

Comparative Studies of *Moringa oleifera* and *Murraya koenigii* Leaf Extracts as a Nutraceutical and a Potent Antibacterial Agent

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Abstract: *Moringa oleifera* and *Murraya koenigii* are in high demand for their nutritional and medicinal value. Their nutritive values have been estimated as 287.5 ± 0.787 and 97.03 ± 0.329 Cal/g respectively. Antibacterial activity of methanolic and aqueous leaf extracts of *Moringa oleifera* and *Murraya koenigii* were tested against five strains of both Gram positive and Gram negative bacteria viz. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Salmonella typhi* by agar disc diffusion method and broth dilution. The susceptibility of the microorganisms to the extracts was compared with each other and with pure antibiotics gentamycin. The result showed that, the methanolic extracts exhibited more pronounced antibacterial activity against the common gastrointestinal pathogens. The minimum inhibitory concentration (MIC) of the extracts was in the range 4-64 mg/ml with *S.typhi* being the most susceptible bacteria showed a zone of inhibition (ZOI) of 9mm and 7mm at 4mg/mL for *M. oleifera* and *M. koenigii*. The phytochemical analysis which was carried out revealed the presence of carbohydrates, proteins, oils, lipids, glycosides, alkaloids, triterpenoids and steroids and absence of tannins, flavanoids and saponins in the leaf extracts.

Key words: Nutraceutical • Antibacterial • Phytochemical • *Moringa oleifera* • *Murraya koenigii*

INTRODUCTION

Nutraceutical can be defined as a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease [1]. Nutraceuticals are non-specific biological therapies that were used to promote wellness, prevent malignant processes and control symptoms. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens [2, 3]. The pharmacological actions and the medicinal uses of aqueous extracts of leaves in folk medicine include the treatment of various type of gastrointestinal disturbances such as vomiting, diarrhoea, inhibition of the peristaltic reflex, gastroenteritis,

spasmodic activity, dysentery, abdominal distention, flatulence and gastric pain [4, 5]. The importance of plant secondary metabolites in medicine, agriculture and industry has led to numerous studies on the synthesis, biosynthesis and biological activity of these substances. It has been estimated that over 40% of medicines have their origins in these active natural products [6-10].

Although the distinction between medicinal plants and nutraceuticals can sometimes be vague, a primary characteristic of the latter is that nutraceuticals have a nutritional role in the diet and the benefits to health may arise from long-term use as foods (i.e. chemoprevention) [11]. In contrast, many medicinal plants exert specific medicinal actions without serving a nutritional role in the human diet and may be used in response to specific health problems over short- or long-term intervals.

Plants like *Moringa oleifera* and *Murraya koenigii* are in high demand for their nutritional and medicinal value. *Moringa* leaves and seeds are used by humans as a good source of vitamins (B and C) and amino acids [12, 13]. *Moringa oleifera* was also claimed to boost immune systems [13, 14]. It has relatively high crude protein, low anti-nutritional factors and antimicrobial

activity [15, 16]. *Murraya* is more popular due to its broad spectrum of medicinal properties and also because of the use of its leaves for centuries as a natural flavouring agent in various curries and food items [17]. Traditional system of medicine in eastern Asia mentions the important uses of this plant. The leaves of *M. koenigii* constitute an important ingredient in the Indian diet to improve appetite and digestion.

In the present study medicinal plants *Moringa oleifera* and *Murraya koenigii* have been screened for their anti-bacterial efficacy against multi-drug resistant bacteria including *S. aureus*, *P. aeruginosa*, *E. coli*, *S. typhi*, *V. cholerae* and their nutraceutical abilities.

MATERIALS AND METHODS

Collection of Plant Material: The fresh and tender leaves were collected, dried in a shade under room temperature for six to seven days and then crushed into coarse powdery substance by using electric grinder. The coarse powdery substance was dried again and was then sieved to get fine powder using the fine plastic sieve, which was then stored in an air tight bottle in the laboratory until it is required.

Extract Preparation: 50 g of the sieved powder was weighed accurately and subjected to extraction in a Soxhlet apparatus at room temperature using ~350 ml methanol and distilled water separately. The extract obtained was filtered, concentrated after dryness in rotary flash evaporator maintained at 45°C. Percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies.

