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Phytochemical, Antimicrobial Activity and Larvicidal Effect of Flower, *Moringa oleifera*

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Abstract

Moringa oleifera is a common plant found all over the world that belongs to the Moringaceae family and comes in a variety of species. It is well-known for its therapeutic properties. The material was extracted using the cold percolation process, which included methanol, ethanol, chloroform, petroleum ether, and aqueous. Moringa's therapeutic impact could be a result of a mixture of bioactive chemicals found in them, as determined by qualitative and quantitative screening. The maximum amount of carbohydrate (238.9 mg/g) was found in the aqueous extract of *M. oleifera* flower compared to the other phytocompounds studied. The extracted material was tested against microorganisms that cause illnesses in humans in everyday life. The crude chloroform *Moringa oleifera* flower extract is highly resistant to the anaerobic, rod-shaped bacteria *Klebsiella pneumonia*, with an inhibition zone of 13 mm, greater than the positive control streptomycin at 11 mm, and the lowest inhibition zone of 8.5 mm for *Escherichia coli*. Ethanolic floral extract is resistant to the majority of microorganisms tested. *Aspergillus flavus* has the maximum zone of inhibition at 11 mm, whereas *Aspergillus niger* has the lowest zone of inhibition at 8 mm. In MIC tests, the results show that the aqueous extract has good activity and that the ethanolic extract has a linear result of 114± 0.00 in all of the bacteria tested. In addition, the aqueous floral extract had a higher value of 609 ± 0.00 and the lowest value of 6.5 ± 0.00 in *Escherichia coli*. The larvicidal activity of *M. oleifera* exhibited in the fourth instar larvae of *Ae. Stephensi* and *A. aeghpti*. The result revealed that the *M. oleifera* flower extract is effective mosquito control agents. Even while ayurvedic treatments employ this plant, it is also used to evaluate chemicals and can be used in modern medications; the current phytocompounds from this species have a lot of therapeutic qualities.

Key words: *Aspergillus flavus, Escherichia coli,* Larvicidal*, Moringa oleifera,* Phytocompounds

Moringa oleifera is one of the most similar and valuable medicinal plants; it has been used to treat pathogens, infections, and other ailments. Infections caused by germs have increased dramatically over the world. When patients have a low immunological level, this life-threatening circumstance has become critical to explain in terms of morbidity and death [1]. *Moringa oleifera* has been a well-known plant for decades. This plant is a member of the Moringaceae family, which includes 13 species [2]. It's commonly referred to as a drumstick. Moringa leaves are utilized as fragrance ingredients, and the leaves are also ingested for their health advantages, since it has been scientifically confirmed to have medicinal capabilities [3]. Every portion of that plant can be utilized to cure a variety of disorders, including liver disease, inflammation, hepatic, peptic ulcers, cardiovascular disease, and others [4]. One of the most well-known uses of Moringa in the West is to use its powdered seeds to flocculate pollutants and purify drinking water [5]. This tree has recently been promoted as an excellent indigenous source of highly digestible protein, calcium, iron, vitamin C, and carotenoids that can be used in many of the world's so-called "developing" nations where malnutrition is a big issue.

Researchers discovered organic compounds like polyphenol in *Moringa oleifera* extract, which plays an important role in human health by regulating metabolism [6]. The desire to discover new effective treatments is on the rise in many countries due to underlying ailments. The motivation for this research utilizing *Moringa oleifera* flower extract was based on previous observations, which revealed a more

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promising presence of antibiotic chemicals. In the long run, it will produce many strains. All of these considerations have made it critical to create new antibiotics that are both effective and environmentally friendly [7]. Phytomedicines are plant therapeutic substances such as alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, carbohydrates, fats and fixed oils, steroids, and glycosides [8]. People commonly trust these bioactive substances, and herbal remedies are quite inexpensive [9-10]. The purpose of this study was to investigate the role of *Moringa oleifera* flower extracts. To test the effectiveness of flowers' larvicidal action and determine whether they may be employed as an antibiotic against some hazardous human germs.

