has increased [26-28]. A great proportion of people around the world tend to use plants in health care [29,30].

The ethanolic extracts of *Moringa oleifera* exerted a strong to moderate to no activity towards the examined fungi. The antifungal activity of *Moringa oleifera* may be attributed to certain constituents including tannins, phenolic compounds and flavonoids [31,27,32,33].

Our finding showed that the ethanolic extracts of *Hyphaene thebaica* exhibited a strong efficiency versus all the tested fungal strains. Similar results were obtained by other research workers [34,35].

The antifungal activity of *Hyphaene thebaica* extract was ascribed to certain compounds present therein such as anthocyanins, saponins, flavonoids, and tannins [36].

Our study has shown that *Moringa oleifera* and *Hyphaene thebaica* possess a promising antifungal activity and it may provide some insights to the possible use of these plants for treating different infectious diseases. Hence further studies should be directed towards bioassay guided -fractionation and characterization of the active principles responsible for the antifungal activity [37].

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