

EVALUATION OF THE ANTIFUNGAL ACTIVITY OF *MORINGA OLEIFERA* SEEDS, LEAVES AND FLOWERS

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ABSTRACT

Background: *Moringa oleifera* is one of multipurpose tree and rich in antimicrobial agents, that can be used for discovery of new classes of compounds that help in the treatment of microbial resistance.

Materials and methods: seeds, leaves and flowers of *Moringa* were extracted by ether, ethanol, and water and tested for its antifungal activity using macrodilution method. **Results:** The nine extracts of *Moringa oleifera* were tested against 16 fungal isolates (*Aspergillus flavus*, *A. fumigatus*, *A. terreus*, *A. niger*, *Candida albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, one dermatophyte *Trichophyton rubrum* and *Madurella mycetomatis*). All of the tested organisms were resistant to seed oil type 1 and 2, leaves and flower of ether extract. It was also found to be resistant to leave water. The best extract was seed

alcohol and seed water. All of the *Moringa* extracts irrespective of their types, in different concentrations did not inhibit the growth of all *Candida* species.

KEYWORD: *Moringa oleifera* (seeds, leaves and flowers), phytoconstituents, minimum effective concentration (MEC), minimum inhibitory concentration (MIC).

INTRODUCTION

In Africa the use of traditional medicinal plants continue to form the basis of rural medicinal care due to the fact that these medicines are easy to obtain and cheap. The extensive use of antibiotics worldwide lead to increase in the microbial resistance which then lead to difficulty in the treatment of various diseases. In addition to the increased costs of hospital stay. Microbial resistance to drugs involved also fungal species. Previous studies reported the

resistance of *Candida* species to azol drug to be as follows: *Candida albicans* 1.0%–2.1%, *Candida parapsilosis* 0.4%–4.2% and 1.4%–6.6% for *Candida tropicalis*.^[1, 2] A plant known as *Moringa oleifera* was confirmed as multipurpose tree which have different uses in the treatment of various disease and as well possesses antibacterial and antifungal activity.^[3]

The aim of the present study was to assess the antifungal activity and to determine the minimum effective concentration of *Moringa oleifera* plant against 16 clinical fungal isolates.

MATERIALS AND METHODS

Plant collection

The different parts of *M. oleifera* were collected from a farm in Sennar State. These were collected during the period from April to October 2013. The plant was identified and authenticated in the Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan. The flowers, leaves and seeds were dried in shade at room temperature. The dried parts were placed in a mortar and pestle until a fine powder was obtained and then used.

Plant material preparation

The plant material was extracted according to Price 2000. One hundred and seventy gram of the seeds, leaves and flowers were extracted by petroleum ether, ethanol and water by using soxhlet apparatus. The extracts were filtered using Whatman filter paper No. 1 and evaporated under reduced pressure using Rota-vap. The extracts were stored at 4° C for phytochemical screening and antimicrobial analysis.^[4]

Phytochemical Screening of different *Moringa* extracts

Phytochemical analysis for qualitative detection of alkaloids, flavonoid, tannins, and saponins, steroid, glycosides and reduced sugars was performed on the extract as described by Evans and Trease.^[5, 6, 7]

Test Microorganisms

Fungal species used in this study included one standard *Candida albicans* ATCC 7596 and 15 isolates (two *Aspergillus flavus*, two *A. Fumigatus*, one *A. terreus*, one *A. niger*, four *Candida albicans*, one *C. glabrata*, one *C. krusei*, one *C. parapsilosis*, one isolate of *T. rubrum* and one *Madurella mycetomatis*, isolated from patients suffering from peritonitis, exit site infection, blood, skin infection and abscesses. *Aspergillus* species were isolated from peritoneal fluid; *Candida albicans* was isolated from exit site, peritoneal fluid and the remaining *Candida* species, *T. rubrum* and *Madurella mycetomatis* were already isolated and

identified in mycology department of the national health laboratory. The chemicals and media were purchased from Hi-Media, Mumbai, India and Scharlau - Spain.

