International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

Phytochemical screening and *in vitro* antibacterial activity of *Moringa oleifera* (Lam.) leaf extract

Bhavana Dnyandeo Tambe * and Pranjali Dnyanshwar Gadhave

SMBT Institute of D. pharmacy, Dhamangaon, Igatputi, Nashik, Maharashtra- 422403 India.

International Journal of Science and Research Archive, 2024, 12(01), 125–130

Publication history: Received on 20 March 2024; revised on 27 April 2024; accepted on 30 April 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.12.1.0759

Abstract

The study aimed to investigate the phytochemical constituents and antibacterial activity of ethanolic extract of *Moringa oleifera* Lam. belonging to family *Moringaceae. Moringa oleifera* is an interesting plant for its use in bioactive compounds. Ethanol and Distilled water were used as a solvent to extract and to detect the phytochemical constituent's and to screen its antibacterial activity from the leaves of *Moringa oleifera*.

The phytochemical constituents were screened by qualitative analysis method. The phytochemical screening finds out the presence of terpenoids, phenols, tannins, flavonoids, glycosides, etc. in leaf extract of *Moringa oleifera*. The antibacterial activity of ethanolic leaf extract of *Moringa oleifera* was examined against gram positive bacteria *(Staphylococcus aureus*) and gram-negative bacteria *(Escherichia coli*). Antibacterial Activity were carried out with ethanolic extract of *Moringa oleifera* using agar well diffusion method. The present study showed that ethanolic extract of *Moringa oleifera* showed more potent antibacterial activity against *S. Aureus* and *E. coli*.

Keywords: *Moringa oleifera* leaf; *Moringa oleifera* leaf extract; Phytochemical screening; Antibacterial activity.

1. Introduction

Moringa (*Moringa oleifera* Lam.) is a type of local medicinal Indian herb belonging to the family of Moringaceae. The tree is often referred to as a "wonder-tree" for its multipurpose usability and also known as "Drumstick-tree", "Horseradish-tree" and "Benoil tree". *Moringa oleifera*, also known as the "tree of life" or "miracle tree," is classified as an important herbal plant due to its immense medicinal and non-medicinal benefits. Traditionally, the plant is used to cure wounds, pain, ulcers, liver disease, heart disease, cancer, and inflammation. *Moringa oleifera* is found in many tropical and sub-tropical regions. Moringa can be grown in the even the harshest and driest of soils, where scarcely anything else will grow. Moringa is nicknamed "never die" because of its staggering capacity to endure harsh climate and even dry season. Traditionally, besides being a daily used vegetable among people of these regions, Moringa is also widely known and used for its health benefits. *Moringa oleifera* is considered as "miracle tree" due to its amazing healing abilities for various ailments and even some chronic diseases because all its parts are used, especially for their pharmacological and nutritional properties.

Moringa oleifera (*M. oleifera*), the "miracle tree", thrives globally in almost all tropical and subtropical regions, but it is believed to be native to Afghanistan, Bangladesh, India, and Pakistan. The Moringa family comprises 13 species (*M. oleifera*, *M. arborea*, *M. rivae*, *M. ruspoliana*, *M. drouhardii*, *M. hildebrandtii*, *M. concanensis*, *M. borziana*, *M. longituba*, *M. pygmaea*, *M. ovalifolia*, *M. peregrina*, *M. stenopetala*), of which *M. oleifera* has become well known for its use in nutrition, biogas production, fertilizer, etc., Nearly all parts of the tree are used for their essential nutrients. *M. oleifera* leaves have a high content of beta-carotene, minerals, calcium, and potassium. Dried leaves have an oleic acid content of about 70%, which makes them suitable for making moisturizers the bark of the tree is considered very useful in the treatment of

* Corresponding author: Bhavana Dnyandeo Tambe

different disorders such as ulcers toothache and hypertension. Roots, however, are found to have a role in the treatment of toothache helminthiasis and paralysis. The flowers are used to treat ulcers, enlarged spleen, and to produce aphrodisiac substances. The tree is believed to have incredible properties in treating malnutrition in infants and lactating mothers.

2. Materials and methods

Collection of plant materials: Plants leaves were collected from rural area of Nashik, India. Moringa leaves were dried in a room temperature for one week, and then to form a fine powder with the help of mortar and pestle. Ethanol and distilled water were used as a solvent in the preparation of Moringa leaves extracts. Agar powder, sterile petri dishes, cotton swabs, sterile saline solution, test tubes was used.

2.1. Preparation of Moringa leaves Extracts

2.1.1. Aqueous Extract of moringa leaves

Take 25 gm of Moringa leaves, triturated in mortar and pestle to form coarse powder, transferred moringa powder into beaker and add 250 ml of sterile water with continues stirring up to 48 hours, after 48 hours heat the extract up to 50-60 ˚C temperature. Then after the cooling. Filtered the extract with whatman filter paper and store in refrigerator for further studies.

