Photochemical Analysis of Moringa and Effect of Extract on Inhibition of *Aspergillum Niger Development*

Hala Yousif Ahmaed Yousif [1],Awad M.Abderahim[2], Fadlelmoula A. Idris[3]

[1, 2.]Faculty of Science, University of Gezira, Wad Medani, Sudan

[3] . Faculty of Industries Engineering and Technology

University of Gezira,Wad Medani,Sudan

E.mail: kadabas_20@yahoo.com

fadlalmawla@uofg.edu.sd

Abstract

The present study investigated the photochemical and activities of the Moringa Rotifera lam plant extracts against the tested microorganisms (Fungi). The mycelia growth method was used for fungi. (Aspergillum Niger), the seeds, pods, leaves, and stem extracts gave 1.3, 3.0, 3.0 and 6.0 mm diameter, respectively. The seed extracts were most effective against Aspergillum Niger; they gave only 0.1 g dry weight and 3.99 g fresh weight.

On the other hand, the stem, leaves and pod extracts were less effective, giving 0.60 g, 0.65g and 0.80g dry weights at the higher concentration (100%), respectively. The chemical analysis showed that the Moringa plant extracts contained flavonoids, tannins, saponins, alkaloids and steroids. From the results, it could be concluded that the Moringa plants' extracts were highly effective against the mycelial growth of fungi (radial growth and weights). The analysis of the active components of the extracts needs to be verified in further studies.

Keywords: Moringa, antifungul,medicinal plants,photochemical,plant extract.

INTRODUCTION

Moringa oleifera (Moringaceae), mainly grown in India sub-continental and Africa also. These countries' nations give special consideration to moringa trees due to their multi-functionality; they can be used for food, medication and the industrial sector (Moyo et al.,2011). In some African country especially in Uganda, Moringa leaves are used to treat malaria and, to some extent, to reduce the pain of HIV deices. (Kasolo et al., 2010). The study conducted by (Vongsak et al., 2013; Al Khateeb et al., 2013) revealed that leaves extracting due to the presence of moringa leaves are contains mineral compounds and vitaminesphenol acids and flavinoids inhabit antioxidant activity. Studies from various countries agreed that M. oleifera leaves are of minerals compound as well as vitamins. (Mbikay, 2012). It has been reported the use of plants in medicines backdated to the beginning of human creation. Ethno-botany (study plant as traditional medicine) is described as the best method to produce other medicine. plants used in medicines have been discovered and implemented along with human civilization. (Sumner, 2000). In 1500 B.C., the Ancient Egyptians wrote the Ebers papyrus, which contains over 850 plant medicines, including garlic, juniper, cannabis, castor bean, aloe, and mandrake (Sumner, 2000). The natural plants used in the rural area as medicines are often more popular due to affordability and less cost than modern pharmaceuticals. The World Health Organization (WHO) reported that 80% of the Asian and African population use traditional treatment (Internet, 2015). Iswar et al. (2010) reported that *Moringa oleifera* is very effective for the treatment of asthma. Studies in the United States and Europe confirmed it is not used widely in clinical settings, but the application increased in recent years(Internet,2015).

Medicinal plants grown in Sudan covered a broad spectrum and available through all seasons, which are based on traditional medicines that are useful for humans and animal treatment. Unfortunately, the user of this treatment unregulated, which may be caused harmful to the user, so it is imperative to be regulated in the form of preparation besides use. Moringa, which is the only genus in the family Moringaceae, initially grown in north India, is now found throughout the tropics region. Moringa is a multi-functional plant, and all of its parts can be used in different ways. It's rich in nutrients, can purify water, and is a valuable source of medicines. Moringa is a good source of various photochemical like alkaloids, flavonoids, carbohydrates, saponins, tannins and Terpenoids, so it has effectively used against some fungi such as *Saccharomyces cerevisiae, Candida albicans* and *Candida tropicelis* (Internet, 2015). Many studies have tested how it can affect blood lipid profiles, although it is not effective in diagnosing, treating, or preventing any human diseases (Sandval *et al.*, 2013). The study by Patel *et al.* 2014) in India was found that *Moringaoleifera* is rich in chemical components such as alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins and terpenoids. Studies conducted by Udupa et al.,1994 and Eilert *et al.* 1981) revealed moringa extract is effectively used for diuretic and antibiotics, respectively.

