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Preliminary Phytochemical Screening and Anti Bacterial Activity of Leaves of *Moringa oleifera*. Lamk

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ST and RS carried out the phytochemical screening. Authors MPV and TT designed the study, wrote the protocol, and wrote the first draft of the manuscript, managed the literature searches. Author ACT carried out anti microbial activity and author TT managed the analyses of the study. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Moringa oleifera is a tree belongs to the family Moringaceae. It is called as Drumstick tree in English. In traditional medicine, the leaves and flowers are used in different ways to cure different ailment. Leaf juice is used for the eye infection. Mixed with honey, it is applied as anjanam to the eyelids in eye disease. Antibacterial activity of extracts of seeds, roots and leaves has been reported previously against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Aspergillus niger* and *Candida albicans.* The aim of this study is to screen the Phytochemicals and antibacterial activity of the decoction and ethanolic extract of obtained from the leaves of *M.oleifera* against Staphylococcus *aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853),

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Escherichia coli (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212). Fresh leaves were collected from Jaffna, Sri Lanka washed and dried under sunshade for one week. The decoction was prepared using water as solvent, and the ethanol extract was prepared using soxhlet apparatus. These extracts were used to screen the phytochemical and test its antibacterial activity. Both were qualitatively tested for the presence of chemical constituents, such as alkaloids, saponins, tannins, steroids, flavonoids, glycosides and triterpenoids. Antibacterial activity was determined by using the standard well diffusion method. The Nutrient Agar (NA) plate was inoculated with 1 mL (about $1x10^6$ CFU/mL) of each liquid bacterial culture was dispersed on the surface of NA plate and allowed to dry at 37°C for 15 min. The wells with 9 mm in diameter and 4 mm in depth were bored into the NA using a sterile cork borer and the well was completely filled with the test extract. Ethanol alone was used as control. Plates were incubated at 37°C for 24 hrs. Inhibition of growth was observed and the diameters of the zones of inhibition (ZOI) were measured. Replicates were made for the entire procedure. Ethanolic extract of leaf showed antibacterial activity against all tested gram positive and gram negative organisms. ZOI was ranged from 12 ± 0 mm to 19 ± 0 mm. Decoction showed antibacterial activity against *S. aureus* and *E.faecalis* the ZOI was 11 ± 0.18 mm. Growth inhibition was highly significant against *P. aeruginosa* and less significant against *E. coli*. in ethanol leaf extract. Degree of antibacterial activity of ethanol leaf extract was higher than decoction among the bacteria tested. Saponin, tannin and cardiac glycoside were present in both extract but terpenoid found only in ethanol leaf extract. It was also found that the extraction of bioactive compounds depend on the type of solvent used and the method of extraction. Ethanolic extract of *M.oleifera* leaf extract exhibited potent antibacterial activity against all tested organisms. It may be due to the presence of terpenoid. It could be used in the treatment of infections caused by these organisms.

Keywords: Phytochemical screening; anti-bacterial activity; leaves of Moringa oleifera; solvent extraction.

1. INTRODUCTION

Moringa oleifera is a tree belongs to the family Moringaceae. It is called as Murungai in Tamil, as Drumstick tree in English and as Murunga in Sinhala. In traditional medicine, the leaves and flowers are used in different ways to cure different ailment. In particular, the leaves are used as a poultice to reduce glandular swellings. The juice of the leaves has purgative and anthelmintic properties [1]. Several uses of Moringa leaves, such as antibacterial, antioxidant, anticancer applications, have been reported. In addition, these leaves can be used as protein supplement due to its high protein content [2]. The young leaves are used as food. Leaves ground into paste with a few pods of garlic, a bit turmeric, salt and pepper are given internally in scurvy. Leaf juice is used in the eye infection. Mixed with honey, it is applied as anjanam to the eyelids in eye disease [1]. The chloroform and ethanol extracts of seeds and leaf of *Moringa oleifera* were investigated previously for antimicrobial activity against some selected food – borne microorganisms, such as Escherichia *coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterobacter aerogenes* [3]. Antibacterial activity of extracts of seeds, roots and leaves has been reported previously against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Aspergillus niger* and *Candida albicans* [4]. However, structure-activity relation is missing in the existing literature. The present investigation mainly aims to screen the Phytochemicals and antibacterial activity of the decoction and ethanolic extract of leaves of *M.oleifera* against Staphylococcus *aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212).

2. MATERIALS AND METHODS

2.1 Collection of Materials

Fresh leaves of *M.oleifera* were collected from Jaffna, Sri Lanka and washed well and dried under sunshade for one week [5]. Then the dried leaves were stored in sterile bottle further analysis.