2.1.2. Ethanolic Extract of moringa leaves

Take 25 gm of Moringa leaves, triturated in mortar and pestle to form coarse powder, transferred moringa powder into beaker and add 250 ml of Ethanol with continues stirring up to 48 hours, after 48 hours heat the extract up to 50-60 ˚C temperature. Then after the cooling. Filtered the extract with whatman filter paper and store in refrigerator for further studies.

Figure 1 Moringa leaves

Figure 2 Moringa leaves Powder

Figure 3 Moringa leaves Extract (Aqueous and Ethanolic)

2.2. Phytochemical screening

The different qualitative phytochemical tests were carried out as per the standard tests for the phyto-constituents such as tannins, saponins, terpenoids, alkaloids, phenols, flavonoids, glycosides, reducing sugars etc. present in the moringa leaves extracts. The positive tests were shows as (+) present and (-) absent.

- **Tannins:** To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for Gallic tannins and green color indicates for catecholic tannins.
- **Saponins:** 1gm of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.
- **Terpenoids:** Four milligrams of extract were treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoids.
- **Alkaloids:** 2 ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids

- **Phenols:** To 2 ml of extract, a few drops of ferric chloride solution were added. The appearance of a greenish yellow color, confirms the presence of phenol.
- **Flavonoids:** 4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.
- **Glycosides:** 25 ml of dilute sulphuric acid was added to 5 ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, then 5 ml of Fehling's solution added. Glycosides are indicated by a brick red precipitate.
- **Reducing sugars:** To 0.5 ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

2.3. Sterility test of the plant extracts

The aqueous and ethanolic extracts were tested for growth or contamination. This was carried out by inoculating 1ml each of them on nutrient agar and incubated at 37 ℃ for 24 hours. The plates were observed for growth. No growth in the extracts after incubation indicates that the extracts were sterile. The extracts were then proceeded for antimicrobial activity.

- **Antimicrobial activity of moringa leaf extract:** The antimicrobial activity test of Moringa leaves extract was carried out by using paper disc diffusion method. In this test take nutrient agar powder suspended in liquid medium and boiled to form a suspended liquid and then agar nutrient medium put into autoclave for sterilization at temperature 121 ℃ for 45 mins.then cooled then nutrient agar medium. The suspended medium poured into petridish and allow to stand for 30-45 minutes for solidify. Poured gram-positive species (*S. aurous*) and gram-negative strain (*E. coli*) .After solidify put the drop of ethanolic extract and aqueous extract and control solution of Ampicillin solution. Put concentration of 50µl and 100 µl Ethanolic extract on nutrient agar medium and concentration of 50µl of aqueous extract and control solution is placed aseptically using sterile tweezers on the surface of the medium with 2-3 cm space among the paper disk from the edge of the petri dish. Then, it was inoculated for 48 hours at 37 ℃.
- **Observation of antimicrobial activity**: Antimicrobial activity was determined by measuring Diameter of Inhibition Zones in mm after incubation for 24 hours. The inhibition zone formed was measured using a calliper the data obtained were then analysed using the ANOVA test and followed by the LSD test.

3. Results and discussion

The qualitative phytochemical analysis of *Moringa oleifera* leaf extracts was done to test for presence of various phytochemicals. The results of the phytochemical analysis of *M. oleifera* leaf extracts using water and ethanol are shown in Table 1. The phytochemical screening indicated the presence of tannins, saponins, terpenoids, alkaloids, phenols, flavonoids, glycosides, reducing sugars etc. in leaf extracts of *Moringa oleifera* that are responsible for its antibacterial activity.

