Journal of Medical Research and Health Sciences
Received 15 June | Revised 28 June 2023 | Accepted 26 July 2023 | Published Online 15 Sept 2023

DOI: https://doi.org/10.52845/JMRHS/2023-6-5

JMRHS 6 (9), 2754–2762 (192023)

ISSN (O) 2589-9031 | (P) 2589-9023

Original Article **Open Access Journal**

In Vitro Effect of Aqueous Extract of Moringa Oliefera Plant on Acanthamoeba Spp.

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Abstract:
Acanthamoeba spp. is a parasite that can significantly destroy vision. It enters eyes through polluted water or contact lenses , and starts to kill eyes epithelial cells.
The aim of this paper is to study the lethal effect of *Moringa oliefera* aqueous plant extract on *Acanthamoeba spp*. In vitro.Samples of *Moringa oliefera* plants were acquired from nurseries in Basra province. Following the creation of plant extracts, (GC-MS) was used to identify the active components. Results revealed the presence of bioactive components like : tannins, glycosides, alkaloids, flavones and phenols in the used extracts . Corneal samples were collected from patients infected with AK in Basrah's teaching hospital. The isolated protozoan has been morphologically classified to the genus level depending on cyst morphology declared by Page F.C. (1967), and it has been given the genus *A. Castellani*. Plant extracts from *M. oliefera* have been examined in vitro for their capacity to destroy *Acanthamoeba* cells. *Acanthamoeba* trophozoites were used in a number of (6 x 10^4)cells per ml. A three separated repeatition experiments' were done to evaluate of *M. oliefera* aqueous plant extract effect on this protozoan.

Key words: *M. oliefera* , plant extract, *acanthamoeba spp*.

Introduction

Keratitis (AK), a dangerous, vision-aggressive opportunistic parasite infection that pass in the eye over polluted or contact lenses and water , primarily belongs to *Acanthamoeba spp*. Infections lead it to swiftly start killing target cells as soon as enters the eye.

According to Lorenzo-Morales *et al.* (2015), AK is a rare corneal infection that might endanger vision if it is not recognized and properly treated. In the 1970s (Naginton *et al*., 1974; Samples *et al*., 1984) Acanthamoeba was first recognized as an eye pathogen that causes chronic keratitis and is commonly resistant to conventional antibiotic therapy. Castellani made the discovery of *Acanthamoeba* in 1930 (Fuerst et al., 2015). It can be found in a variety of environments, such as: the mucosa of nasopharynx's , skin lesions, contact lenses (CL), surgical tools, drainages,

sediments, dialysis units, air, dirt, and dust, (Shanmuganathan, 2019).

Oddo (2006) asserts that these creatures are also capable of existing beyond the bodies of their infected hosts. According to Nicholas (2017). These organisms, which can exist as active trophozoites or latent cysts, are known to bloom in hot tubs , swimming pools ,ponds , and Contact lenses .

The well-known herb *Moringa oliefera*, belongs to the Asteraceae family (Mankar et al., 2022). This herb because of its multiple aromatic and therapeutic qualities, according to Bigagli et al. (2017). These plants' essential oils (EOs) are crucial for aromatherapy, medicines, and natural food flavor (Rathore and Kumar, 2021). *M. oliefera* is the most widely used is an annual or perennial plant that is indigenous to temperate regions of Asia, according to Dai et al. (2022).

It is frequently planted all over the world, including in nations like western Xinjiang, China, Germany, Hungary, France, and Russia.

The goal of this study is to find out how effective plant extracts from *M. oliefera* are against amoebae that cause AK.

Material and methods

Preparing of plant extract:

Plant After being carefully cleaned, it was ground into a fine powder using a mechanical grinder , kept in sterile containers until they were needed. Plant extracts as an aqueous form have been used , depending on Harbone (1984). A glass flask holding 400 ml of hot, distilled water (500°C) and (20) g of dry plant powder was continuously turned for (12) hours and for 30 minutes. the filtrate was put in petri dishes and dried in the lab until it was needed at a temperature of (40°C). A (45°C) rotary evaporator (Orem Scientific Ltd., Switzerland) was used to concentrate the plant.

Gas contact mass spectrometry:

For quality control reasons labs of Nahran Omar-Basra Oil Company, the Gas Chromatography-Mass Spectrograph (GC-MS) method was used to find out what chemicals were made from the extract of *M. oliefera*.

