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Research Article

Majidea zanquebarica- A Potential Source of Antidermatophytic Agent

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ABSTRACT

Development of more effective and less toxic antifungal agents is required for the treatment of dermatophytosis. Plants and their extract preparations have been used as medicines against infectious diseases. The fractionized Hexane and methanolic extracts taken from the aerial parts (bark, pod, seed and leaf) of *Majidea zanqubarica* have been evaluated against two human pathogenic dermatophytes namely *Trichophyton metagrophytes* (ATCC - 28185), *T. rubrum* (ATCC - 2818810) and one opportunistic fungus *Candida albicans* (ATCC -10231). The antibiotic ketoconazole was used to compare the inhibitory activity of the extracts. In the antifungal activity, the hexane bark extract of *M. zanqubarica* showed good inhibitory effects in almost all the fungi tested. The activity of the different part extracts against the test pathogens in terms of inhibition zone diameter in decreasing order was as follows: Bark > Pod > Seed > Flower > Leaf. These results show that the hexane bark extract of *M. zanqubarica* is promising in the treatment of dermatophytosis, as an alternative in the development of a new therapy.

KEY WORDS: Antifungal agents, Antidermatophytes activity, cellular changes, Dermatophytes, Dandruff, *Majidea zanqubarica*

Introduction

Plant materials remain an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds1.

Infectious fungal skin diseases are most common throughout the tropical and subtropical region of the world [2, 3].

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'ringworm' or 'tinea', caused by a group of keratinophilic fungi 'dermatophytes' pose a serious concern3. It involves superficial infections of the keratinized tissue of the skin, hair and nails of animals and human beings3. Owing to limited antimicrobial spectrum of most of the drugs, emergence of multidrug resistant strains and serious ill effects of the current drugs, treatment of such cutaneous infections is quite challenging. Plants are considered as a major source of medicinal constituents. The efficacy and safety of herbal medicines have turned the scientific community towards medicinal plant's research. To check the microbial diseases, extensive research on herbs, carried out throughout the world has revealed that some plants possess antimicrobial ingredients in them [5 – 8].

Microbial resistance to antibiotics in use nowadays, provides the need for the search of new compounds with potential effects against pathogenic bacteria [9,10]. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immune suppression etc.) and are major burning global issues in treating infectious diseases [11]. Although pharmacological industries

had produced considerable number of commercial antibiotics time to time but resistance in pathogens towards these drugs too has increased at high rate and multi drug resistant microorganisms have improved the situation. In the present scenario, there is an urgent and continuous need of exploration and development of cheaper, effective new plant-based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines [12, 13].

Sapindaceae family is known for its traditional medicinal uses as a diuretic, stimulant, expectorant, natural surfactant, sedative, vermifuge and against stomachache and dermatitis in many parts of the world. Chemical investigations of this family have led to the isolation of saponins, diterpenes and flavonoids, among other secondary metabolites. Several saponins and acyclic sesquiterpene and diterpene oligoglycosides have been isolated as main secondary metabolites of several Sapindaceae species used in traditional oriental medicine [14]. With the above background, hither to unexploited species of Sapindaceae, *Majidea zanquebarica* J. Krik. ex Oliv. (Sapindaceae) syn. *Harpullia zanquebarica* has been selected for the antidermatophytic activity. *Harpullia* are used as fish poison, as antirheumatic, and to prevent leech bites [15] skin troubles, gastrointestinal complaints, rheumatic pains [16], since there is no report available about this valuable indigenous plant, antidermatophytic activity has been carried out on various aspect to like know the efficacy of *M. zangubarica*.

Material and Methods

Collection and identification of the plant

The source of material is *Majidea zanquebarica* J. Krikex Oliv. (Sapindaceae) was collected from in and around Coimbatore District, Tamil Nadu, India and authenticated by Botanical Survey of India (No. BSI/SRC/5/23/10-11/tech-671).

Preparation of extracts

The dried powders of aerial parts (barks, pod, leaf and seeds) (2 Kg) were soaked individually at room temperature in hexane for 72 hrs. The extract was suction filtered using Whatmann filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and concentrated at 40°C to 45°C under reduced pressure using vacuum rotary evaporator type 350. The concentrated crude methanol extract was subjected to antidermatophytic studies [17, 18]. In vitro Antidermatophytic activity was done by agar well diffusion method.

