



Synergistic Effect of Moringa Leaves and Antifungal on *Candida tropicalis*

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ABSTRACT

Background: Increased interest in the medical properties of *Moringa oleifera*, which is widely utilised as an antibiotic, antioxidant, and nutrition because to its high mineral and vitamin content. **Objective:** The present research aims to extract active components from *oleifera* leaves and apply them as ant microbes. **Materials and Methods:** The active components from *oleifera* leaves were extracted using several procedures, including cold water, hot water, a combination of water and alcohol in various ratios, and hot alcohol. Suspensions of fungal and bacterial strains were also created for testing. **Results:** The results demonstrate that the most active components were extracted using hot alcohol, followed by a hot water method, and then cool alcohol. The extracts exhibit varied bioactivities depending on the amount of active components in the extract solution. **Conclusions:** *Oleifera* leaf extract may be utilised as a bioactive agent against *Candida tropicalis*.

Keywords: *Oleifera* leaf, extract, *C. tropicalis*, water, alcohol extraction

INTRODUCTION

Drought resistant The *Moringa* tree (scientific name: *Moringa oleifera*) is part of the Moringaceae family. This tree is indigenous to India and features horseradish-like roots and enormous drum-shaped seed pods, giving it the common names horseradish tree and drumstick tree. *Moringa* is well-known and used over the world for its anticancer, antidiabetic, antitumor, and antibacterial effects. *Moringa oleifera* has been used in ancient diets in South America, Southeast Asia, the Arabian Peninsula, and Africa (Abdullah et al., 2023). *Moringa* also contains high levels of vitamin C, tocopherol, beta carotene, minerals, and vital amino acids. *Moringa oleifera* has fourteen times the calcium than milk, 9 times more iron than spinach, 7 times more vitamin C than oranges, 4 times more potassium than bananas, and twice as much vitamin A as carrots. In addition to its nutritional value, it improves the flavour and aroma of food. Furthermore, the presence of phytochemicals such as flavonoids and other phenols in their leaf extracts can prevent the growth of harmful bacteria and prolong the shelf life of food (Arif et al., 2022).

***Candida tropicalis*:** Fungal infections are seen as posing an increasingly serious threat to human health worldwide. According to data, roughly 1.7 billion individuals suffer from fungal infections, the majority of which are superficial infections of the skin and mucosa (reviewed by). *Candida* species are the leading cause of nosocomial fungal infections, ranking fourth among all hospital-acquired illnesses. *Candida* species cause around 400,000 yearly bloodstream infections worldwide, with the causative fungus resulting in death rates of more

than 40%. *Candida albicans* is the most often isolated *Candida* species; nevertheless, the incidence of non-*albicans* species such as *Candida tropicalis*, *Candida parapsilosis*, and *Candida glabrata* has grown in recent decades due to the long-term usage and limited options of antifungal drugs (Bing et al., 2020).

Crucially, reports of *C. tropicalis*'s increasing treatment resistance have emerged in recent years. According to the 2013 global SENTRY antifungal monitoring report, 11.60% of the 31 countries studied exhibit *C. tropicalis* resistance to fluconazole. Furthermore, the fluconazole resistance rate of *C. tropicalis* increased from 11.20% in 2009 to 42.70% in 2014, according to data from the China Invasive Fungal Resistance Monitoring Network (CHIF-NET) (Wang et al., 2021).

Candida tropicalis, one of the genus' most notable species, has a remarkable epidemiology in tropical regions, including South America and Asia. In fact, India is now host to the species' first *Candida* epidemic. In recent years, *Candida tropicalis* has also demonstrated a worrying antifungal resistance profile. It is crucial to highlight that each pathogenic species in the genus *Candida* has its own distinct biology; nonetheless, the majority of research on *Candida* virulence factors is concentrated on studies done on *Candida albicans* (Moreira & Ishida, 2023).

Materials and Methods

Preparing of plant extracts

There extracts of moringa leaves are prepared as fallowing:

Cold Water Extract

A grinder was used to dry, weigh, and powder the plant components. A macerator was used to soak 500 g of each powdered material in 70% V/V ethanol for 72 hours at room temperature. After that, the extract was examined to find any secondary metabolites (Kusuma et al., 2022).