MATERIALS AND METHODS

Collection of plant material and extraction procedure

Moringa oleifera flowers (Fig 1) were collected in March of 2021 from Kurunthancode, Tamil Nadu, India. The samples were botanically identified by Dr. P. Nagerndra Prasad, Head of the Department of Biotechnology, Sri Paramakalyani College, Alwarkuruchi, Tirunelveli district. The extraction of the *Moringa oleifera* flower was performed by following the procedure in accordance to Napoleon *et al*. [11] with five different solvents such as Ethanol, Methanol, Chloroform, Petroleum ether and Aqueous.

Kingdom	:	Plantae
Class	:	Angiosperms
Order	:	Brassicales
Family	:	Moringaceae
Genus	:	*Moringa*
Species	:	*oleifera*

Fig 1 Flower of *Moringa oleifera*

Phytochemical analysis

Qualitative analysis of phytochemicals

A quantitative phytochemical test was performed on the flower extract of *M. oleifera* to ensure the presence of several chemical constituents such as alkaloids, fat and fixed oil, tannin, carbohydrate, steroids, flavonoid, phenol, glycosides, and saponins [12].

Quantitative analysis of phytoconstituents

Phtoconstituents were estimated by using different methods like Steroids [13], Saponin [14], Tannin [15], Terpenoids [16], Amino acids [17], Alkaloids, Flavonoids and Phenol [18], Carbohydrate [19] and Glycosides [20].

Antimicrobial screening

The Antibacterial activities of *Moringa oleifera* flower extract were investigated against Gram (-) and Gram (+) bacteria. The pathogenic bacteria are *Klebsiella pneumonia*, *Escherichia coli, Bacillus subtilis*, and *Staphylococcus aureus*. The Antifungal activities of *Moringa oleifera*flower extract were performed under three fungal strains known as *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger*. The antimicrobial activity of five different solvents (like Ethanol, Methanol, Chloroform, Petroleum ether, and Aqueous) of *Moringa oleifera* flower extract was determined using an Agar disc diffusion method followed by the protocol of [21].

Each one of the microbial plates was prepared under sterile conditions. The sterile disc was soaked in the respective solvents and kept in a vortex mixer for 15 minutes. The sterile swab is used to equally spread the microbes in the plates. The sample-loaded discs were placed in the plates at labeled positions. At 37°C, all the bacterial plates were incubated for 24 hours. The fungal plates were observed for 48 hours at room temperature (Approx. 31°C). The inhibition clear zone against microbes is measured in the millimeter (mm) range.

Minimum inhibitory concentration (MIC)

Triplication in the experiment was done. A 96-well microtiter plate was used to perform the Minimum Inhibitory Concentration (MIC) assay. Each well-containing nutrient broth (90 μL) inoculated with bacterial culture 10 μL. In each well, the extract of *Moringa oleifera* was added separately with the concentration ranging from 900 μL to 6.25 mg/mL. After 12 hours of incubation of the plate at 37°C, in each plate p-iodonitrotetrazolium, Violet was added (0.4 mg/mL) and again incubated for 6 hours. After incubation, in viable bacteria, the color change from yellow to red to purple was observed and no color change in a lower concentration. The result was noted as MIC. The procedure was determined and described by [22].

Larvicidal activity

The larvicidal activity of the *M.oleifera* flower extract was evaluated as per methods recommended by [37]. Different concentration (65.5, 125, 250, 500 and 1000 mg/ml) of the test samples were used. The 10 ml of distilled water was filled in each petri plates, early second stage of *Aedes aeghpti* and *Anopheles stephensi* (100) were introduced to each of the test solution as well as the control. The control consists of distilled water only. For each experiment all the six replicates were maintain at a time in room temperature. The larvicidal activity was calculated after every 24 hours by prohibit analysis up to three days [23].