Test organisms (dermatophytes)

The cultures of dermatophytes *Microsporum gypseum* (ATCC 241025) and *Trichophyton rubrum* (ATCC 2818810) were procured from the American type culture collection (ATCC) Rockville, U.S.A, and the pathological strains were procured from the Department of Dermatology, Sri Ramachandra Medical College and Research Institute, Porur, Chennai, Tamilnadu, India. *Trichophyton mentagrophytes* (MTCC 8476), *Malassizia furfur* (MTCC 1374) and *Candida albicans* (MTCC 227) obtained from Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Type Culture (IMTECH), Chandigarh, India. The cultures were maintained on freshly prepared Sabouraud's Dextrose Agar (SDA) medium, procured from Hi Media Laboratories Pvt. Ltd, Mumbai, India at 4°C on slants and subcultured at regular intervals.

Procedure

The antidermatophytic activity of the crude hexane and 70% methanolic extract of aerial parts (barks, pod, leaf and seeds) were determined by agar well diffusion method [19]. Sabouraud's Dextrose Agar plates were inoculated with 0.2 ml of overnight grown culture of each test fungal / suspension containing 1.0 X 109 cells. The plates were evenly spread out with the help of a sterile cotton swab. Agar wells were prepared by scooping out the media with a sterile cork borer (7 mm in diameter). Each wells were loaded with 100 µl of the crude extract dissolved in DMSO. Ketoconazole was used as positive control while, agar wells filled with only DMSO was used as negative control. The plates were incubated at 37±1°C for 24 h. The inhibition percentage was calculated as

$$\text{Inhibition percentage} = \frac{I}{90} \times 100$$

Where, I = Diameter of the inhibition zone

90 = Diameter of the medium used

For the hexane and 70% methanolic extracts of bark, pod, leaf and seed 50 µg/ml concentrations were used. The same concentrations of the antibiotic ketozonazole were used for comparison. The same amount of DMSO used in the preparation of the concentrations of the extract was added

to the control. The experiments were carried out in triplicates.

Antifungal activity was determined by measuring the diameter of the inhibition zone surrounding microbial growth. For each strain, controls were included that comprised pure solvents instead of the extract [20].

Zone of inhibition	Inhibitory activity
>17	+++ Strong
12-16	++ Moderate
7-11	+ Weak
6 or 0	- Negative

Poison plate method

Extracts that showed excellent activity were further tested against the fungal pathogens using different concentrations. 25, 50, 75 and 100 µg/ml of extracts as used by Poison plate method by [21], was spread over each SDA plate using an L-shaped sterile glass rod under aseptic condition in a laminar air flow. Then, a mycelial plug of 6 mm in diameter was taken from a 7 day old culture, using a sterile cork borer. Then, the culture was seeded in the center of each SDA plate. The inoculated plates were incubated for 72 h at 37°C. The stock solutions of the extract (1000 µg/ml of DMSO) were prepared. From the stock solution of the extract, the required amount was added to the SDA medium in the bearable warmth to get required concentrations. For comparison, ketoconzole was used in 50 µg/mL concentration for the extracts. The antifungal activity of the extracts was determined by measuring the diameter of the inhibition zone around the well that was filled with the extract.

Activity index determination: the activity index of the extracts was determined by the following formula [22].

$$\text{Activity index} = \frac{\text{Inhibition zone of extract}}{\text{Inhibition zone of standard}}$$

Percentage inhibition: Percentage inhibition was calculated according to the following formula [23].

$$\% \text{ inhibition} = \frac{\text{Inhibition zone in mm}}{\text{Control*}} \times 100$$

*Growth zone is equal to plate diameter i.e. 90 mm as growth occurs all over the agar plate.

1.3.4. Microscopic examination

The high activity extract of *M. zanquebarica* before and after treatment with extracts cells were stained with Aman's lactophenol cotton blue solution and were micro photographed in compound microscope to view the changes in the hyphal and conidial cells of sporulating colonies.

Aman's Lactophenol cotton blue solution

Phenol crystals	- 20.0 g
Lactic acid	- 20.0 g
Glycerine	- 40.0 ml
Cotton blue	- 0.05 g
Distilled water	- 20 ml

For the extract treated hyphal cells, amount of cotton blue was doubled as the cells poorly stained with the normal solution. Semi-permanent slides were prepared and micro photographed under a bright field microscope. The control and treated samples were mounted in dist. water on a clean, dust free, grease free thin slide and micro photographed under a compound microscope.