Hot Water Extract

After being hand-picked, the leaves were rinsed under running water and drained. After being thermally dried for 48 hours at 40 °C in an oven (Memmert, Buchenbach, Germany), the samples were crushed into a fine powder using a food grinder (Philips, Jakarta, Indonesia). *M. oleifera* 2.5% decoction was prepared by weighing approximately 2.5 g of dried leaves and soaking them in 100 mL of distilled water that was kept at 90 °C (within ± 2 °C) for 30 minutes. heated water was poured immediately onto the sample to achieve 100 mL after the mixture had been filtered under heated circumstances over a Buchner funnel. *M. oleifera* 5.0% underwent the same process (Natsir et al., 2023).

Cold Alcoholic Extract

One thousand milliliters of 70% ethanol were used to macerate the dried powdered flowers (100 grams) for three days at room temperature, shaking occasionally. Until the plant materials were used up, the extract was filtered and the marc was extracted again using the same procedure. To create the dry extracts (yield w/w: 8.69%), the collected filtrates were combined, evaporated to dryness under low pressure, and then kept at 4 °C until needed (Alhakmani et al., 2013).

HOT Alcohol Extract

To make the aqueous solution, 100 grammes of moringa leaves were weighed, placed in a piece of gauze, and placed inside the soxhlet apparatus. 700 millilitres of 99% ethanol alcohol were then applied to the leaves during a 24-hour period. A glass funnel and a piece of gauze were used to filter the sample, which was then transferred to a glass beaker with a specified weight after the operation was repeated several times to ensure an acceptable quantity of the active component. It was maintained in an incubator set at 40-45 degrees Celsius until it dried and was ready to use (Ladd et al., 1978).

Preparation of 70% alcoholic water extract

400 milliliters of the extract were produced by weighing 100 grams of Moringa leaves and putting them in 70% ethanol alcohol (420 milliliters) and distilled water (180 milliliters) (Vongsaka et al., 2013).

Effect of the synergistic morenga extracts with anti-fungals against *C. tropicalis*

- The Veterinary Drugs Research and Production Center's methodology states that the following antifungals have been used: miconazole nitrite, clotrimazole, and nystatin. This is how it operated:
- To ensure a complete dissolving procedure, 0.1 gm of dried moringa extract samples were dissolved in 10 ml of distilled water before being added to the Vortex device.
- Ten millilitres of distilled water (pH:6) are used to dissolve 0.01 grammes of the three antifungals employed in this investigation (nystatin, clotremazole, and miconazole nitrate). To guarantee effective dissolving, a vortex device and a tiny quantity of heat are applied, and we then make the following dilutions:
 - 1- First, 50 ul of moringa extract and 50 ul of antifungals are taken and placed on culture medium.
 - 2- The second dilution is 75 ul of moringa extracts and 25 ul of antifungal and placed on culture medium.
 - 3- The third dilution is 95 ul of Moringa extracts and 5 ul of antifungal and put to culture media. The plates are placed in the incubator for 24 hours, after which we inspect the results and measure the diameters.

Biochemical Diagnosis (Vitek Test)

The following Vitek tests are performed on the fungal isolates to verify them:

To suspend the microorganism in 3.0 mL of sterile normal saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 × 75 mm transparent plastic (polystyrene) tube, transfer a suitable number of clean colonies from selective media using an applicator wooden stick. The DensiChek turbidity meter is used to adjust the turbidity level. Gram-positive bacteria have a McFarland turbidity range of 0.50 to 0.63.

Inoculation: Bacterial suspensions are inoculated onto reagent cards using an integrated vacuum mechanism. The reagent card is inserted into the adjacent slot, the transfer tube into the corresponding suspension tube, and a can tube with the bacterial suspension is placed in a specific rack (cassette). A vacuum chamber station accepts the loaded cassette. The organism suspension is pushed via the transfer tube into micro-channels that fill each test well after the vacuum is removed and air is restored into the station.