Phytochemical Analysis: Freshly prepared extracts of the powdered leaves were subjected to phytochemical analyses to find the presence of the following phytoconstituents such as flavanoids, alkaloids, carbohydrates, glycosides, tannins, saponins, steroids, proteins, lipids, oils by standard methods [18, 19].

Anti-bacterial Analysis

Test Microorganisms: The micro-organisms namely *S. aureus* (MTCC 3160), *P. aeruginosa* (MTCC 7837), *E. coli* (MTCC 1554), *S. typhi* (MTCC 3216), *V. cholerae* (MTCC 3906) that used during the present experiment were procured from Hi-media which are potential causative pathogen for different diseases.

Concentrations Screened: 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 mg for agar diffusion method and for broth dilution method up to 64 mg/ml concentrations were used according to the sensitivity of samples.

Agar Diffusion Method: Media used: Peptone-10 g, NaCl-10g and yeast extract 5g, agar 20g in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 h. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µL, 10⁴ cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentrations of the samples. The control wells were filled with gentamycin along with solvent. All the plates were incubated at 37°C for 24 h and the diameter of inhibition zones were noted.

Broth Dilution Method: Media Used: Peptone-10 g, NaCl-10g and yeast extract 5g, in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 h. The tubes containing the above media were prepared, autoclaved and respective concentrations of the samples were added. Each tube was inoculated with 18 h old cultures (100 µL, 10⁴ cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37°C on a shaker with 140 rpm for 24 h and the growth was measured at 660 nm. The % of inhibition was calculated by using the formula below

$$\% \text{ inhibition} = 100 \left[\frac{\text{OD of culture with sample (test)}}{\text{OD of culture with sample (control)}} \right]$$

Biochemical Estimations and Nutritive Value: The fresh leaves were collected, washed, dried and then crushed in a mortar and pestle and were subjected to various biochemical analyses. The moisture content was determined by taking the fresh plant samples in petri dishes and kept overnight in an air oven at 100-110°C till they attained a constant weight. The loss in weight was regarded as a measure of moisture content [20]. The total carbohydrates content was estimated by anthrone method [21]. Total protein was estimated by Lowry's method [22]. The lipids were determined by method of Indrayan *et al.* [20]. The nutritive value was calculated by the method of Indrayan *et al.* [20]. Crude fibre was calculated by the method of Nile and Khobragade [23].

Table 1: Nutritive value of *M. oleifera* and *M. koenigii*.

Dried Leaf powder	Carbohydrates (g)	Proteins (g)	Fats (g)	Crude fibre (g)	Moisture content	Nutritive value (Cal/g)
<i>M. oleifera</i>	38.4±0.909	27.1±0.725	2.2±0.081	7.26±0.173	76.50±0.763	287.5±0.787
<i>M. koenigii</i>	16±0.408	9.03±0.205	1.3±0.124	3.70±0.294	62.54±0.605	97.03±0.329

Table 2: Zone of inhibition (in mm) of methanolic leaf extracts of *M. oleifera* and *M. koenigii*

Concentration (mg/mL)	<i>E. coli</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>		<i>V. cholera</i>		<i>S. typhi</i>	
	MO	MK	MO	MK	MO	MK	MO	MK	MO	MK
0.125	-	-	-	-	-	-	-	-	-	-
0.25	-	-	-	-	-	-	-	-	-	-
0.50	-	-	-	-	-	-	-	-	-	-
1.0	-	-	-	-	-	-	-	-	-	-
2.0	-	-	-	-	-	-	-	-	-	-
4.0	-	5	-	-	3	-	-	2	9	7
MIC(mg/mL)	NF*	4	NF*	NF*	4	NF*	NF*	4	4	4

MO- *M. oleifera*, MK- *M. koenigii*, NF*- Not found

Table 3: Zone of inhibition (in mm) of aqueous leaf extracts of *M. oleifera* and *M. koenigii*

Concentration (mg/mL)	<i>E. coli</i>		<i>S. aureus</i>		<i>P. aeruginosa</i>		<i>V. cholerae</i>		<i>S. typhi</i>	
	MO	MK	MO	MK	MO	MK	MO	MK	MO	MK
0.125	-	-	-	-	-	-	-	-	-	-
0.25	-	-	-	-	-	-	-	-	-	-
0.50	-	-	-	-	-	-	-	-	-	-
1.0	-	-	-	-	-	-	-	-	-	-
2.0	-	-	-	-	-	-	-	-	-	-
4.0	-	3	-	-	2	-	-	-	6	5
MIC(mg/mL)	NF*	4	NF*	NF*	4	NF*	NF*	NF*	4	4

MO- *M. oleifera*, MK- *M. koenigii*, NF*- Not found

RESULTS AND DISCUSSION

The use of nutraceuticals, as an attempt to accomplish desirable therapeutic outcomes with reduced side effects, as compared with other therapeutic agents has met with great monetary success [24, 25]. The nutritive value of *M. oleifera* is 287.5±0.787 Cal/g and of *M. koenigii* is 97.03±0.329 Cal/g. The results are shown in Table. 1.