Moringa oliefera qualitative reagents

Each *Moringa oliefera* seeds was put through a series of quality checks using different chemical reagents made in a lab: the Phenols test (Harbone, 1984), the Flavonoids test, the Glycosides test, the Al-Khazarji test, the Alkaloids test, the Peach and Tracey test, the Saponins test, the Bargah test, and the Tannins test (Jawad test).

Parasite specimens collection :

73 corneal specimen from the Basra Teaching Hospital that were allegedly taken from AK-infected people included 27 people who wore contact lenses and 46 people who did not.

Samples were obtained from the lower conjunctiva and the eye using a sterile cotton swab that was rolled from one side to the other. Then a light microscope was used to examine these samples. According to Anisah *et al.*

Parasite cultures:

Using PYGA media, *Acanthamoeba* growth has been accomplished. Many petri plates were sub-cultured to produce enough trophozoites and cysts to conduct the study.

Staining and examination methods:

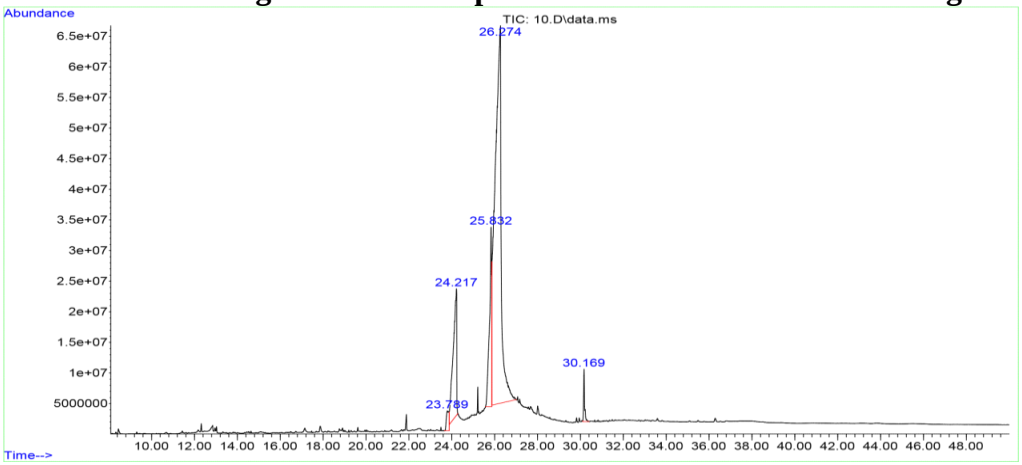
Prior to the investigation using the wet mount technique, two drops (25 l) of the *Acanthamoeba spp*. suspension were spread out on a glass slide, and the material was then covered off with a coverslip (El-Sayed & Hikal, 2015). The final classification method used was morphological level-based (Page, 1967). Transient stains like eosin and methylene blue are used in wet-mount staining techniques.

Cell viability test

The "Trypan Blue" dye (0.4%) was used in a 1:1 ratio to test the vitality of *Acanthamoeba* cells (Geisa et al., 2020). *Acanthamoeba* cells were taken out of the growth medium after being centrifuged at 2000 rpm for five minutes and being cleaned twice with sterile PBS saline. They were then counted using a Neubauer hemocytometer after being examined under a light microscope and injected into the mice's eyes.

Results:

Table 1. GC-MS showing bioactive composites of a crude extract of Moringa oleifera.



Precise tests of therapeutic plants have been used for the aqueous extracts of crude *Moringa oleifera* plant. Saponins , either, Alkaloids, phenols, tannins, glycosides, and flavones were found to be the majority compounds that are active in the *Moringa oliefera* aqueous plant extract.

Table 2 . Qualitative reagents of aqueous extract Moringa oleifera .

Reagents used	Moringa oleifera
Dragendroff Reagent / Alkaloids	+
Ferric Chloride Reagent / phenols	+
Saponins	+
Benedicts reagent/ glycosides	-
Lead Acetate / Tannins	+
Flavonoids/ alcoholic potassium hydroxide	+

(+) means extract have an active compounds (-)have no active compounds.

lethal activity of plant extract :

the effect of plant extract were indicated that the aqueous extract have been shown the greater amoebicidal activity compared with other extracts used .