Incubation and card sealing: Before loading into the incubator, infected cards are passed via a device that shuts the card and disconnects the transfer line. Every card is incubated at $35.5 (\pm 1.0)$ degrees Celsius. Every fifteen minutes, each card is removed from the incubator, transferred to the optical system for reaction readings, and returned to the incubator until the next read time. The full study set's results were obtained in 4-8 hours.

Results and Discussion

Incubation and card sealing: Prior to loading into the incubator, contaminated cards are passed via a mechanism that closes the card and disconnects the transfer line. Each card is incubated at 35.5 ± 1.0 degrees Celsius. Every fifteen minutes, each card is withdrawn from the incubator, brought to the optical system for reaction readings, and then returned to the incubator until the next reading time. The entire research set's findings were achieved in 4-8 hours (Arora , 2021).

Table 1: Shows the Chemical constituents of leaf extracts of Moringa Oleifera

	Detection type	Cold Alcoholic Extract	Cold Water Extract	Hot water Extract	Hot Alcohol Extract	70% Alcoholic water Extract
1	Tannins Test	+	+	+	+	+
2	Carbohydrate Test	—	—	—	—	—
3	Glycosides Test	+	+	+	+	+
4	Phenols Test	+	+	++	—	+
5	Resins Test	+	—	—	+	—
6	Flavonoids Test	+	+	+	+	+
7	Saponin Test	+	+	+	+	+
8	Alkaloid Test	+	+	+	+	+
9	Protein Test	+	+	+	+	+
10	Coumarins Test	+	+	+	+	+
11	Terpenes Test	—	—	—	—	—
12	Steroids Test	—	—	—	—	—

Table 2: Synergistic effect of Moringa extracts with the antifungal nystatin on *C. tropicalis*

Dilution for Nystatin on *C. tropicalis*

Number	Moringa extract	50:50	75:25	95:5
1.	Cold Alcoholic Extract	25	29	24
2.	Cold Water Extract	23	27	21
3.				
4.	70% Alcoholic water Extract	27	24	25
5.	Hot alcohol Extract	33	23	23
6.	Hot water Extract	21	30	25

Table 2 shows that the hot alcoholic extract had the greatest synergistic effect when nystatin and moringa were diluted in a 50:50 ratio. This was followed by a 70% alcohol water extract, cold alcoholic extract, cold water extract, and hot water extract. When nystatin and moringa were diluted 75:25, the results showed that the hot water extract had the most synergistic action. This was followed by the cold alcohol extract, 70% alcohol water extract, and the hot alcohol extract.

The results of diluting Nystatin and moringa at a 95:05 ratio indicated that the strongest synergistic action was displayed by 70% alcoholic water extract and hot water extract, followed by cold alcohol extract, hot alcohol extract, and cold water extract.

Table 3: The synergistic efficacy of Moringa leaf extracts with the antifungal Miconazole nitrate on *C. tropicalis*

Dilution for miconazole nitrate on *C. tropicalis*

number	Moringa extract	50:50	75:25	95:5
1	Cold Alcoholic Extract	25	23	21
2	Cold Water Extract	24	24	22
3	70%Alcoholic water Extract	27	24	16
4	Hot alcoholExtract	35	36	30
5	Hot waterExtract	20	25	18

The results are reported in Table 3. When Miconazole Nitrate and Moringa were mixed 50:50, the Hot Alcohol Extract had the strongest synergistic action, followed by 70% alcoholic water extract, Cold Alcoholic Extract, Cold Water Extract, and Hot Water Extract. When

Miconazole Nitrate and Moringa were diluted 75:25, the Hot Alcohol Extract had the strongest synergistic action, followed by the Hot Water Extract, 70% alcoholic water extract, Cold Water Extract, and Cold Alcoholic Extract. The findings of diluting Miconazole nitrate and moringa at a ratio of 95:05 revealed that the hot alcohol extract had the most synergistic impact, followed by the cold alcohol extract, the hot water extract, and the 70% alcohol water extract.