Antibacterial properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and triterpenes and reducing sugars [26-29]. The results of the evaluation of phytochemical screening of the extracts of revealed the presence of glycosides, steroids, alkaloids, triterpenoids, oils and flavanoids and absence of tannins and saponins.

The antimicrobial efficacy of the extracts of *M. oleifera* and *M. koenigii* leaves was quantitatively assessed on the basis of inhibition zone (in mm) and the results are shown in Table. 2 and 3 following the agar disc diffusion method and minimum inhibitory concentration by broth dilution method. The tested micro- organisms were also inoculated with pure antibiotics- Gentamycin to compare the efficacy of leaf extract for their antimicrobial properties and the results are shown in Table 4.

Table 4: MIC of Gentamycin against the tested micro- organisms

Microorganisms	MIC(µg/ml)	ZOI(mm)
<i>E.coli</i>	25	18
<i>P.aeruginosa</i>	100	1
<i>S.aureus</i>	25	13
<i>S.typhi</i>	25	2
<i>V.cholerae</i>	25	13

In the present investigation the extracts were found to be effective against all the tested pathogens. When the above pathogens were screened by agar disc diffusion method the zone of inhibition (ZOI) observed for the methanolic and aqueous extracts of both the plants were in the range 2-8mm at 2-4mg/ml concentration of the extracts. *S.typhi* was found to be highly susceptible as it showed an inhibition zone of 9 mm and 7 mm at 4mg/ml for methanolic extract of *M. oleifera* and *M. koenigii* respectively whereas the aqueous extract showed 6 mm and 5mm ZOI. *E. coli* showed no ZOI for *M. oleifera* (both extracts) but it exhibited 5 mm and 1 mm ZOI for *M. koenigii* at 4mg/mL concentration. *P. aeruginosa* was comparatively less sensitive and only *M. oleifera* (methanolic) showed a ZOI of 3mm at 4mg/ml concentration. *S. aureus* and *V. cholerae* did not show any zone of inhibition reflecting their insensitiveness towards the methanolic as well as aqueous extracts of the leaf.