Table 3. The lethal effect of Moringa oleifera extract on Trophozoite stage.

Time	concentration	*Ethanol*	*Ethyl acetate*	*Hexane*	*Mix*	Aqueous	$LSD_{0.05}$
2 min	0.625 mg/ml	0.80 ± 0.01^{c}	0.64 ± 0.05^{d}	0.91 ± 0.02^{b}	0.47 ± 0.06^{e}	1.00 ± 0.00^{a}	0.016*
	0.3125 mg/ml	0.13 ± 0.01^{a}	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	
	0.1562 mg/ml	0.74 ± 0.04^{c}	0.58 ± 0.02^{d}	0.80 ± 0.01^{b}	0.43 ± 0.02^{e}	1.00 ± 0.01^{a}	
	0.02 % w/v (Drug)	0.13 ± 0.01					
4 min	0.625 mg/ml	0.75 ± 0.01^{b}	0.53 ± 0.03^{c}	0.78 ± 0.01^{b}	0.37 ± 0.05^{d}	1.00 ± 0.00^{a}	0.041*
	0.3125 mg/ml	0.93 ± 0.06^{b}	0.79 ± 0.01^{c}	0.93 ± 0.03^{b}	0.57 ± 0.06^{d}	1.00 ± 0.00^{a}	
	0.1562 mg/ml	0.22 ± 0.03^{a}	0.35 ± 0.02^{a}	0.35 ± 0.02^{a}	0.35 ± 0.02^{a}	0.35 ± 0.02^{a}	
	0.02 % w/v (Drug)	0.22 ± 0.03					
6 min	0.625 mg/ml	0.60 ± 0.05^{c}	0.46 ± 0.02^{d}	0.73 ± 0.02^{b}	0.30 ± 0.02^{e}	0.97 ± 0.03^{a}	0.036*
	0.3125 mg/ml	0.81 ± 0.04^{c}	0.71 ± 0.01^{d}	0.90 ± 0.01^{b}	0.54 ± 0.05^{e}	1.00 ± 0.00^{a}	
	0.1562 mg/ml	0.96 ± 0.05^{b}	0.83 ± 0.04^{c}	0.96 ± 0.01^{b}	0.71 ± 0.05^{d}	1.00 ± 0.00^{a}	
	0.02 % w/v (Drug)	0.53 ± 0.03					

Different letters significant difference with (p value < 0.05).

Table 4. Shows Moringa oleifera aqueous extracts effect on cyst stage.

Time	concentration	*Ethanol*	*Ethyl acetate*	*Hexane*	*Mix*	Aqueous	$LSD_{0.05}$
2 min	0.625 mg/ml	0.74 ± 0.04^{c}	0.59 ± 0.02^{d}	0.84 ± 0.03^{b}	0.36 ± 0.05^{e}	1.00 ± 0.00^{a}	0.026*
	0.3125 mg/ml	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	0.22 ± 0.02^{a}	
	0.1562 mg/ml	0.59 ± 0.06^{c}	0.50 ± 0.01^{c}	0.71 ± 0.03^{b}	0.30 ± 0.01^{d}	0.98 ± 0.01^{a}	
	0.02% w/v (Drug)	0.13 ± 0.01					
4 min	0.625 mg/ml	0.64 ± 0.04^{c}	0.51 ± 0.02^{d}	0.70 ± 0.01^{b}	0.30 ± 0.01^{e}	1.00 ± 0.00^{a}	0.035*
	0.3125 mg/ml	0.83 ± 0.05^{c}	0.70 ± 0.01^{d}	0.90 ± 0.02^{b}	0.45 ± 0.04^{e}	1.00 ± 0.00^{a}	
	0.1562 mg/ml	0.35 ± 0.02^{a}	0.35 ± 0.02^{a}	0.35 ± 0.02^{a}	0.35 ± 0.02^{a}	0.35 ± 0.02^{a}	
	0.02% w/v (Drug)	0.22 ± 0.03					
6 min	0.625 mg/ml	0.53 ± 0.05^{c}	0.43 ± 0.05^{d}	0.62 ± 0.03^{b}	0.25 ± 0.05^{e}	0.96 ± 0.02^{a}	0.042*
	0.3125 mg/ml	0.77 ± 0.11^{b}	0.59 ± 0.01^{c}	0.78 ± 0.07^{b}	0.34 ± 0.02^{d}	1.00 ± 0.00^{a}	
	0.1562 mg/ml	0.89 ± 0.03^{b}	0.79 ± 0.02^{c}	0.90 ± 0.00^{b}	0.60 ± 0.04^{d}	1.00 ± 0.00^{a}	
	0.02% w/v (Drug)	0.53 ± 0.03					

Different letters shows significant difference with (p value < 0.05).