Selection of mosquito species

The selected of Mosquito species taken for studies are, *Aedes aeghpti* and *Anopheles stephensi. A. aeghpti* (L) mosquito spreads vector of several fevers, yellow fever, chikungunya fever, dengue fever and Zika fever. This *A. aegypti* transmitting several tropical virus or fever and the bites of female for blood help them a need to mature her eggs. *A. stephensi* is a primary vector to course malaria in India and Africa including the same sub genes as *Anopheles gambiae*. This species is also able to endure high levels of salinity and have been found to breed in water and even salinity in equal found in sea water.

Mosquito culture

The all of test were carried out against reared *Aedes aeghpti* and *Anopheles stephens* they were free to exposed to pathogen and insecticides in the open environment. The growth of the larvae was observed in the container where the water stored from long time, some decayed leaves and yeast were disposed in it which was required nutrient for growth. The female mosquito breeds the eggs in water and sucked blood were help them to lay number of eggs. The pre-mature larvae were observed and collected.

Larvicidal assay

It was determined according to standard procedure and instruction of the WHO [24]. Bio-assay with Larvae was performed according to [25] were incubated with *M. oliefera* flower extract solution of different concentration was prepared in the range (65.5, 125, 250, 500 and 1000 mg/ml). Bio-assay were performed using *M. oleifera* flower extract in third instar larvae of *Aedes aeghpti* and *Anopheles stephens* were placed in each test solution to observe the larvicidal properties as per following procedure. The group of 100 larvae were placed in the each of the plate containing with the test distilled water solution the death of each larva in plate was recorded. The negative control experiment without extract was run parallel L. The mortality rate (%) was determined after incubated at 27 ± 17°C for each 24 hours up to three days under 12-12 hours (light-dark). The number of death larvae was counted after 24 hours of exposure, and percentage mortality was recorded from the average of five replicates. Mortality was recorded and calculated when control mortality ranged from 5-20 percent evaluated by *Abbott's* formula.

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical screening is a technique for assessing the potential of a phyto-compound found in *Moringa oleifera* flower (MOF) extract using a variety of solvents (Ethanol, Methanol, Chloroform, Petroleum ether, and Aqueous). Phytochemical screening reveals the presence of several chemicals such as alkaloids, flavonoids, saponin, terpenoids, carbohydrates, glycosides, fat and fixed oils, and steroids. More phyto chemicals were found in ethanol, methanol, and aqueous extracts than in chloroform and petroleum ether extracts (Table 1).

Alkaloids are chemical compounds with basic nitrogen atoms that exist naturally. They are frequently used as pharmaceuticals and recreational substances and have pharmacological effects [26]. Flavonoids work as antioxidants and boost the benefits of Vitamin C. They're also known to fight liver toxins, cancers, viruses, and other microorganisms physiologically [27]. Tannins appear to have antiviral, antibacterial, and antiparasitic properties. Saponins cause red blood cell hemolysis [28]. Several studies have detailed the antioxidant effects of medicinal plants rich in phenolic compounds [29-30]. Plant extracts were also found to contain saponins, which are known to have an anti-inflammatory impact [31]. Saponins have the ability to coagulate and precipitate red blood cells. Terpenoids from plants are often used for their fragrant properties. They're used in traditional herbal remedies and are being studied for antibacterial, anticancer, and other pharmaceutical purposes [32]. Steroids have been shown to have bactericidal effects [33], and they are extremely essential molecules due to their interactions with sex hormones [34]. Glycosides have been shown to reduce blood pressure in numerous studies [35]. Because they have hypoglycemic, hypolipidemic, anticholesterolemic, antioxidant, anti-inflammatory, and detoxifying properties, carbohydrates obtained from plants are highly essential active substances for the prevention and treatment of diabetes mellitus and diabetic angiopathies. The findings of this study indicate that the detected phytochemical substances may be bioactive constituents, and that these plants are proving to be a valuable reservoir of bioactive compounds with significant medical value.