2.1.1 Preparation of aqueous and ethanolic extract

50 g of dried leaves were placed in a sterile container. 600 mL of distilled water was added

and boiled in low flame. Heating was continued until getting 20 mL of the aqueous extract. 50 g of dried *Moringa* leaves was extracted with 200 mL of ethanol using soxhlet apparatus [6]. The solvent was evaporated using rotatory evaporator. Aqueous and ethanolic extracts obtained were used to screen the phytochemicals and study the antibacterial activity.

2.1.2 Screening the Preliminary phytochemical analysis

The freshly prepared Ethanolic extract and water extract were chemically tested separately to check the presence of bioactive constituents, such as alkaloids, saponins, tannins, steroids, flavonoids, glycosides and triterpenoids. The qualitative identification was done by observing the characteristic colour changes using standard procedures described [7-10] below:

2.2 Test for Alkaloids

A few drops of 2N hydrochloric acid was added to 2 mL of the ethanolic extract and heated in a water bath at 50°C. The solution was filtered and Wagner's reagent added to the filtrate.

2.3 Test for Tannins

Sodium chloride solution (0.9%) was added to 2 mL of the ethanolic extract and heated in a water bath at 50°C. The solution was then filtered and 1% $FeCl_3$ solution added to the filtrate.

2.4 Test for Saponins

Aqueous extract (1 mL) was taken in a measuring cylinder, 9 mL of distilled water was added and shaken vigorously for 15 s. The extract was allowed to stand for 10 min. and test for the stable foam formation.

2.5 Test for Steroids

10 mL of Chloroform was added to 2 mL of the ethanolic extract. 1 mL of acetic anhydride was added, followed by the addition of 2 mL of concentrated sulphuric acid along the sides of the test tube.

2.6 Test for Triterpenoids

The test for triterpenoids is same as that for steroids. However, the colour formation is red or brown here for the triterpenoids.

2.7 Test for Cardiac Glycosides

A few drops of glacial acetic acid, ferric chloride and 3-4 drops of concentrated sulphuric acid were added to 1 mL of the extract. The appearance of blue-green colour was tested to check the glycosides.

2.8 Test for Flavonoids

The metal Magnesium (0.5 g) and 10 drops of conc. hydrochloride acid were added to 1 mL of the extract to check the red colour formation

2.9 Antibacterial Assay

The antibacterial activity of the leaf decoction and ethanolic extract was determined by using the standard well diffusion method [7,9,11]. Each isolated bacterial colony was taken separately and smeared on the inner wall of sterile Universal bottle, containing approximately 2 mL of sterile normal saline. The bottle was vortexed for five seconds and the turbidity of the liquid culture was made similar to that of the Mac Farland 0.5 standard solution.

The Nutrient Agar plate was inoculated with 1 mL (about $1x10^6$ CFU/mL) of each liquid bacterial culture. Then, it was dispersed on the surface of NA plate and allowed to dry at 37°C for 15 min. The wells with 9 mm in diameter and 4 mm in depth were bored into the NA using a sterile cork borer and the well was completely filled with the test extract. Ethanol alone was used as control. The plates were left on the bench for 30 min. and incubated at 37°C for 24 hrs. The inhibition of growth was observed and the diameters of the zones of inhibition (ZOI) were measured. Replicates were made for the entire procedure.

3. RESULTS AND DISCUSSION

Preliminary phytochemical screening showed that the Ethanol extract of the leaves contain flavonoid, terpenoid, steriod, tannin and cardiac glycocides; whereas, the terpenoid was absent in the aqueous extract (Table 1). In comparison with the study done by Bukar et al. in Nigeria, our findings show somewhat different phytochemicals in the extracts of leaves may be due to the geographical differences. The ethanol extracts in their studies contain saponin and flavonoid, whereas the extract obtains in this study consist five different phytochemicals

Table 1. Preliminary Phytochemical screening of leaves of *Moringa oleifera*

Extract	Saponin	Flavonoid	Terpenoid	Steroid	Alkaloid	Cardiac glycoside	Tannin
Ethanol extract	-	+	+	+	-	+	+
Decoction	-	+	-	+	-	+	+

Table 2. Mean and SD of Inhibition zone (mm) of decoction and ethanolic extract of leaves of *Moringa oleifera using* the Cut well method

Organisms	Diameter of Inhibition Zone (mean ± SD) mm		
	Decoction (aqueous extract)	Ethanolic extract	Ethanol control
S. aureus ATCC 25923	12 ± 0	14 ± 0	nil
P. aeruginosa ATCC 27853	nil	19 ± 0	nil
E. coli ATCC 25922	nil	12 ± 0	nil
E.faecalis ATCC 29212	11 ± 0	13 ± 0	nil

Note: No inhibition zone was observed in control

in particular, terphenoid and tannin. Further, the water extract does not contain terpenoid, whereas the other phytochemicals present was found to be same as the ethanol extract.