Fungal species preparations

The *Candida* species were cultured in Sabouraud dextrose agar for 24 hour. Inoculum was prepared by dissolving 5 colonies in 5ml of 0.85 normal saline and vortexed for 15 seconds. The turbidity of the suspension was adjusted to 0.5 McFarland standards this yield a yeast suspension of approximately 1×10^6 to 5×10^6 cells /ml.^[8]

Aspergillus species were cultured on Sabouraud dextrose agar for 7 days at 35 °C to obtain a good sporulation. The spores were transferred to 1ml of normal saline (0.85%), mixed gently to allow heavy particles to settle for 5 minutes and was adjusted to 0.5 McFarland standards. Using automatic pipette the upper homogeneous layer was transferred to a sterile test tube, closed well and mixed for 15 seconds.^[9]

Antifungal activity

From the different extracts, of the 3 parts of *Moringa*, 7 different concentrations (1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml and 15.6mg/ml) were prepared by adding 4g of *Moringa* plant to 4ml of D.W for water extract and 1% dimethyl sulfoxides (DMSO) for ether and alcohol extracts. Then two fold dilutions were prepared for each extract two plates of Mueller Hinton agar were used. The sensitivity of *Candida* species was done, medium in each plate were punched out by a sterile cork borer to create 4 wells, each with a diameter of 7 mm. 100 µl of each extract type was then added to the corresponding well punched out in the medium. The plates were left for 1 hour to allow for diffusion of extract before incubation at 35°C for 24 hours. The diameter of inhibition zone around the well was measured by a ruler. The MIC was taken as the lowest concentration that prevented of fungal growth. The antifungal activity of 4 concentrations (100%, 75%, 50%, 25%) v/v of *Moringa* seed oil type 1 (oil extracted by ether), type 2 (ready to use oil) was done as follows: one colony of *Candida* species was emulsified in 1ml of undiluted seed oil (100%), for the remaining concentrations the 750 µl, 500 µl and 250µl of oil was mixed with 250 µl, 500 µl, 750 µl of *Candida* broth culture respectively after addition of 5 drops of ether to help in emulsification. The mixture was incubated at 35°C for 24h and then subcultured on Sabouraud agar plates after 24 hour and 48 hours. Each of the tests mentioned above was performed in duplicates.^[8]

For *Aspergillus* species the test was done using broth macrodilution method. Briefly The test was done by adding 500µl of the extract and 500µl of the organism suspension in Brain heart infusion broth media. This step was found to achieve the required concentration of 0.4×10^4 to 5×10^4 CFU/ml. The seed oil type 1 & 2 was added as method mentioned above. Incubated without agitation at room temperature for 1hour then incubated for 24 hour at 35°C or after 46 hour still sufficient growth is seen covering the bottom of the test tube. They were examined for the presence or absence of growth. Two control groups were used negative control containing the extract with no fungal suspension, second one was suspension of the fungi with no extract and incubated at 35°C for 46 hours. DMSO solution was tested for its toxicity. By adding the same amount of the above suspension to 500µl of the 1%DMSO and incubate at 37 ± 2 °C for three days and proved to be safe to use at that concentration. The MEC was taken as the lowest concentration that allowed the growth of rounded compact hyphae.

The test for *Madurella mycetomatis* was done as for *Aspergillus* species except that the incubation was 3 days. Each of the tests was done in duplicates.^[9]

The *Trichophyton rubrum* colonies were cultured and suspended on 1ml of sterile normal saline. Mixed gently to allow heavy particles to settle for 5 minutes. Using automatic pipette the upper homogeneous layer was transferred to a sterile test tube, well closed and mixed for 5-10 minutes. A broth macrodilution method was used; the test tube was inoculated with 500µl of the extract and 500µl of the suspension after diluting it in BHI broth media. This step was found to achieve the required concentration of 1×10^3 to 3×10^3 CFU/ml. The seed oil was done as method mentioned above. Incubated without agitation at room temperature for 1hour then put in the incubator and incubated for 5 days at 30°C or until a good conidial growth was obtained. They were examined for the presence or absence of growth. Two control groups were used, negative control containing the extract with no fungal suspension, second one was suspension of the fungi with no extract and incubated at 30°C for 5 days. DMSO solution was tested for its toxicity. By adding the same amount of the above fungal suspension to 500µl of the 1%DMSO and incubate at 37 ± 2 °C for three days. Each of the tests was placed in duplicates.^[9]

RESULTS

Phytochemical analysis showed presense of saponin, flavonoids, alkaloids, steroids and tannin in the three parts of the plant but no glycosides and reduced sugars table 1.