Statistical analysis

The data were reported as mean±standard deviation (n=3). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5% level significance was employed. The P value <0.05 were considered as significant [24]. All the data were subjected to Duncan's Multiple Range Test (DMRT). It was done using the SPSS Version 2007 WINSAT software.

Sharp and Dange, 2020

Results

The activity of plant extract may vary according to their nature of active ingredients in the plant. Successful prediction of bioactive components from plant materials is largely dependent on type of solvents used in the extraction procedure. Two different extracts (hexane and 70% methanol) from bark, pod, seed and leaf of *M. zanquebarica* were tested against dermatophytic fungal pathogens *M. furfur*, *T. rubrum*, *T. mentagrophytes*, *M. gypseum* and *C. albicans* using agar well diffusion method. *M. zanquebarica* exhibited broad spectrum of antifungal activity (Table 1 and 2; Figure I and II). The activity of plant extract may vary according to their nature of active ingredients in the plant. Successful prediction of bioactive components from plant materials is

largely dependent on type of solvents used in the extraction procedure.

Table 1 Antidermatophytic activity of bark and pod hexane and 70% methanol extract of *M. zanquebarica*

Extract 50mg/ml	Zone of inhibition (mm)				
	Malassezia furfur	***Trichophyton mentagrophytes***	***Candida albicans***	***Trichophyton rubrum***	***Microsporum gypseum***
Hexane bark	35.56±0.49	21.4±0.96	19.6±0.52	21.23±0.25	24.5±0.5
Hexane pod	12.12±0.41	0.0	16±0.2	0.0	0.0
70% methanol bark	31.63±0.40	17.46±0.35	20.56±0.51	18.46±0.41	23.43±0.40
70% methanol pod	12.26±0.30	10.96±0.45	18.63±0.40	6±0	8±0
Positive control (Ketoconazole)	34.56±0.49	23±0.2	16.36±0.40	20.06±0.20	22.43±0.40
Negative control (DMSO)	-	-	-	-	-

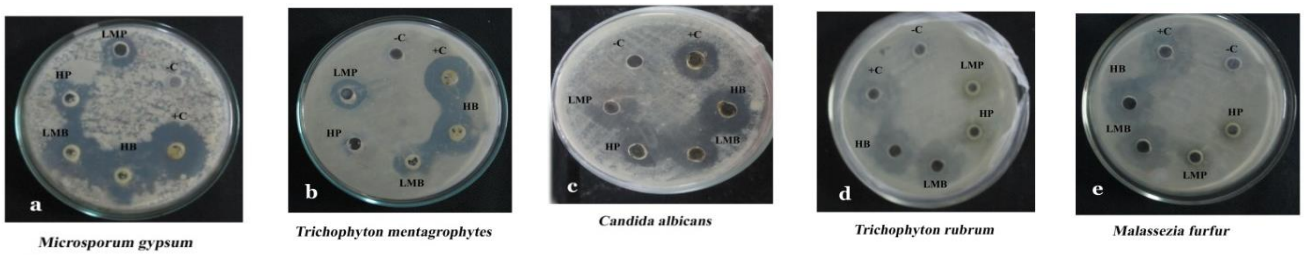


Figure I Antidermatophytic activity of *Majidea zanquebarica* Bark and Pod extract

Table 2 Antidermatophytic activity of leaf and seed hexane and 70% methanol extract of *M. zanquebarica*

Extract 50mg/ml	Zone of inhibition (mm)				
	Malassezia furfur	***Trichophyton mentagrophytes***	***Candida albicans***	***Trichophyton rubrum***	***Microsporum gypseum***
Hexane leaf	7.2±6 I	7±0	9.23±0.25	0	0
Hexane seed	8±0	7±0	8.16±0.28	0	0
70% methanol leaf	12.26±0.25	7±0	7±0	0	0
70% methanol seed	8.23±0.20	7±0	8±0	0	0
Positive control (Ketoconazole)	22.43±0.40	16.43±0.40	20.23±0.23	20.06±0.20	22.43±0.40
Negative control (DMSO)	-	-	-	-	-