MATERIALS AND METHODS:

Leaves and stems of *M. olifera* were brought from Atra Moringa farm, while the seeds were purchased from Moringa Research Center in Wad Medani. The blender is used to crushed dry samples into powder. Five different concentrations.(0, 25, 50, 75 and 100%) were taken for each plant part separately. The media used and cultures of microorganisms were obtained from the Food Science and Technology Laboratory, Faculty of Science and Technology, University of Gezira. They were using Oxiod Corporation substances. The media Potato Dextrose Agar (PDA) was used for isolation and maintenance of fungi, consists of the following materials, Potato

peeled and diced(200g), D-Glucose(20 g), Agar (15 g), and Distilled water (1000 ml).

Preparation of the medium:

The potato samples 200 g were peeled, diced, and boiled for 1 hour in 800 ml distilled water, and then the extract was filtered and made up to 1 liter, and then 20 g glucose and 15 g of Agar were added. The medium was then dispensed in 100 ml samples in conical flasks covered with cotton plugs and aluminum foil before being sterilized. The medium was then stored at 4^0 C in a refrigerator. When needed, it was melted in a water bath and poured into sterilized Petri dishes.

Preparation of the medium:

The potato samples 200g were peeled, diced, and boiled for 1 hour in 800 ml distilled water, then the extract was filtered and made up to 1 liter. The medium was then dispensed in 50 ml samples in conical flasks covered with cotton plugs and aluminum foil before being sterilized in the autoclave 121^0 C (15 lb./in^2) for 15 minutes. However, glucose was sterilized separately by filtration and then added to the medium under aseptic conditions. The medium was used as a liquid culture for fungal experiments.

The fungal radial growth test:

The five extract concentrations were mixed with medium, sterilized, and poured using a petri dish and left to dry at room temperature (28-37^0 C). Each solidified Petri-dish was inoculated by a fungal growth disk cut by a sterile cork-borer (5.0 mm diameter) from the edge of an actively growing culture of both fungi (*A. Niger*) grown on PDA. The inoculated Petri dishes were then incubated at room temperature for eight days. The growth diameter was measured every 48 hours by taking the average of two crossed dimensions for each disc in a Petri-dish. The radial growth was calculated as a percentage from the diameter of the Petri-dish. All treatments were done in triplicates. For mycelia fresh and dry weight test. The potato dextrose broth (PDB) dispensed in a 100 ml conical flask (50 ml in each flask), five concentrations (0.0,25.50.75 and 100mg/ml) were made by the

serial dilution of the extract with the medium. All dilutions were sterilized in the autoclaved at 121^0 C ($151/in^2$) for 15 minutes, cooled at room temperature. Each flask was inoculated by three discs made by a sterile cork-borer (2.0 mm diameter) from the edge of the actively growing culture of *A. Niger* on a solidified PDA medium and incubated at room temperature ($28\text{-}37^0$ C) for eight days. After incubation, the cultures were filtered through Whitman (No.1) filter papers, and the mycelial mats were collected, weighed (fresh weight), and dried at 80^0 C for 24 hours before being reweighted (dry weight). All treatments were done in triplicates.

Photochemical screening:

The dry extract portion was subjected to the Photochemical screening using the method adopted by Trease, Evans, and Harbourne. The phytochemical screening was performed to test for alkaloids, saponins, glycosides, flavonoids, triterpenoids, and tannins.

Statistical Analysis:

To evaluate associations between variables (antifungi profiles), the data were analyzed statistically using the SPSS package.

RESULTS

The study was conducted to investigate the effect of moringa plant extract against fungi (*A. Aiger*); the photochemical screening tests were also conducted to investigate the chemical compounds in ethanol moringa plant extracts. Results in Table (1) show that flavonoids and alkaloids were found in all tested parts of Moringa, while flavenoids and Saponins were found in the leaf extract only. Tannins and sterols were only found in seed extracts. The test indicated that Moringa is a good source of phytochemical compounds. The found results agree with the study carried by Patel *et al*. (2014), demonstrated that the *moringaoleifera* is a good source of various photochemical.

Table (1): Chemical Screening test of different ethanol extracts of the Moringa plant parts

Parts / Chemical compounds	Leaf	Pod	Seed	Stem
Flavoids	+	+	+	+
Flavenouns	+	-	-	-
Saponins	+	-	-	-
Sterol	-	-	+	-
Alkaloids	+	+	+	+

+ found - Not found

The effect of the leaves, pods, seeds, and bark extracts of Moringa on radial growth of *A. niger* are shown in tables (2). From this Table, it was found that the leaves extracts were effective in inhibiting growth compared to the control, and it was significantly effective, especially at the 100% concentration, where it gave a 3 mm diameter compared to 8 mm at the control treatment (0.0%).