Discussion

Few compounds that are effective against parasitic infections are used as antiseptics or treatment agents due to many factors : the failure of this chemicals to enter the (blood-brain barrier) and the toughness of (cyst stage) furthermore, its resistance to severe circumstances (Mungroo et al., 2021).

But chemists have created several substances that could be utilized to treat a number of endoparasites.

Furthermore, the discovery of plant extracts and their application for AK therapies, as employed with many other parasites, have not received much attention in recent investigations. Due to the rarity of illnesses brought on by free-living amoebae and the limited information of physicians, the discovery of antiparasitic disease drugs is not adequately rewarded (Wink, 2012).

The popular aromatic medicinal herb *Moringa oliefera* is mostly utilized for its therapeutic effects. The most popular items are dried flowers, which have a variety of medical characteristics, including analgesic, anti-inflammatory, antibacterial, anti-spasmic, and sedative effects (Gardiner, 2007). The current study's findings indicate that antibacterial properties can be found in substances such as : chamazulene, , bisabolol umbelliferon and cyclic ethers. This is comparable to Ali and Alattar (2018) study ,it was found that the components in the extracted chamomile flower were the same. The cytotoxic effects of plant extracts on mice and their anti-amoebic abilities against Acanthamoeba castellani were assessed in vivo. The results of the present study show that these compounds considerably retard the proliferation of pathogenic Acanthamoeba.

There aren't many studies on how well *Moringa oliefera* plant extracts work against *Acanthamoeba*. One of them has been discovered to provide a variety They may be effective against infections caused by free-living amoebae because of their variety of therapeutic benefits, which include anti-inflammatory, antiviral, antibacterial,

antidiabetic, and antiprotozoal properties (Siddiqui et al., 2022). Because the cyst stage of *Acanthamoeba* spp. has the strongest layers of cyst walls, it is widely known to be resistant to a number of environmental stressors, such as extreme weather (Mitsuwan *et al.*, 2020; Sanchez & Gustavo, 2021). According to recent research, M. chamomilla plant extracts inhibited Acanthamoeba castellani trophozoites and cyst forms, and aqueous extracts demonstrated potent anti-Acanthamoebic activities.

A thorough destruction and inhibition have been shown to occur when using *Moringa oliefera* aqueous plant extracts.

According to Singh *et al*. (2011), certain plant species contain bioactive chemicals that have been shown to have strong amoebic effects on *Acanthamoeba castellani*, preventing its encystation and excitation. Oleic acid, has an antimicrobial activity and can induce cell death , is present in medicinal plant extract, according to a recent study. This is like a study of Desbois and Smith., (2010), that proposed that (oleic acid) is significantly obstructs the growth of the trophozoites because of the critical role in causing the destruction of the structure in membrane's lipid. Additionally, the results imply that *Acanthamoeba castellani* proliferation may be inhibited by oleic acid therapy by inducing apoptosis and trophozoite autophagy. The current study's findings imply that the high flavonoid concentration of these plant extracts contributes to the destruction of *Acanthamoeba* spp.'s wall stability and inhibits both stages of the organism. This is comparable to a 2013 study by Dzoyem et al. that showed flavonoids can stop microbe growth by depleting the membrane and preventing the creation of DNA, RNA, and even proteins. According to the estimation of the LD50 of used aqueous plant extracts, that appears with no lethal properties on mice (no proportion of mice died across all groups).