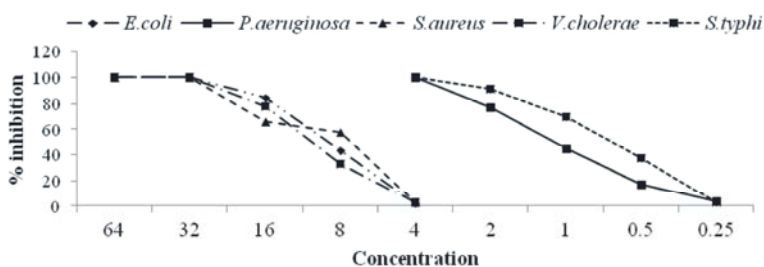


Fig. 1: % inhibition by the Methanolic extracts of *M. oleifera*

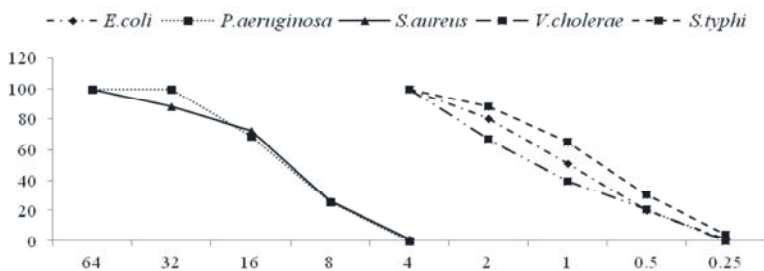


Fig. 2: % inhibition by the Methanolic extracts of *M. koenigii*

The broth dilution method of the extracts showed more pronounced antimicrobial activity through 100% inhibition for all the pathogens in the range of 0.25-64mg/mL concentration. The methanolic extract proved to be more effective than the aqueous extract. The MIC of *M. oleifera* (methanolic) for *S. typhi* and *P. aeruginosa* was 4mg/mL and for *E. coli*, *V. cholerae* and *S.aureus* it was 32mg/ml. Similarly the MIC of *M. koenigii* (methanolic) for *E.coli*, *V.cholerae* and *S.typhi* was 4mg/ml and for *P. aeruginosa* and *S. aureus* it was 64mg/ml (Fig. 1 and 2). Several workers have reported similar results for various medicinal plants such as *Psidium guajava* and *Mangifera indica* [26, 27]. *Agele marmelos* and *Cinamammoum tamala* [30], *Vitex negundo* and *Adhatoda vasica* [31] etc.

The plant *Murraya koenigii* Spreng. (Rutaceae) has been used in folk remedies by Indians and is reported to have a broad range of therapeutic effects, including analgesic, anti-inflammatory, alexiteric, febrifuge activity and is useful in leucoderma and blood disorders [32-33]. Similarly, *Moringa oleifera* is useful as a cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective agent [34]. Various surveys have been reported about the medicinal properties of both the plants but the exact mechanism of action and concentration to be taken for complete inhibition of the microorganisms are reported in the present study. Therefore, the results of the present study support the medicinal usage of the methanolic leaf

extracts of *M. oleifera* and *Murraya koenigii* and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation. The estimated nutritive value of the plants proves them to be a potent, abundant and a cheap nutraceutical.

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REFERENCES

1. Brower, V., 1998. Nutraceuticals: poised for a healthy slice of the healthcare market? Nat Biotechnol, 16: 728-731.
2. Clardy, J. and C. Walsh, 2004. Lessons from natural molecules. Nature, 432: 829-837.
3. Verpoorte, R., Y.H. Choi and H.K. Kim, 2006. Ethnopharmacology and system biology: a perfect holistic match. J. Ethnopharmacol, 100: 53-56.
4. Lutterodt, G.D., 1992. Inhibition of Microlax-induced experimental diarrhea with narcotic-like extracts of *Psidium guajava* leaf in rats. J. Ethnopharmacol., 37(2): 151-157.