Table 1 Qualitative analysis of phytochemical

Phyto compounds	Solvents				
	Ethanol	Methanol	Chloroform	Petroleum ether	Distilled water
Alkaloids	-	-	+	-	-
Flavonoids	-	+	+	-	+
Tannin	-	-	-	-	-
Phenol	-	-	-	-	-
Saponin	+	-	-	-	-
Terpenoids	+	+	+	+	+
Carbohydrate	+	+	-	-	+
Glycosides	+	+	-	-	+
Fat and fixed oils	+	+	+	+	+
Steroids	+	+	+	+	-

'+' - Present, '-'- Absent

Table 2 Quantitative analysis for phytochemical

Test	Extracts				
	Ethanol	Methanol	Chloroform	Petroleum ether	Aqueous
Flavonoids	-	11.0 ± 0.19	34.5 ± 0.3	-	1.3 ± 0.06
Carbohydrate	55.3 ± 0.4	191.8 ± 1.3	-	-	238.9 ± 1.5
Glycosides	45.3 ± 0.3	41.0 ± 0.3	-	-	13.3 ± 0.2
Terpenoids	12.0 ± 0.2	12.7 ± 0.26	14.7 ± 0.2	60.7 ± 0.4	28.1 ± 0.3
Steroids	3.2 ± 0.1	2.2 ± 0.1	13.1 ± 0.2	1.3 ± 0.1	12.9 ± 0.6
Saponin	33.4 ± 0.3	-	-	-	-
Amino acid	19.8 ± 0.2	19.0 ± 0.1	8.6 ± 0.5	6.7 ± 0.4	20.7 ± 0.2

The (Table 2) shows the quantitative phytochemical analysis of *M. oleifera* flower using various extracts. The maximum quantity of carbohydrate (238.9 mg/g) and amino acid (20.7 mg/g) were found in the aqueous extract of *M.*

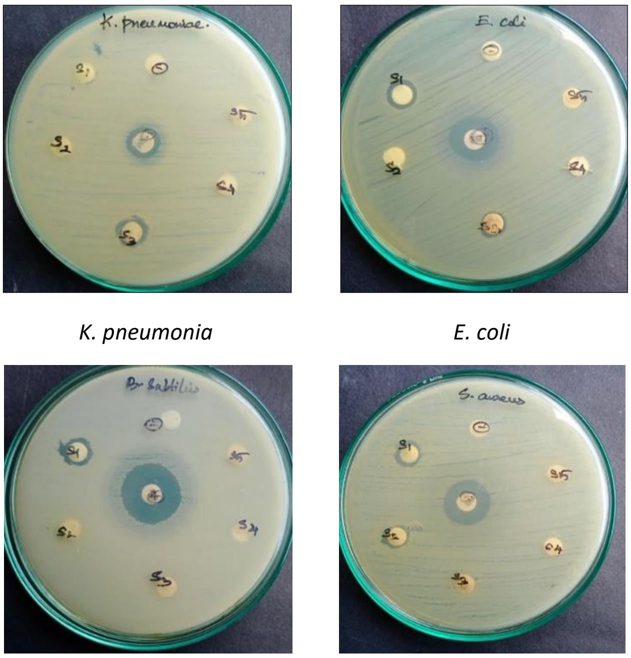
oleifera flower. Petroleum Ether has the highest concentration of Terpenoids (60mg/g). The presence of the highest quantity of Glycosides (45.3mg/g) and Saponin (33.4mg/g) was found in the Ethanol extract. The maximum amount of Flavonoid (34.5mg/g) and Steroids (13.1mg/g) were found in the Chloroform extract.

There are only a few research studies available on the plant's possible nutritional benefits in India. However, because dietary supplements are natural substances, the medical benefits linked with MOF (*Moringa oleifera* Flower) have gotten a lot of attention in the recent decade. Suryawanshi and Umate [34] investigated the hexane and ethanol extracts of MOF and found a large number of phytochemicals in the bloom.