Moringa oleifera bioactive Compounds:

Due to the bioactive chemicals found in its leaves, roots, and seeds, *Moringa oleifera* is a curative plant with a diversity of calming qualities (anticancer, antihypertensive , antiproliferative, anti-inflammatory and antibacterial) (Pop *et al*., 2022). Influence's of these medicinal compounds largely depends on two things : their activity and their stability. According to Pop et al. (2022), these characteristics are directly correlated with their components and capacity to incorporate and sustain their bioactive qualities. n-Hexadecanoic acid, 6-octadecenoic acid , carbohydrates, amino acids, alkaloids, glycosides, flavonoids, steroids saponins, tannins and proteins have all been found in *M. oleifera* seed samples. The same was found by Adhere *et al.* (2020) study , when they found the same component in seeds.

In vitro effect of plant extracts on *Acanthamoeba spp*.

The study investigated that the cytopathic effects of plant extracts against *Acanthamoeba spp.* in vitro. analysis had been detected that these substances have potent lethal properties against *Acanthamoeba*.

There are few studies on the effectiveness of *Moringa oleifera* extracts on Acanthamoeba spp. Studies have been found a wide various types of curing , such as : antiviral , anti-inflammatory, antidiabetic, antibacterial and finally antiparasitic action , which explain their effectiveness on amoebae.

The cyst stage of *Acanthamoeba spp.* is generally known to be resistant to a variety of environmental stressors, including harsh conditions like hunger, pH ,heat, osmolarity, dryness', chlorination , antimicrobial substances and drugs resistance), because it has the double walls in cyst stages (Mitsuwan et al., 2020; Sanchez & Gustavo, 2021).

Acanthamoeba spp. trophozoites and cysts stages were inhibited by *M. oleifera* aqueous plant extracts, and had a strong lethal effects, when compared to the control, cysts treated with aqueous plant extracts from *M. oleifera* have been showed a complete destruction and suppression.

Also it has , lethal activity of (0.22 -+ 0.02) for trophozoites and (0.22 _+0.02) for cysts, . It was also found in Singh et al.'s (2011) study that the presence of essential active units in this extracts shown important amoebicidal action for *Acanthamoeba spp*., by blocking its cyst and trophozoite formation yelding in effectiveness inhibiting ability of *Acanthamoeba spp*. growth is likely due to the (bioactive) units in this plant that destroy the cell membrane of the organism. Also the study suggested that , medicinal plant act as a strong lethal factor for *Acanthamoeba spp*. According to oleic acid compound which has lethal activity causing apoptosis.

This is comparable to a study by Desbois and Smith .,(2010), which suggested that (oleic acid) can inhibits the trophozoites growth by its role in ruining the structure of membrane's lipid.

Additionally, the results of this study imply that oleic acid administration may restrict Acanthamoeba spp. growth by causing apoptosis and trophozoites to engage in autophagy.

This is comparable to Wu et al.'s (2018) study, which showed the significance of oleic acid in the activation of amoebic autophagy. Also, contrasts with Manna *et al*. (2013), who claimed that , only function of oleic acid in *Acanthamoeba spp*. was to block the electron transport and act as an active amoebicidal .

The results are consistent with Dzoyem *et al*., (2013), that found (flavonoids) can prevent the growth of microorganisms by depleting membrane proteins.

Furthermore, the high flavonoid content in these plant extracts appears to contribute to the destruction of Acanthamoeba spp.'s wall stability and to the inhibition of both of its developmental stages.

However, there is no research on Acanthamoeba species. Phenolic chemicals, have also been shown lethality on parasites, in a study by Ramanandraibe *et al*., (2008) on their lethal effect on Plasmodium. Phenols have a significant role in the fight against Entamoeba species by inhibiting the enzymes responsible for oxidizing chemicals produced by the parasites (Lal et al., 1996).

The results show that aqueous extracts of *M. oliefera* at a concentration of 0.625 mg/ml are highly efficient in killing the amoeba stages. This extract was highly efficient in eliminating both parasite stages when used in previously mentioned quantities.

The findings suggest that Moringa oliefera aqueous plant extracts at a dosage of 0.625 mg/ml might be helpful for eliminating the protozoan in Finally, it may be claimed that more research on this parasite is really necessary.

Conflict of Interest:

The authors declare no conflict of interest

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Cite this: Abdulbaki , Z. A., Alsamir , S. A., & Almansour, N. A. A. (2023). In Vitro Effect of Aqueous Extract of Moringa Oliefera Plant on Acanthamoeba Spp. Journal of Medical Research and Health Sciences, 6(9), 2765–2762. https://doi.org/10.52845/JMRHS/2023-6-9-5