Table 1: Phytochemical screening of *M. oleifera* extracts.

Seeds	Saponin	Flavonoids	Alkaloids	Steroid	Tannins	Glycosides	Reduced sugars
Ether extract	N	N	P	P	N	N	N
Alcoholic extract	P	P	P	N	P	P	N
Aqueous extract	P	P	N	N	N	N	N
Leaves							
Ether extract	N	N	P	P	N	N	N
Alcoholic extract	P	P	N	T	P	N	N
Aqueous extract	P	T	N	N	T	N	N
Flowers							
Ether extract	N	N	T	P	N	N	N
Alcoholic extract	N	T	T	P	T	N	N
Aqueous extract	N	T	N	N	T	N	N

*P: Positive, N: Negative, T: Trace.

The alcohol extract of *Moringa* seed, leaf and flower showed antifungal activity against all the tested species of fungi. The best inhibitory effect of alcohol against the fungi tested was shown by the seed followed by seed water extract and leaf alcohol table 2. All fungal isolates were resistant to seed oil type1 and 2; leaves ether, leaves water and flower ether table 2. *Candida* species tested and control were resistant to all types of the extract at different concentration.

Table 2: Minimum effective concentration of different extract activity of *M. oleifera* against Fungal isolates.

Fungal species	MEC of the Plant extract in mg/ml										
	No tested	S oil 1	S oil 2	SA	SW	LE	LA	LW	FE	FA	FW
<i>Aspergillus flavus</i>	2	R	R	62.5	125	R	250	R	R	250	250
<i>A. Fumigatus</i>	2	R	R	15.6	125	R	125	R	R	250	250
<i>A. terreus</i>	1	R	R	31.25	125	R	125	R	R	250	250
<i>A. niger</i>	1	R	R	125	250	R	250	R	R	250	250
<i>Trichophyton rubrum</i>	1	R	R	31.25	62.5	R	250	R	R	250	250
<i>Madurella mycetomatis</i>	1	R	R	7.8	7.8	R	7.8	R	R	7.8	R

1: seed oil extracted by ether, 2: ready to use oil, SA: seed alcohol, SW: Seed water, LE: leaf ether, LA: leaf alcohol, LW: leaf water, FE: flower ether, FA: flower alcohol FW: flower water. R: Resistant.

DISCUSSION

The presence of various types of secondary metabolites or compounds on *Moringa* leaves, seeds, and flowers such as glycosides, saponins, tannins, flavanoids and alkaloids explained its activity against some fungal species. *Moringa* leaves ethanol extract revealed presence of

flavonoids and saponins and *Moringa* seed ethanol extract contains alkaloids this is in agreement with bukar^[10]. *Moringa* seed ethanol extract was found to contain tannins and saponins which is in agreement with Napolean.^[11]

It can be safely presumed that a major part of traditional medicine involves the use of plants and their derived active principles, through their use is not always verified by the scientific mean especially in the developing country. *Moringa* is witnessing these days a wide use, especially in Sudan. Some use it for the treatment of disease; according to Ezaldeen who studied the effect of *Moringa* against mycetoma as treatment. There are about 18.9% of the patients who use the plant as hot drink some of those were not satisfied with the result obtained, others might have benefited from the plant. In our study we applied one isolates of Mycetoma against leaves, seeds and flowers. The most active part is the seed alcohol and aqueous extract; also leaves alcohol showed activity in high concentration. *Madurella mycetomatis* was not inhibited by seed ether, leaf ether, leaf water, flower ether and flower water. Seed water and seed alcohol showed the best activity against eumycetoma. There are no enough studies for the treatment of mycetoma by *Moringa*. Further studies needed in different parts of *Moringa oleifera*.^[12]

Saddabi reported that seed water did not inhibit the growth of *Candida albicans* in 5, 10 & 20% concentration, but inhibited their growth in 40 % concentration with inhibition zone of 11 mm, while *A. niger* was slightly inhibited by the extract. He also reported that the ether extract was inactive against *Candida*, *A. niger*^[13]. These results are in close agreement with our findings obtained that all fungi tested was not inhibited by leaves ether extract. Anthonia reported that the aqueous leaves extract was inactive against *Candida albican*, *A. Flavus*, *Trichophyton* species but the ethanolic extract inhibited the growth of *A. flavus* producing zone of growth inhibition (15mm) at 30mg/ml,^[14] Nwosu and Chuang reported that *T. rubrum* have been inhibited by ethanolic extract of *M. oleifera* leaf.^[15 and 16]