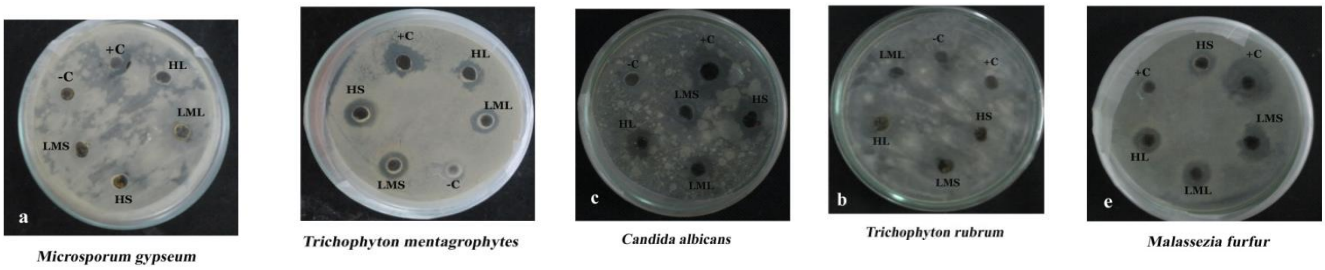


Figure II Antidermatophytic activity of *M. zanquebarica* Leaf and Seed extract

Antidermatophytic activity of bark hexane extract of *M. zanquebarica* exhibited maximum inhibition zone of 35.56±0.49 mm against Malassezia furfur at 50 µg/ml concentration. The inhibition zones formed by this extract at the same concentration were 24.5±0.5 mm, 21.23±0.25 mm, 21.4±0.96 mm and 19.6±0.52 mm against *M. gypseum*, *T. rubrum*, *T. mentagrophytes* and *C. albicans* respectively. The inhibition zone range formed by 70% methanol extract was 31.63±0.40 mm to 23.43±0.40 mm against *M. furfur* and *M. gypseum* whereas 20.56±0.51 mm against *C. albicans*, 18.46±0.41 mm in *T. rubrum* and 17.46±0.35 mm on *T. mentagrophytes*. The antifungal activity of hexane extract was recorded as highest than 70% methanol extract against *M. furfur*, *M. gypseum* and *T. rubrum*.

70% methanol extract of pod showed highest activity against *C. albicans* (18.63±0.40 mm), followed by *M. furfur* (12.26±0.30 mm) and *T. mentagrophytes* (10.96±0.45 mm). Pod hexane extract exhibited moderate activity against *C. albicans* (16±0.2 mm) and *M. furfur* (12.12±0.41 mm) while no activity was found against *T. mentagrophytes*, *T. rubrum* and *M. gypseum*. The hexane and 70% methanol extract of seed and leaves exhibited less activity against *C. albicans* and *M. furfur*. Whereas they showed no activity against *T. mentagrophytes*, *T. rubrum* and *M. gypseum*. The antibiotic ketoconazole showed high activity against all the tested organisms. The observation can be rationalized in terms of the polarity of compounds being extracted by each solvent and in addition to their intrinsic bioactivity ability to dissolve or diffuse in culture media used in the study. Hexane bark extract exhibited greater activity against all the organisms than the other parts. Since, it was further tested against the fungal pathogens using 25 µl, 50 µl, 75 µl and 100 µl showed similar highest zone of inhibition in increasing concentration (Table 3 and Figure III) against *M. furfur* (35.4±0.45 mm), whereas the extracts inhibited *C. albicans* (30.33±0.41 mm), *T. rubrum* (23.5±0.50 mm), *M. gypseum* (14.4±0.40 mm) and *T. mentagrophytes* (24.6±0.52 mm) to a considerable level in µg/ml concentration. Standard Ketoconazole (positive control) showed low activity against all the fungi 16.63±0.55 mm, 16.13±0.32 mm, 8.4±0.45 mm, 6.6±0.52 mm and 8.6±0.65 mm zone of inhibition against *M. furfur*, *C. albicans*, *T. rubrum*, *M. gypseum* and *T. mentagrophytes* respectively.