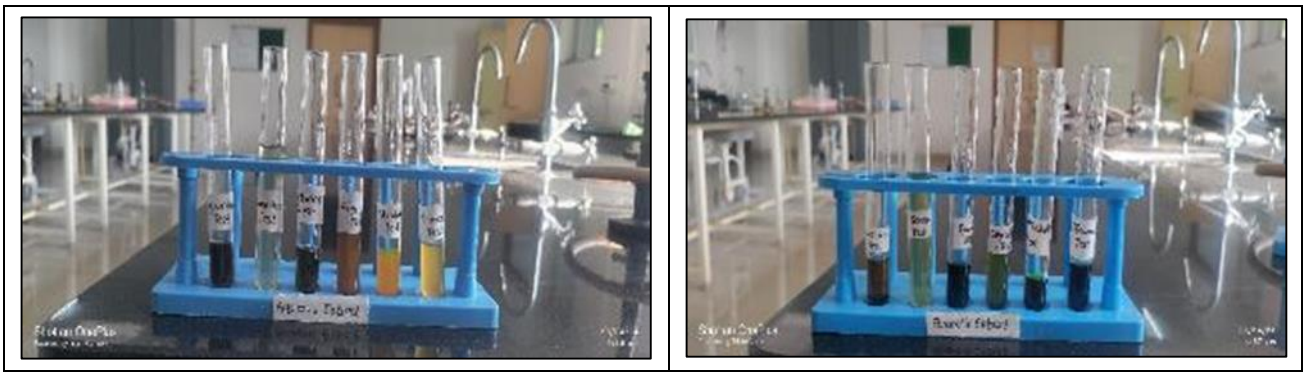

Figure 4 Phytochemical Screening of *Moringa oleifera* Lam Leaf extracts. (Aqueous and Ethanolic)

Table 1 Phytochemical Screening of *Moringa oleifera* Lam Leaf extracts

Test	Aqueous Extract	Ethanolic Extract
Tannins	+	+
Saponins	-	-
Terpenoids	+	+
Alkaloids	-	-
Phenols	-	+
Flavonoids	+	+
Glycosides	+	+
Reducing sugars	-	-

*Note : (+) Shows present and (–) Shows absent

The result showed that from the three concentrations of *Moringa oleifera* L. leaf extract can inhibit the growth of S. Epidermidis which is characterized by the formation of transparent and circular inhibition zones around the paper disk for incubation period along 24 hours at 37ºC (figure1). The transparent circle around the reservoir is caused by the extract of *Moringa oleifera* L. leaf which inhibits the growth of bacteria that cause infection. [13]

The antibacterial activity of ethanolic leaf extract of *M. oleifera* (1.0 g/ml) against each strain was studied by agar well diffusion method using nutrient agar medium. Agar plates was prepared and kept for sterilization. After sterilization the media was poured in to sterile petriplates and were allowed to solidify for 30-40 minutes. After the medium was solidified, it was inoculated with 24 hours old cultures of the given test gram-positive species (*S. Aureus*) and gram-negative strain (*E. coli*) by spreading the bacterial inoculums on the over the nutrient agar medium using sterile cotton swab horizontally and vertically to get a uniform microbial growth. Wells of 6 mm were punched in the agar and filled with Aqueous and ethanlolic *Moringa oleifera* leaf plant extracts. The control plates were made by using Ampicillin (10 mg/ml) as positive control to determine the sensitivity of bacterial strains. The plates were incubated at 37°C for 48 hours. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria.

Figure 5 Antimicrobial activity of ethanolic leaf extract of *Moringa oleifera* against gram-positive species (*S. Aureus*) and gram-negative strain (*E. coli*)

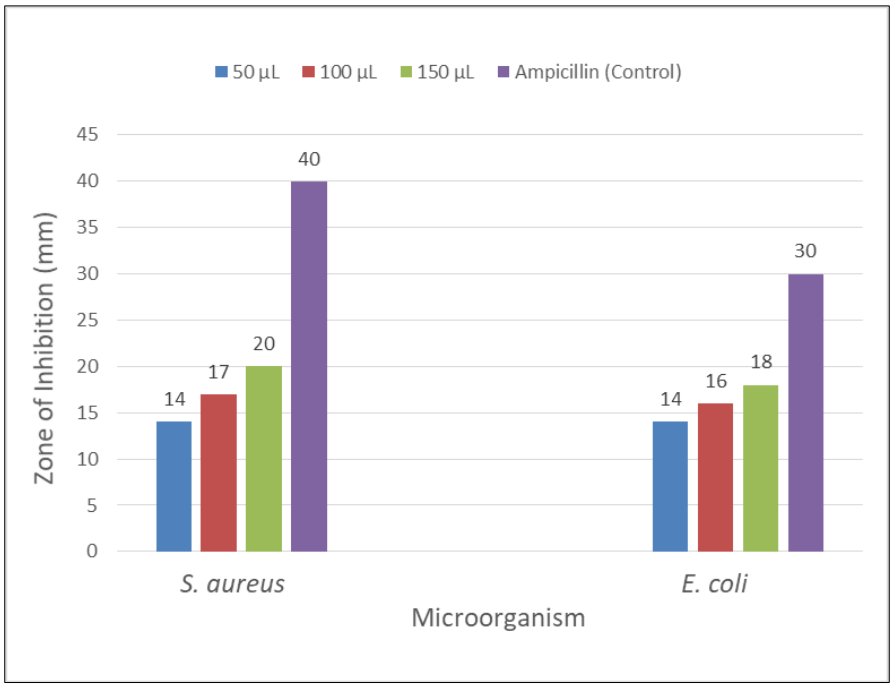

Figure 6 Comparision of Zone inhibition of Gram-positive species (*S. Aureus*) and gram-negative strain (*E. coli*) of extract of *M. oleifera*