Antimicrobial activity

Antibacterial activity

Bacteria decrease infection in different ways. Gram-negative (*Klebsiella pneumonia* and *Escherichia coli*) and Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria were tested and analyzed with each *Moringa oleifera* flower extract. The inhibitory zone was calculated and tabulated (Table 1, Fig 2). This image depicts the inhibited zone of pathogenic bacteria. In both the petroleum ether and aqueous extracts of *Moringa oleifera* flower, there is no zone of inhibition. The Ethanolic extract of *Moringa oleifera* has the moderate inhibition zone of 10 mm in *Staphylococcus aureus* and 9 mm in *Bacillus subtilis*. The chloroform extract of *Moringa oleifera* shows the maximum inhibition zone at 13 mm in *Klebsiella pneumonia*.



K. pneumonia *E. coli*

B. subtilis *S. aureus*

1 - Negative control, Center disc - Positive control
S_1 - Ethanolic extract, S_2 - Methanol extract, S_3 - Chloroform extract, S_4 - Petroleum ether extract, S_5 - Aqueous extract

Fig 2 Antibacterial activity of various extracts of *Moringa oleifera* flower

Table 3 Antibacterial activity of *Moringa oleifera* flower extract of five different solvents by agar disc diffusion method

Bacterial strains	*Moringa oleifera* flower extract					Positive control (Streptomycin)
	Ethanol	Methanol	Chloroform	Petroleum ether	Aqueous	
Klebsiella pneumoniae	nz	nz	13mm	nz	nz	11mm
Escherichia coli	9.5mm	nz	8.5mm	nz	nz	15mm
Bacillus subtilis	9mm	nz	nz	nz	nz	21mm
Staphylococcus aureus	10mm	9mm	nz	nz	nz	16mm

*(nz - no zone of inhibition)

Antifungal activity

Antifungal activities are also known as medication for fungal infection, which causes by various ways like touch, sneeze, dye degradation, degrade trees, ringworm, etc. and their response is the view as irritation, etching swelling, etc. and cryptococcal meningitis. The Antifungal activities of *Moringa oleifera* flower extract were performed with three fungal strains known as *Candida albicans*, *Aspergillus flavus*, and *Aspergillus niger*. The results of antifungal activity were captured after 48 hours of incubation. *Moringa oleifera* flower extract of different solvents shows the maximum zone of inhibition zone against *Aspergillus flavus* (11mm). Chloroform, Petroleum ether and Aqueous extract showed that, there was no inhibition against *C. albicans, A. flavus* and *A. niger* (Table 4, Fig 3).

C. albicans *A. flavus* *A. niger*

1 - Negative control, Center disc - Positive control
S_1 - Ethanolic extract, S_2 - Methanol extract, S_3 - Chloroform extract, S_4 - Petroleum ether extract, S_5 - Aqueous extract

Fig 3 Antifungal activity of various extracts of *Moringa oleifera* flower

Table 4 Antifungal activity of *Moringa oleifera* flower extract of five different solvents by agar disc diffusion method

Fungal strains	*Moringa oleifera* flower extract					Positive control (Amikacin)
	Ethanol	Methanol	Chloroform	Petroleum ether	Aqueous	
Candida albicans	8 mm	nz	nz	nz	nz	12 mm
Aspergillus flavus	11 mm	8 mm	nz	nz	nz	25 mm
Aspergillus niger	7.5 mm	nz	nz	nz	nz	13 mm

*(nz - no zone of inhibition)

Minimum inhibitory concentration (MIC)

With five different solvents, the maximum inhibition of *Moringa oleifera* flower extract against all three microorganisms *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* was achieved. The ethanolic floral extract of *Moringa oleifera* was likewise found to be more tolerant in all three bacteria among the solvents. The MIC is approximately 112 mg/mL. *Bacillus subtilis* had the lowest MIC value of 7 mg/mL. The aqueous extract *of Moringa oleifera* had the highest MIC value of 609 mg/mL (Table 5).