Table 4: The synergistic efficacy of Moringa leaf extracts with the antifungal clotremazole on *C. tropicalis*

Dilution for clotremazole on *C. tropicalis*

Number	Moringa extract	50:50	75:25	95:5
1	Cold Alcoholic Extract	14	13	11
2	Cold Water Extract	12	12	7
3	70%Alcoholic water Extract	16	11	13
4	Hot alcohol Extract	20	14	24
5	Hot water Extract	17	11	18

Table 3 shows that the hot alcohol extract had the strongest synergistic impact when clotremazole and moringa were diluted 50:50. This was followed by hot water extract, 70% alcoholic water extract, cold alcohol extract, and cold water extract. The results of diluting clotremazole and moringa at a 75:25 ratio showed that the hot alcohol extract had the greatest synergistic impact, followed by the cold alcohol extract, the cold water extract, the 70% alcohol water extract, and the hot water extract. When clotremazole and moringa were diluted 95:05, the findings showed that the hot alcohol extract had the greatest synergistic impact, followed by the hot water extract, 70% alcoholic water extract, and cold alcoholic extract. Cold Water Extract has the least amount of synergy.

Table 5: The results of vitek test for *Candida tropicalis*

Biochemical Details

3	LysA	-	4	IML Ta	+	5	LeuA	+	7	ARG	+	10	ERYa	-	12	GLY La	-
13	TyrA	+	14	BNA G	-	15	ARB a	-	18	AM Ya	-	19	dGAL a	+	20	GEN a	-
21	DGL Ua	+	23	LAC a	-	24	MAd Ga	-	26	dCE La	-	27	GGT	-	28	dMA La	+
29	DRA Fa	-	30	NAG A1	-	32	dMN Ea	+	33	dME La	-	34	dMLZ a	+	38	ISBE a	-

39	LRH Aa	-	40	XLT a	+	42	dSOR a	+	44	SAC a	+	45	URE	-	46	AGL U	+
47	DTU Ra	+	48	dTR Ea	+	49	NO3a	-	51	lAR Aa	-	52	dGAT a	+	53	ESC	-
54	LGL Ta	+	55	dXY La	+	56	LATa	-	58	ACE a	+	59	CITa	+	60	GRT as	+
61	LPR Oa	-	62	2KG a	+	63	NAG a	+	64	dGN Ta	+						

The results of the vitek examination of *Candida tropicalis* showed that it is positive for the following compounds (TyrA, dGLUa, dTURa, lGLTa, lPROa, lMLTa, XLTa, dTREa, dXYLa, 2KGa, LeuA, dMNEa, dSORa, NAGa, ARG, SACa, ACEa, dGNTa, dGALa, dMLZa, dGATa, CITa, dMALa, AGLU and GRTas).

The results of the vitek examination of *Candida tropicalis* showed that it is negative for the following compounds (LysA, dRAFa, lRHAA, BNAG, LACa, NAGA1, ARBa, MAdGa, NO3a, LATa, AMYa, dCELa, dMELa, lARAa, ERYa, GGT, URE, GLYLa, GENa, lSBEa and ESC).

Examination of the efficacy of moringa extracts with antifungal against *Candida* spp.

Table No. (6) showed that Moringa extracts, and nystatin had significantly different effects on *Candida tropicalis* than other extracts such as cold alcoholic extract, cold water, and hot water extract (25.67, 23.67, and 21.67 mm, respectively). The 50/50 concentration of hot alcoholic extract had the greatest inhibition Zone 33 mm, followed by the 70% alcoholic extract 27 mm.

Furthermore, it was shown that there were substantial variations in the extracts at 25/75 concentration. The hot water extracts had the largest inhibitory zone on *Candida tropicalis*, measuring 30.33 mm, followed by the cold alcoholic extract at 29 mm. This differed from the other extracts, which were cold water, 70% alcoholic, and hot alcoholic extract (27, 24, and 23.67 mm, respectively).

While at 95/5 concentration, it was discovered that the hot water extract 25.33 showed the highest inhibitory activity against *Candida tropicalis* 25.33, followed by the 70% alcoholic extract 25mm in compared to the other extracts: cold alcohol, hot alcohol, and cold water extract (24,23.67,21.33 mm, respectively).