Table 2 Antimicrobial activity of ethanolic leaf extract of *Moringa oleifera* against *S. Aureus* and *E.Coli*

Sr.No	Bacterial strains	Zone of Inhibition (mm)			
		Ampicillin (Control)	**50 µL**	**100 µL**	**150 µL**
1	Gram-positive species (*S. Aureus*)	40	14	17	20
2	Gram-negative strain (*E. coli*).	30	14	16	18

4. Conclusion

The ethanolic leaf extract of *M. oleifera* used in this experiment showed maximum antibacterial activity against test pathogens, this thus supports the fact that *M. oleifera* contain contains active phytochemicals with wide-spectrum antibacterial activity, capable of inhibiting the growth of gram-positive and negative bacteria. The antibacterial activity of ethanolic leaf extract of *M. oleifera* could be due to the better solubility of its components in organic solvent, which indicates that the active components responsible for the bactericidal activity are more soluble in organic solvents. Detailed study is needed to investigate the active compounds present in these plant parts having antibacterial activity that may help us to design more effective chemotherapeutic agent to heal bacterial infection.

Compliance with ethical standards

Acknowledgments

I would like to acknowledge and give my warmest thanks to SMBT Institute of D.Pharmacy for providing facilities. Finally I would like to thanks my family and friends.

Disclosure of conflict of interest

No conflict of interest.

References

[1] Hannan M.A., Kang J.Y., Mohibbullah M., Hong Y.K., Lee H., Choi J.S., Choi I.S., Moon I.S. *Moringa oleifera* with promising neuronal survival and neurite outgrowth promoting potentials. J. Ethnopharmacol. 2014; 152:142–150. Doi: 10.1016/j.jep.2013.12.036.

[2] Tayo G.M., Pone G.W., Komtangi M.C., Yondo G., Ngangout A.M., Mbida M. Anthelminthic Activity of *Moringa oleifera* Leaf Extracts Evaluated *in Vitro* on Four Developmental Stages of Haemonchus contortus from Goats. AJPS. 2014; 5:1702–1710. Doi: 10.4236/ajps.2014.511185.

[3] Aekthammarat D., Pannangpetch P., Tangsucharit P. *Moringa oleifera* leaf extract lowers high blood pressure by alleviating vascular dysfunction and decreasing oxidative stress in L-NAME hypertensive rats. Phytomedicine. 2019; 54:9–16. Doi: 10.1016/j.phymed.2018.10.023.

[4] Posmontier B. The medicinal qualities of *Moringa oleifera*. Holist. Nurs. Pr. 2011; 25:80–87. Doi: 10.1097/HNP.0b013e31820dbb27.

[5] Choudhury M.K., Bodakhe S.H., Gupta S.K. Assessment of the antiulcer potential of *Moringa oleifera* root-bark extract in rats. J. Acupunct. Meridian Stud. 2013; 6:214–220. Doi: 10.1016/j.jams.2013.07.003.

[6] Ansari M.M., Kumar S.D. Fortification of Food and Beverages with Phytonutrients. Public Health Nutr. 2012; 2:241–253.

[7] Anwar F., Ashraf M., Bhanger M.I. Interprovenance variation in the composition of *Moringa oleifera* oilseeds from Pakistan. J. Am. Oil Chem. Soc. 2005; 82:45–51. Doi: 10.1007/s11746-005-1041-1.

[8] Kasolo J.N., Bimenya G.S., Ojok L., Ochieng J., Ogwal-Okeng J.W. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. J. Med. Plant Res. 2010; 20104:753–757.

[9] Gopinath L.R., Jeevitha S., Gokiladevi T., Archaya S. Isolation and Identification of therapeutic compounds from *Moringa oleifera* and its antimicrobial activity. IOSR-JPBS. 2017; 12:1–10.

[10] Chaudhary K., Chourasia S. Nutraceutical properties of *Moringa oleifera*: A review. EJPMR. 2017; 4:646–655.

[11] Gandji K., Chadare F.J., Idohou R., Sialkot V.K., Assogbadjo A.E., Glèlè R.L.K. Status and utilisation of *Moringa oleifera* Lam: A review. Afr. Crop Sci. J. 2018; 26:137–156. Doi: 10.4314/acsj.v26i1.10.

[12] Fuglie L.J. Producing Food without Pesticides: Local Solutions to Crop Pest Control in West Africa. 1st ed. Church World Service; Dakar, Senegal: 1998. pp. 1–158.

[13] Bukar, A., Uba, A. and Oyeyi, T. (2010). Antimicrobial profile of *Moringa oleifera* Lam. extracts against some food–borne microorganisms. Bayero Journal of Pure and Applied Sciences, 3(1).