Table (2): Effect of leaves extract on radial growth (mm) of *Aspergillum Niger*

Extract concentrations%	Days			
	Radial growth			
	2.0	4.0	6.0	8.0
0	4.0	5.9	7.5	8.0
25	3.8	5.4	6.5	7.5
50	1.6	3.2	4.0	5.0
75	1.4	3.0	3.6	4.5
100	1.2	2.8	2.8	3.0

Table (3): ANOVA Table

K-value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Prob
2	Factor A	3	114.064	38.021	332.5481	0.0000
4	Factor B	4	190.432	47.608	416.3965	0.0000
6	AB	12	9.216	0.768	6.7172	0.0000
-7	Error	60	6.860	0.114		
	Total	79	320572			

Table (3) shows the effect of pods extracts of Moringa on radial growth of *A. Niger*. From the results, it is clear that the pod extract was less effective in inhibiting this fungus's radial growth. The pod extracts gave a 3.0 mm diameter similar to that given by the leaf extracts.

Table (4): Effect of pods on radial growth (mm) of *Aspergillum Niger*

Extract concentrations%	Days			
	Radial growth			
	2.0	4.0	6.0	8.0
0	3.1	4.0	5.6	8.0
25	3.0	3.2	3.4	7.5
50	2.4	2.8	3.0	7.0
75	2.1	2.8	2.8	6.0
100	1.9	2.5	2.5	3.0

Table (5): ANOVA Table

K-value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Prob
2	Factor A	3	174.388	58.129	645.8815	0.000
4	Factor B	4	61.673	15.418	171.3139	0.000
6	AB	12	35.167	2.931	32.5620	0.000
-7	Error	60	5.400	0.090		
	Total	79	276.628			

The effect of seed extracts of Moringa on radial growth of *A. Niger* is shown in table (4). The results indicated that the seed extracts were significantly effective, especially at the higher concentrations (75, 100%). The seeds were highly effective compared to the other parts (only 1.3 mm diameter).

Table (6): Effect of seed on radial growth (mm) of *Aspergillum Niger*

Extract concentrations%	Days			
	Radial growth			
	2.0	4.0	6.0	8.0
0	3.1	4.5	6.1	8.0
25	2.0	4.1	4.6	6.0
50	1.4	3.1	3.2	3.6
75	1.2	2.0	2.2	2.4
100	1.0	1.4	1.4	1.4

Table (7): ANOVA Table

K-value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Prob
2	Factor A	3	74.799	24.933	809.7362	0.0000
4	Factor B	4	193.317	48.329	1569.5602	0.0000
6	AB	12	39.743	3.312	107.5575	0.0000
7	Error	60	1.847	0.031		
	Total	79	309.707			

The results in Table (5) show the effect of the stem extracts on radial growth of *A. Niger*. The results indicated that the stem extracts were less effective than the other parts; they gave a 6.0 mm diameter, although, at the high concentrations, the stem extracts were significantly effective in inhibiting the growth of *A. Niger* compared to the control.

Table (8): Effect of stem on radial growth (mm) of *Aspergillum Niger*.

Extract concentrations%	Days			
	Radial growth			
	2.0	4.0	6.0	8.0
0	3.1	4.0	5.6	8.0
25	2.0	3.9	4.2	8.0
50	1.2	1.5	2.3	7.5
75	1.0	1.5	1.5	7.0
100	0.5	0.7	1.0	6.0

Table (9): ANOVA Table

K-value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Prob
2	Factor A	3	42.118	14.039	979.4887	0.000
4	Factor B	4	176.633	44.158	3080.8092	0.000
6	AB	12	19.507	1.626	113.4128	0.000
-7	Error	60	0.860	0.014		
	Total	79	239.118			

From the results, it is clear that the seed extracts were the best in inhibiting the growth *of A. Niger* and the stem extracts were the least effective than the other parts. Although, at the higher concentrations, all parts of the Moringa plant affect the development of A. Niger significantly compared to the control treatment.

DISCUSSION

The study's ultimate goal was to determine the effect of moringa plant parts extracts on the development of fungi(A.Niger). The study also investigates the presence of phytochemical compounds.). All extracts of Moringa parts (leaves, pods, seeds, and stem)were found effective in reducing mycelia weights and radial growth of (A. Niger) in different ranges and at different concentrations. However, higher concentrations (75,100%) always were more effective. Nweke (2015)reported that the leaves and stem bark of Moringa was effective against A. niger.