The Ethanolic extract of the leaves showed the antibacterial activity against all tested gram positive and gram negative organisms. ZOI was ranged from 12 ± 0 mm to 19 ± 0 mm (Table 2). Decoction of leaves of *M oleifera* showed antibacterial activity against *S. aureus* ATCC 25923 and *E.faecalis* ATCC 29212, and the ZOI obtained was 12 ± 0 and 11 ± 0 mm, respectively. The reason for this observation may be the collective effect of terpenoid along with the other phytochemicals presence in the ethanol extract of Moringa Leaves. Although the decoction prepared by using the traditional method, the advanced extraction technique, such as soxhlet extraction lead to extract most of the chemical constitutions from the plant materials [12], and, thus, showed the highest activity.

S. aureus is the commonest organism, which causes eye infections [13]. In this study, the antibacterial activity was attained by the decoction and ethanol leaf extract against *S.aureus*. However, the decoction failed to inhibit the growth of both *E. coli* and *P.aeruginosa*. A similar effect was observed with *S. aureus* and *E. coli* like the previous report against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Aspergillus niger* and *Candida albicans* with the ethanol leaf extract of *M.oleifera* [4]. Notably, the growth inhibition was found to higher against *P. aeruginosa* and less against *E. coli*. with the ethanol leaf extract. In addition, the degree of antibacterial activity of ethanol leaf extract was higher than that of decoction among the bacteria tested.

4. CONCLUSIONS

The results from this study indicate that the extraction of the bioactive compounds depends on the type of solvent and method used for extraction. The highest degree of antibacterial activity observed with the ethanol extract may be due to the presence of terpenoid. Overall, this study indicate that the ethanol leave extract of *M. oleifera* can be used in the treatment of infections caused by these organisms.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jeyaweera DMA. Medicinal plants (indigenous and exotic) used in ceylon, part IV. A Publication of the National Science Council of Sri Lanka, Colombo. 1982;101-102.
2. Ali EN. International Journal of Chemical. Environmental & Biological Sciences (IJCEBS). 2014;2(2):141-144.

3. Bukar A, Uba A, Oyeyi T. Antimicrobial profile of *Moringa oleifera* lam. Extracts againstsome food – born microorganisms. Bayero Journal of Pure and Applied Sciences. 2010;3(1):43-48.
4. Pal M, Saha BP. Antimicrobial action of the leaf extract of the *Moringa oleifera* lam, Ancient Science of Life. 1995;14(3):197-199.
5. Guidelines on good field collection practices for indian medicinal plants. National Medicinal Plants Board, Department of Ayush, Ministry of Health and Family Welfare Govt. of India in collaboration with WHO Country Office for India, New Delhi; 2009.
6. Handa SS. An overview of extraction techniques for medicinal and aromatic plants. International Centre for Science and High Technology. 2008;1:21-52.
7. Thayalini T, Thevanesam V, Kathirgamanathar S, Gamage T. Comparative study of antibacterial activity and preliminary phytochemical screening of leaves and seeds of *Myristica fragrans* 1st International conference of Unani, Ayurveda, Siddha and Traditional medicine, University of Colombo, Sri Lanka. 2013;28.
8. Farnsorth NR. Biological and phytochemical screening of plants. J. Pharm. Sci., 1966;55:225-276.
9. Thayalini T, Thevanesam V, Kathirgamanathar S, Gamage T, Antibacterial activity and preliminary screening of phytochemicals of whole plant of *Enicostemma littoralae*. – Tropical Agricultural Research. 2012;24(1):99.
10. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005;4(7):685-688.
11. Mohamed SSH, Hansi PD, Kavitha T. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharma Sciences and Research. (IJPSR) 2010;1 (10):430-434.
12. Rockwood JL, Anderson BG, Casamatta, DA. Potential uses of *Moringa oleifera* and an examination of antibiotic efficacy conferred by *M. oleifera* seed and leaf extracts using crude extraction techniques available to underserved indigenous populations. International Journal of Phytothearpy Research. 2013;3(2): 61-71.
13. Greenwood D, Barer M, Slack R, Lrving W. Medical microbiology, A guide to microbial infections, Pathogenesis, immunity, laboratory investigation and control. Churchill Livingstone, Elsevier. 2012;18:176.

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