Table 5 Minimum inhibitory concentration value of flower extract of *Moringa oleifera*

Bacterial strains	Solvent extracts				
	Ethanol	Methanol	Chloroform	Petroleum ether	Aqueous
Bacillus subtilis	112 ± 0.00	7 ± 0.00	13 ± 0.00	10.5 ± 0.00	609 ± 0.00
Staphylococcus aureus	112 ± 0.00	12 ± 0.00	11 ± 0.00	10.5 ± 0.00	609 ± 0.00
Escherichia coli	112 ± 0.00	6.5 ± 0.00	47.5 ± 0.00	11 ± 0.00	609 ± 0.00

*The mean value of three different trials

Medicinal plants' antimicrobial capabilities are rapidly being reported from all over the world. Plant extracts or active ingredients are estimated to be utilized as folk medicine by 80 percent of the world's population in traditional therapies, according to the World Health Organization [1]. The extracts produced from *Moringa oleifera* exhibit high efficacy against the majority of the investigated bacterial and fungal strains in this study. The findings were matched to those of regular antibiotics. Extracts of *Moringa oleifera* were found to be active against all organisms tested, including Gram-positive, Gram-negative, and fungal strains, which were all resistant to all *Moringa oleifera* extracts.

The above findings indicate that ethanol extracts of *Moringa oleifera* have potent antibacterial and antifungal properties. This research also reveals the presence of many phytochemicals with biological activity that may have therapeutic value. The phytochemical results in this study revealed that the plant includes similar components such as saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Plants high in tannin and phenolic compounds have been demonstrated to have antibacterial properties against a variety of microbes, according to the findings.

Larvicidal activity of Moringa oleifera

The (Table 6) showed the larvicidal effect of *M. oleifera* flower extract of LC_{50} and LC_{90} in terms of time. The extract exerted high toxicity on larvae died within the period of time. This study's findings will help with the integration of this bioactive chemical into vector management and control.

Table 6 Larvicidal effect of *Moringa oleifera* extract against fourth instar larvae of *Aedes aegypti* and *Anopheles stephensi*

Fourth instar larvae	Conc. (ppm)	24 hours % mortality	LC_{50} (ppm)	LC_{90} (ppm)
Aedes aegypti	100	80	79.03	237.19
	75	65	(89.86- 69.50)	(246.07- 214.59)
	50	43		
	25	29		
	12.5	22		
Anopheles stephensi	100	95	35.36	107.42
	75	71	(44.95- 27.81)	(120.09- 91.47)
	50	63		
	25	42		
	12.5	38		

Many studies have been carried out on plant-derived compounds that are non-toxic to humans and domestic animals and can be used to build safer and more selective mosquito pesticides [36]. Compared to other herbal extracts, *M. oleifera* flower extract is a larvicidal agent, and research on water-extracted *M. oleifera* flower against *Aedes aegypti* larvae and *An. stephensi* have been described. The results of *M. oleifera* being treated against *An. stephensi* were promising. Quercetin and kaempferol are flavonoids, which are antioxidant chemicals made up of phenolic hydroxyl groups found in *Moringa oleifera* [37].

Much earlier research has shown that *M. oleifera* extract can cleanse water. *M. oleifera* is a natural coagulant (primary coagulant) that can be employed in both household and community water treatment systems [38]. Plants are abundant sources of bioactive organic compounds, and they have an advantage over synthetic pesticides in terms of toxicity, resistance development, and biodegradability. The flower extract of *Moringa oleifera* will play a critical role in mosquito control.

CONCLUSION

One of the most extensively grown species of the Moringaceae family is *Moringa oleifera*, an essential medicinal plant. Different sections of it have been utilized for different human illnesses, and extracts exhibited varying degrees of antibacterial and antifungal action on the microorganism examined, according to pharmacological reports. The presence of phytochemicals suggests that MOF may have both preventative and curative functions. Also, the findings have much larvicidal effect on *An. stephensi*. Despite the fact that various plant-derived chemicals have been described as larvicides, there is still a lot of room for additional effective plant products to be discovered. More pharmacological testing of the extracts is needed to substantiate *M. oleifera*'s antibacterial and larvicidal efficacy. More pharmacological investigations are needed to support the use of *M. oleifera* as a therapeutic plant, as our study indicated that folk medicine can be as effective as modern medication in combating pathogens and may useful for control of vector borne diseases.

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