On the other hand, in the present study, the fungi show resistance against the stem extracts, while leaves solvent extract was not effective. It was continued growing after the eighth day despite being inhibited in the first days. Similar results were also found by Vinoth et al. (2012), who reported that the increase in fungal infections and the frequency resistance and therapeutic failure were found with herbal screening for compounds with antifungal properties. Inhibition zone test for fungi shows that ethanol and methanol extracts were effective

against A. niger. However, seeds extracts were the best in inhibiting growth, but it was not effective against A. flavus, and the highest zone was found against the yeast C. krusei. While leaves extracts have no zone with (A. Niger). These were similar to the results of Moyo et al. (2012), who studied the effect of acetone and aqueous extract of Moringa leaf against some bacteria and fungi, and found that both acetone and aqueous extracts did not exhibit any antifungal activity against P..notatum, A. flavus as well as A. Niger. Patel et al. (2014) in India reported that the antifungal activity of Moringa oleifera was clearly shown against various fungi.

CONCLUSIONS

From the present study, it could be concluded that: the seed extracts were the best part of the moringa plant, which reduced the growth *of A. Niger* and the stem extracts were the least effective than the other parts. Although, at the higher concentrations, all parts of the Moringa plant affect the development of A.Niger significantly compared to the control treatment. Therefore it is recommended to use the seed part of Moringa to inhabit the development of A.niger.

REFERENCES

Moyo, B., Masika, P.J., Hugo, A., Muchenje, V., (2011). Nutritional characterization of
Moringa (Moringa oleifera Lam.) leaves. Afr. J. Biotechnol. 10, 12925–12933.

Kasolo, J.N., Bimenya, G.S., Ojok, L., Ochieng, J., Ogwal-Okeng, J.W.,(2010).
Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities. J. Med. Plants Res. 4, 753–757.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y.,
Gritsanapan, W., (2013). Maximizing total phenolics, total flavonoids contents
and antioxidant activity of Moringa oleifera leaf extract by the appropriate
extraction method. Ind. Crops Prod. 44, 566–571

Al Khateeb, W., Bahar, E., Lahham, J., Schroeder, D., Hussein, E., (2013). Regeneration
and assessment of genetic fidelity of the endangered tree Moringa peregrina
(Forssk.) Fiori using inter simple sequence repeat (ISSR). Physiol. Mol. Biol.
Plants 19, 157–164.

Mbikay, M., 2012. Therapeutic potential of Moringa oleifera leaves in chronic
hyperglycemia and dyslipidemia: a review. Front. Pharmacol. 3, 24.

Sumner, J. (2000). The Natural History of Medicinal Plants. Timber Press, P:16-17- ISBNO-88192-483-0.

Internet, (2015).http://www.trafic.org/medicinal-plants.

Iswar Chandra G, Shamim Q, Safwan AK, et al. Short Communication Evaluation of the Anthelmintic Activity of Moringa oleifera seeds. International Journal of Pharma Professional's Research, 2010; 1(1): 88–89.

Murkami, A., Kitazona, Y., Jiwajunda, S., Koshimizu,K., Nkya, J.W. ; Erasto, P. ; Kilambo, D. and Chacha, M. (2014). *In vitro* evaluation of antifungal activity of *Moringaoleifera (*lam) extracts against coffee with pathogen, Giberellaxylarioides and Saccas. American Journal of Research Communication. 2(12):53-62.

Patel, D. ;Desal, Sh. and Meshram, D.H.(2014). Pgytochemical analysis and anti fungal activity of *Moringaoleifera.*International Journal of Pharmacy and Pharmaceutical Scie.6,(5):144-147.

Udupa, A., S.L.; Udupa, A.l. and Kulkarni, D.I. (1994).Studies on the antiinflammatory and wound heading properties of *Moringaoleifera* and aeglemarmacolsfitoterapia. 65:119-12.

Eilert, U.B. and Nahested, A. (1981). The Antibiotical principle of seeds of *Moringaolieferaand**Moringastendopetala*.J.ofPlantaMedica, 42:55-61.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y.,

Gritsanapan, W., 2013. Maximizing total phenolics, total flavonoids contents

and antioxidant activity of Moringa oleifera leaf extract by the appropriate

extraction method. Ind. Crops Prod. 44, 566–571.