In Poison plate method the results are shown in (Table 4; Figure IV). The inhibition zone range formed by 100 µl concentration of hexane extract was 77.4±0.32 mm, 77.56±0.28 mm, 88.23±0.14 mm and 80.33±0.17 mm against *T. rubrum*, *M. gypseum*, *M. furfur* and *T. mentagrophytes* respectively. The effect of hexane extract of 50 µg/ml *M. zanquebarica* on fungal growth is showed in Figure V for those non treated fungal cells, mycelia were abundant and having the intact appearance. However, the growth of mycelia in the treated fungal cells is remarkably decreased. The fungus *M. furfur* was found in the form of mycelia fragments (or) conidia spores.

Table 3 Antidermatophytic activity of bark hexane extract of *M. zanquebarica*.

Name of the organisms	Zone of inhibition (mm)					
	Negative control (DMSO)	Positive control (Ketoconazole)	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Malassezia furfur	0	16.63±0.55	20.6 ±0.52	24.6±0.52	28.4±0.37	35.4±0.45
Candida albicans	0	16.13±0.32	19.66±0.61	22.43±0.45	26.53±0.47	30.3±0.41
Trichophyton rubrum	0	8.40±0.45	14.4±0.36	16.4±0.45	18.6±0.55	23.5±0.50
Microsporeum gypseum	0	6.60±0.52	7.40±0.41	8.40±0.40	9.40±0.41	14.4±0.40
Trichophyton mentagrophytes	0	8.60±0.65	10.80±0.76	12.60±0.6	16.5±0.50	24.6±0.52

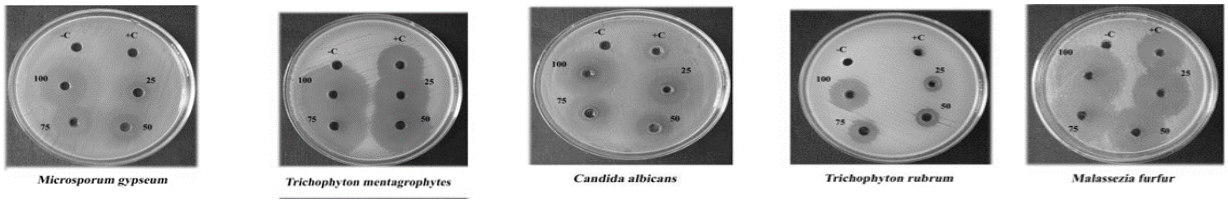
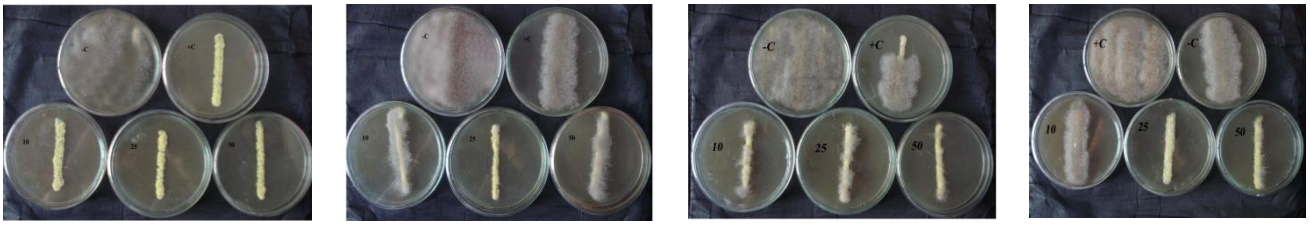


Figure III Antidermatophytic activity of *M. zanquebarica* Bark hexane extract

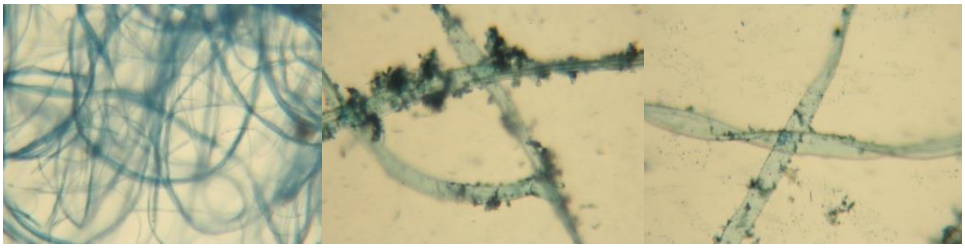
Table 4 Antidermatophytic activity of bark hexane extract of *M. zanquebarica* using Poison plate method