CONCLUSION

The results of the present study have shown the potentials of *M. oleifera* seeds on ethanol and aqueous extracts against the tested fungi. This comparative study exhibits the information about the medicinal properties of these plants which can be used as medicine. Further studies should be done to identify the active ingredients which can be used for discovery of new classes of antibiotic that could serve as selective agents against different types of microorganisms and to determine the toxicity and the optimum dose to be used as effective as

antibiotics. These results indicate possible use of certain plant extracts in the treatment of subcutaneous mycosis in humans.

REFERENCES

1. Pfaller MA, Diekema DJ. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. *Clin Microbiol Infect*, 2004; 10(Suppl 1): 11–23.
2. Pfaller MA, Diekema DJ, Rinaldi MG, *et al.* Results from the ARTEMIS DISK Global Antifungal Surveillance Study: a 6.5-year analysis of susceptibilities of *Candida* and other yeast species to fluconazole and voriconazole by standardized disk diffusion testing. *J Clin Microbiol*, 2005; 43: 5848–59.
3. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother Res*, Jan 2007; 21(1): 17-25.
4. Price, M.L, The *Moringa* Tree purification and activity assay of the coagulant protein from *Moringa oleifera* seed. *Water Res*, 2000; 39: 2338-2344.
5. Evans, W.C., Trease and Evan's Pharmacognosy. 5th ed., Haarcourt Brace And Company, 2002; 336.
6. Talukdar AD, Choudhary MD, Chakraborty M and Dutta BK: Phytochemical screening and TLC profiling of plant extracts *Cyathea gigantea* (Wall. Ex. Hook.) Haltt. and *Cyathea brunoniana*. Wall.ex.Hook. (Cl.& Bak.). Assam University *Journal of Science & Technology: Biological and Environmental Sciences*, 2010; 5(1): 70-74.
7. Siddiqui, A.A., Ali, M., Practical Pharmaceutical chemistry. Ist ed., CBS Publishers and Distributors, New Delhi, India, 1997; 126-131.
8. National Committee for Clinical Laboratory Standards. Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts; document M44-A. Wayne PA: CLSI, 2004.
9. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of Filamentous Fungi; second edition. Document M38-A2. Wayne PA: CLSI, 2008.
10. Bukar, A., Uba, A. and Oyeyi, T. I. Antimicrobial profile of *Moringa oleifera* Lam. Extracts against some food-borne microorganisms. *Bayero Journal of Pure and Applied Sciences*, 2010; 3(1): 43-48.
11. Napoleon, P., Anitha, J. and Emilin, R.R. Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam. *Current Biotica*, 2009; 3(1): 33 – 37.

12. Ezaldeen EA, Fahal AH, Osman A. Mycetoma herbal treatment: the Mycetoma Research Centre, Sudan experience. *PLoS Negl Trop Dis*, Aug 2013; 7(8): e2400.
13. Abdulmoneim M. Saadabi and I.E.Abu Zaid. An invitro antimicrobial activity of *Moringa oleifera* seed extracts against different groups of microorganisms. *Australian Journal of Basic and Applied Sciences*, 2011; 5(5): 129-134.
14. Anthonia Olufunke Oluduro, Evaluation of Antimicrobial properties and nutritional potentials of *Moringa oleifera* .*Malaysian Journal of Microbiology*, 2012 Vol; 8(2): 59-67.
15. Nwosu, M. O. and Okafor, J. I. Preliminary studies on the antifungal activities of some medicinal plants against *Basidiobolus* and some other pathogenic fungi. *Mycoses* 1995; 38(5-6): 191-195.
16. Chuang, P. H., Lee, C. W., Chou, J. Y., Murugan, M., Shieh, B. J. and Chen, H. M. *In vitro* antifungal activity of ethanolic extract of the leaves of *Moringa oleifera* against dermatophytes. *Bioresources Technology* 2007; 98: 232–236.