Table (6): The synergistic efficacy of Moringa leaf extracts with the antifungal Nystatin on *Candida tropicalis*

Moringa extract	50:50:00	75:25:00	95:05:00	P value
Alcoholic cold leaves	25.67 ±1.15 A a	29 ±2 A b	24 ±1 A a	Sig
Watery cold powder	23.67 ±1.53 B a	27 ±2 A b	21.33 ±1.53 B a	Sig

Alcoholic watery 70%	27 ±2 A a	24 ±2 B a	25 ±1 A a	Not Sig
Hot alcohol	33 ±3 C a	23.67 ±1.53 B b	23.67 ±0.58 A b	Sig
Hot watery	21.67 ±1.53 B a	30.33 ±2.52 A b	25.33 ±1.53 A a	Sig
P value	Sig	Sig	Sig	

When compared to other extracts, such as cold alcohol, cold water, and hot water extract (25.33, 24, and 20.33 mm), the 50/50 hot alcohol extract had the greatest inhibition Zone 35 mm, followed by the 70% alcoholic extract at 27.33 mm. According to Table No. 7, there were significant differences in the effects of Moringa extracts and miconazole on *Candida tropicalis*. Significant differences were also found between the extracts at the 25/75 concentration; the hot alcohol extract exhibited the biggest inhibitory zone on *Candida tropicalis*, measuring 36.67 mm, followed by the hot water extract, measuring 25.67 mm. This contrasted with the other extracts, which were 70% alcoholic, cold water, and cold alcoholic extract (24.67, 24.33, and 23 mm, respectively). When compared to the other extracts—cold alcohol, hot water, and 70% alcoholic extract (21, 18, and 16.67 mm, respectively), it was discovered that the hot alcoholic extract had the highest inhibitory activity against *Candida tropicalis* at 95/5 concentration for 30.67 mm, followed by the cold water extract for 22.33 mm.

Table (7): The synergistic efficacy of Moringa leaf extracts with the antifungal Miconazole on *Candida tropicalis*

Moringa extract	50:50:00	75:25:00	95:05:00	P value
Alcoholic cold leaves	25.33 ±1.53 A a	23 ±1 A b	21 ±1 A b	Sig
Watery cold powder	24 ±2 A a	24.33 ±0.58 A a	22.33 ±2.08 A a	Not Sig
Alcoholic watery 70%	27.33 ±1.15 A a	24.67 ±1.53 A a	16.67 ±1.53 B b	Sig
Hot alcohol	35 ±2.65 B a	36.67 ±2.52 C a	30.67 ±1.15 C b	Sig
Hot watery	20.33 ±1.53 C a	25.67 ±0.58 A b	18 ±1 B c	Sig
P value	Sig	Sig	Sig	

Table No. (8) shows that the effects of Moringa extracts and clotremazol on *Candida tropicalis* differed significantly. The 50/50 concentration of hot alcohol extract had the largest inhibition zone, measuring 20.33 mm, followed by the hot water extract, which measured 17 mm. This contrasted with the other extracts, which were 70% alcoholic, cold alcohol, and cold water (16.33, 11.11 mm, respectively).

Significant variations were also seen between the extracts at the 25/75 concentration; the hot alcoholic extract exhibited the biggest inhibitory zone on *Candida tropicalis*, measuring 14.67 mm, followed by the cold alcoholic extract, measuring 13 mm. This contrasted with the other extracts, which comprised 70% alcoholic extract, hot water, and cold water (12.67, 11.33, and 11 mm, respectively). In contrast to the other extracts, which were 70% alcoholic, cold alcohol, and cold-water extract (13, 11.33, and 7 mm, respectively), the hot alcoholic extract had the greatest inhibitory activity against *Candida tropicalis* at 95/5 concentration, measuring 24.67 mm, followed by the hot water extract (18.33 mm).