Hexane extract	Concentration of the extract (µg/ml)	Trichophyton rubrum	Microsporeum gypseum	Malassezia furfur	Trichophyton mentagrophytes
Inhibition zone in mm	10	70.6±0.30	44.33±1.20	76±0.57	72.5±2.25
% of inhibition		77.77	46.66	84.44	77.77
Activity index		1.21	0.72	0.95	1.35
Inhibition zone in mm	25	76.4±0.30	72.5±0.36	78.16±0.44	77.93±0.17
% of inhibition		84.44	80	87.77	86.66
Activity index		1.31	1.89	0.99	1.5
Inhibition zone in mm	50	77.4±0.32	77.56±0.28	88.23±0.14	80.33±0.17
% of inhibition		86.66	85.55	97.77	88.88
Activity index		1.34	2.03	1.1	1.54
+C	50	64.44	42.22	88.88	57.77

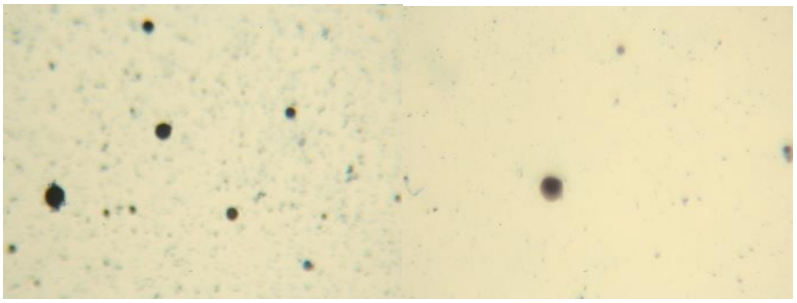


a) *Malassezia furfur* B) *Trichophyton mentagrophytes* C)*Trichophyton rubrum* D) *Microsporeum gypseum*

Figure IV Antidermatophytic activity of bark hexane extract of *M. zanquebarica* using Poison plate



A) Microscopic view of *Malassizia furfur* before treatment with hexane extract



B) Microscopic view of *Malassizia furfur* After treatment with hexane extract

Figure V Microscopic view of *Malassizia furfur* before and after treatment with Bark hexane extract of *M. zanquebarica*

Discussion

The antibiotic ketoconazole was used to compare the inhibitory activity of the extracts. The extracts and the antibiotic were used in 50 µg/ml of the medium. The dermatophytes tested for their sensitivity in the present study were Malassezia furfur, Trichophyton rubrum, *T. mentagrophytes*, Microsporum gypseum and Candida albicans. This antidermatophytic effect might be exerted due to the presence of several bio-active compounds in various extracts of M. zanqubarica as evident by the findings of others [25 – 27].

The inhibitory activity of 50 µg/mL concentration of methanol and hexane of *M. zanquebarica* on *M. gypseum* sterile colony was comparable to the effect of antibiotic and also effective in controlling the growth. Aerial parts of *Gentianella nitida* [28] and *Acorus calamus* [29] have inhibitory activity against *M. gypseum*. The methanol extract of *M. zanquebarica* had very low activity when compared to hexane extract against *T. mentagrophytes*. Similar findings were reported for *Catharanthus roseus* [30, 31].

In the antifungal activity, the hexane bark extract of *M. zanqubarica* showed good inhibitory effects in almost all the fungi tested. Similar results were obtained in *Cardiospermum halicacabum* [32].

In the present study, the organic solvent extracts of the bark exhibited very good inhibitory activity on the *M. furfur*, *M. gypseum*, *T. rubrum* and *T. mentagrophytes*. In conclusion, *M. zanquebarica* may play a highly beneficial role in dermatophytoses patients. Any treatment given to an organism will cause changes in the cellular and biochemical levels. The treatment of hexane extract of *M. zanquebarica* with sporulating colony with the hexane extract of *M. furfur* produced significant changes in the cell morphology. The treated cells became bulged, the contents rounded up and also changes in the cell wall and inclusions were observed. The cell wall of the treated macro conidia had ruptures here and there. The wall layers were also slightly disorganized.

Plant extracts producing changes in the cells of the dermatophytes. Aqueous onion extract targeted the cell membrane of *T. mentagrophytes* with extrusion of materials into surrounding medium [33].

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