5. Mittal, P., V. Gupta, G. Kaur, A.K. Garg and A. Singh, 2011. Phytochemistry and pharmacological activities of *Psidium guajava*: a review. Int. J. Pharmaceu. Sci. Res., 1(9): 9-19.
6. De Fatima, A., K.N. Silva, I.J.L.D. Basilio, P.F. De Freitas and J.M.B. Filho, 2008. Survey of medicinal plants used in the region northeast of Brazil. Braz. J. Pharmacog, 18(3): 472-508.
7. De Fátima, A., L.V. Modolo, L.S. Conegero, R.A. Pilli, C.V. Ferreira, L.K. Kohn and J.E. De-Carvalho, 2006. Lactones and their derivatives: biological activities, mechanisms of action and potential leads for drug design. Curr. Med. Chem., 13: 3371- 3384.
8. Vohra, K. and V.K. Gupta, 2011. *Murraya koenigii* (Linn.) Spreng (Rutaceae): A precious Gift from the Nature. Int. J. Pharma. Rec. Res., 3(1): 18-25.
9. Ijaz, F., Z. Iqbal, J. Alam, I.U. Rahman, Sohail, S. Rehman and S. Manan, 2015. Ethno medicinal study upon folk recipes against various human diseases in Sarban Hills, Abbottabad, Pakistan. Global Journal of Pharmacology, 9(1): 56-63.
10. Kumar, A., S. Dandapat, M. Kumar and M.P. Sinha, 2013. Antipathogenic efficacy and aemolytic activity of *Calotropis procera* leaves. World Journal of Zoology, 8(4): 366-370.
11. Korver, O., 1998. Functional foods: the food industry and functional foods: some European perspectives. In T Shibamoto, J Terao, T Osawa, eds, Functional Foods for Disease Prevention: II. Medicinal Plants and Other Foods. American Chemical Society, Washington, DC, pp: 22-25.
12. Makkar, H.P.S. and K. Becker, 1996. Nutritional value and an nutritional components of whole and extracted *Moringa oleifera* leaves. Animal Feed Sci.Tech., 63: 211-228.
13. OlugbemI, T.S., S.K. Mutayoba and F.P. Lekule, 2010. Effect of *Moringa (Moringa oleifera)* Inclusion in Cassava based diets to broiler chickens. Int. J. Poultry Sci., 9(4): 363-367.
14. Jayavardhanan, K.K., K.. Suresh, K.R. Panikkar and D.M. Vasudevan, 1994. Modular potency of drumstick lectin on host defense system. J. Expt. Clin. Cancer Res., 13: 205-209.
15. Makkar, H.P.S. and K. Becker, 1997. Nutrients and anti-quality factor in different morphological part of *Moringa oleifera* tree. Journal of Agric. Sc., 128: 211-322.
16. Dahort, U.M., 1998. Anti-microbial activity of small protein of *Moringa oleifera* leaves, Journal of Islamic Acad. Sci, 11(1): 27-32.
17. Nayak, A., S. Mandal, A. Banerji and J. Banerji, 2010. Review on chemistry and pharmacology of *Murraya koenigii* Spreng (Rutaceae) J. Chem. Pharmaceu. Res., 2(2): 286-299.
18. Trease G.E. and W.C. Evans, 2002. Pharmacognosy. 15th Ed. Saunders Publishers, London, pp: 42-393.
19. Sofowora, A., 2008. Screening Plants for Bioactive Agents. In: Medicinal Plants and Traditional Medicinal in Africa. 3rd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, pp: 134-156.
20. Indrayan, A.K., S. Sharma, D. Durgapal, N. Kumar and M. Kumar, 2005. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. Curr. Sci., 89(7): 1252-1255.
21. Sadasivam, S. and A. Manickam, 1996. Biochemical method. 2nd Ed. New age International (P) Limited, New Delhi.
22. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
23. Nile, S.H. and C.N.N. Khobragade, 2009. Determination of nutritive value and mineral elements of some important medicinal plants from western part of India. Journal of Medicinal Plants, 8(5): 79-88.
24. Nelson, N.J., 1999. Purple carrots, margarine laced with wood pulp? Nutraceuticals move into the supermarket. J. Natl. Cancer Inst., 91: 755-757.
25. Whitman, M., 2001. Understanding the perceived need for complementary and alternative nutraceuticals: lifestyle issues. Clin. J. Oncol. Nurs., 5: 190-194.
26. Choudhury, S., L. Sharan and M.P. Sinha, 2012a. Phytochemical and Antimicrobial Screening of *Psidium guajava* L. Leaf Extracts against Clinically Important Gastrointestinal Pathogens. J. Nat. Prod Plant Resour, 2(4): 524-529.
27. Choudhury, S., L. Sharan and M.P. Sinha, 2012b. Antibacterial efficacy and Phytochemistry of methanolic leaf extracts of *Mangifera indica* Linn. The Ecoscan, special issue, (1): 419-423.
28. Choudhury, S., L. Sharan and M.P. Sinha, 2013a. Pharmacological efficacy of some medicinal plants used for treatment of gastrointestinal diseases. The Ecoscan, Special issue, (3): 111-116.
29. Choudhury, S., L. Sharan and M.P. Sinha, 2013b. Phytochemical and antimicrobial standardization of the methanolic leaf extracts of *Murraya koenigii* Linn. Archives des Sciences, 66(6):

30. Choudhury, S. and M.P. Sinha, 2014. Effects of *Psidium guajava* aqueous extract on testosterone and serum lipid profile of albino rats. Middle East J. Sci. and Res., 21(10): 1893-1897.
31. Dandapat, S., M. Kumar, A. Kumar and M.P. Sinha, 2013. Antipathogenic efficacy of ethanolic extract of *Cinnamomum tamala* and *Agele marmelos* (L.) with their nutritional potentiality. The Bioscan 8(2), Supplement on Medicinal Plants, pp: 689-693.
32. Kumar, M., S. Dandapat, A. Kumar and M.P. Sinha, 2013. Anti-typhoid activity of *Adhatoda vasica* and *Vitex negundo*. Persian Gulf Crop Protection, 2(3): 64-75.
33. Kirtikar K.R. and B.D. Basu, 1994. Indian Medicinal Plants. Edition 2, Vol. III, Bishen Singh Mahendrapal Singh, Dehradun, pp: 472-473.
34. Anwar, F., S. Latif, M. Ashraf and A.H. Gilani, 2007. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytother. Res., 21: 17-25.