Table (8): The synergistic efficacy of Moringa leaf extracts with the antifungal Clotremazol on *Candida tropicalis*

Moringa extract		50:50:00	75:25:00	95:05:00	P value
Alcoholic leaves	cold	14.33 ± 1.53 A a	13 ± 1 A a	11.33 ± 1.53 A a	Not Sig
Watery leaves	cold	12 ± 1 B a	12.67 ± 1.15 A a	7 ± 1.73 B b	Sig
Alcoholic 70%	watery	16 ± 1 C a	11 ± 1 B b	13 ± 2.65 A b	Sig
Hot alcohol		20.33 ± 1.53 D a	14.67 ± 1.15 A b	24.67 ± 1.53 C c	Sig
Hot watery		17 ± 1 C a	11.33 ± 1.53 B b	18.33 ± 1.53 D a	Sig
P value		Sig	Sig	Sig	



Figure. (1). The inhibition zone of *C. tropicalis* against moringa extract and nystatin

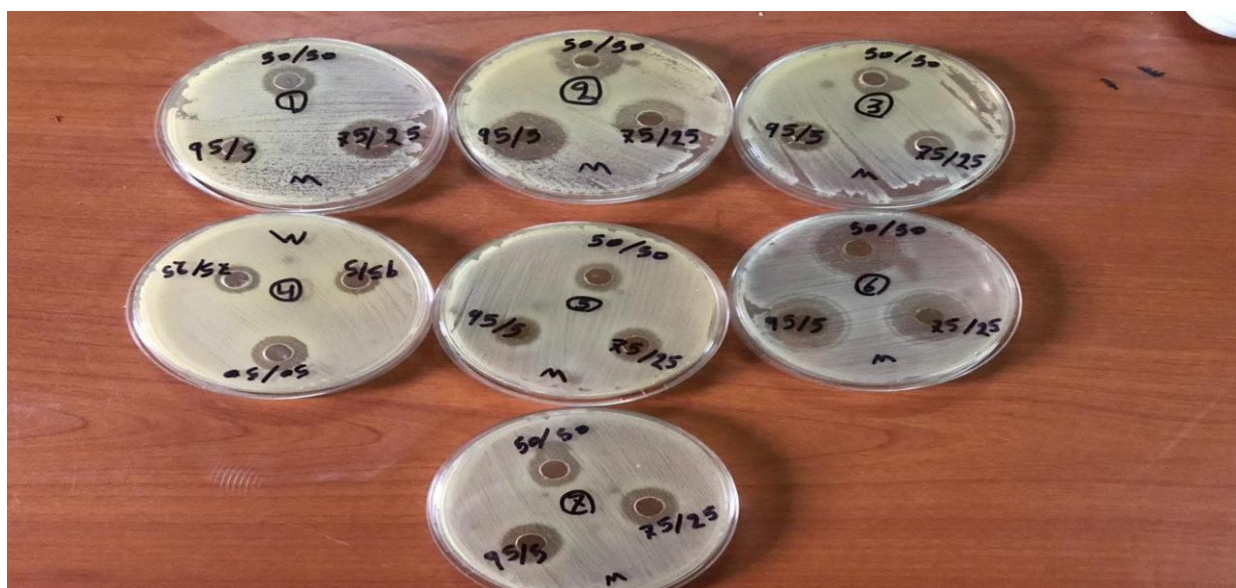


Figure. (2). Inhibition zone of *C. tropicalis* against moringa extract and Miconazole nitrate



Figure. (3). The inhibition zone of *C. tropicalis* against moringa extract and clotremazole

Conclusions

The leaf of *Moringa Oleifera* has been shown to have certain bioactive components that have action against particular germs, which validates traditional healers' usage of *Moringa oleifera* leaf to treat some diseases. However, the appropriate dose and purity remain a hurdle. The hot alcoholic extract of *Moringa* leaves demonstrated the highest effectiveness.

Recommendation

- Further study is needed on the dose and in vivo assessment of the leaf extract.
- Moringa extracts and antifungal medications (*Nystatin*, *Miconazole Nitrate*, *Clotremazole*) are particularly effective against *Candida tropicalis*.

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