International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2021; 9(6): 28-31
Received: 06-09-2021
Accepted: 18-10-2021

Febitha M Das
Student, Department of Botany, M E S Asmabi College, Thrissur, Kerala, India

Aruna MV
Faculty, Department of Wood Science and Technology, Kannur University, Kannur, Kerala, India

Corresponding Author:
Febitha M Das
Student, Department of Botany, M E S Asmabi College, Thrissur, Kerala, India

Phytochemical screening, antioxidant and antibacterial activity of leaf extract of *Morinda citrifolia* L. against *Escherichia coli* & *Pseudomonas aeruginosa*

Febitha M Das and Aruna MV

DOI: https://doi.org/10.22271/flora.2021.v9.i6a.781

Abstract
Morinda citrifolia is used traditionally for the treatment of infectious diseases. This study aims to investigate phytochemical screening and examines the antioxidant and antibacterial property of leaves. The shade dried leaf powder was extracted serially using hexane, acetone, and water. All three extracts were subjected to phytochemical screenings and only water extract is subjected to antibacterial and antioxidant analysis. Preliminary phytochemical screening was carried out by different chemical tests. Amino acid, Carbohydrate, Protein were the primary metabolites found to be present. The Alkaloids, Flavonoids, Terpenoids, Steroids, Saponins, Phenol, Tannin, and Cardiac glycosides showed to be present as the secondary metabolites. The terpenoids were found only in acetone extract. The evaluation of antibacterial activity of the aqueous extract of *Morinda citrifolia* against Escherichia coli and Pseudomonas aeruginosa at concentrations 10, 50,100µl. The bacterial strains are sub-cultured on nutrient broth. The aqueous extract of *Morinda citrifolia* shows maximum antibacterial activity in 100µl concentrations, in *Pseudomonas aeruginosa* it will be 61.46% and in the case of Escherichia coli 52.24%. The antioxidant activity of aqueous extract of *Morinda citrifolia* was carried out by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Different concentration of the extract was taken and the percentage of inhibition was calculated. Maximum radical scavenging activity was observed in 100µl concentration i.e. 68.94% and minimum in 50µl concentration. i.e., 49.47%. So as the concentration increases the antioxidant activity also increases. The data generated as a result of this investigation has provided the scientific basis for its use as therapeutic in traditional medicine.

Keywords: Phytochemical screening, antioxidant, antibacterial, leaf extraction, *Morinda citrifolia* l., *escherichia coli, pseudomonas aeruginosa*

1. Introduction
Plants induce a vast variety of chemicals that can have remedial use in the flattening of medical fields. The antioxidant advantage of medicinal plant lifestyles is often credited with these therapeutic blessings [1, 2]. More than eighty percent of the world's population, in line with the World Health Organization (WHO), rely on traditional medicine, which has a big spectrum of drugs that can be used to deal with persistent and infectious ailments [3]. Currently bacteria cause around 250 kinds of infection. *Escherichia coli* is most commonly remoted pathogenic bacterium in these infections and intoxications [4, 5]. Otherwise, in line with, Gram-negative microorganisms like *E. coli* and *Pseudomonas aeruginosa* species are the most ordinary pathogenic microorganism that reasons wound infections in diabetics and contaminated wounds after operations [6-7]. These microorganisms are in normal answerable for the generation of toxins. The plant leaves are immoderate in alkaloids, flavonoids, tannins, phenols, saponins, and glycosides [8-12]. These compounds are identified to have medicinal and physiological use [13]. Noni (*Morinda citrifolia*) leaves have been used to deal with topical burns, fever, menstrual cramps, gonorrhoea, virus infestation, ringworm and different illnesses for heaps of years and are now used to deal with helminthic infections, oxidative pressure, open wounds, and as an anti-allergen in current instances [14-15]. In the leaf extract of *Morinda citrifolia*, phytochemical analyses exhibit the presence of flavonoids, saponins, diterpenes, and phorbol esters [16-21]. Considering the vast potentiality of plants as sources for antimicrobial drugs, the present research aims to carry out preliminary phytochemical screening and examines the antioxidant and antibacterial activity of *Morinda citrifolia* leaf extracts.

2. Materials and Methods
2.1 Plant Material
The leaves of the *Morinda citrifolia* were collected separately from the natural habitat of Kodungallur, Thrissur district. They were washed thoroughly in running tap water and finally, wash with sterile distilled water. The leaves were shade dried for 3 weeks.

2.2 Preparation of Plant Extracts
Three different types of extracts including acetone extract, aqueous extract, and hexane extract were prepared. The solvent was selected in the increasing order of polarity. The dried leaves were ground to fine powder mechanically in an electric grinder. Powdered leaves (20g) were added in three flasks of 250 ml volume and 200 ml of each solvent was added to each flask separately. The flasks were kept in an incubator at 37ºC overnight with shaking at 180 rpm. The contents of the flask were filtered. The filtrate was evaporated in a water bath at 50ºC. The weight of residues was recorded. The resulting extracts were stored at 4ºC for further use in experiments.

2.3 Phytochemical Screening
Phytochemical screening was enacted by the standard methods [22-24]. The phytochemical constituents included carbohydrates, protein, amino acid, alkaloids, flavonoids, phenols, cardiac glycosides, tannins, saponins, terpenoids, and steroids were tested in this study.

2.4 Test Organisms
To determine the antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* were provided from Uni Biosys research lab. Each organism was plated into Nutrient broth.

2.5 Antibacterial Assay
Microtiter plates were prepared under aseptic conditions. A sterile 96 well plate was labeled. A volume of 10 µl, 50 µl, and 100µl of the test material was pipetted into the wells. 100µl of nutrient broth was added to each well. Finally, 100µl of microbial suspension was added to each well. Control dilutions of test material were also kept.
The plate was wrapped loosely with cling film to ensure that organism did not become dehydrated. Each plate had a set of controls: a column with all solutions except the test compound, and a column with all solutions except the organism adding 100µl of nutrient broth instead of a column with 200µl nutrient broth. The plates were incubated at 370ºC for 24 hours and OD reading was taken (OD600) after sufficient incubation. Optical density was obtained by subtracting the Extract OD from the Test OD. The % of inhibition was calculated as:

$$\% \text{ of inhibition} = \frac{(\text{Control OD - Final Test OD})}{\text{Control OD}} \times 100\ \% \quad \text{- (1)}$$

2.6 Antioxidant Assay Using DPPH Assay
The different volume of extract, 0.5 ml of methanolic solution of DPPH was added and made up to 2ml using methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and a tube without the extracts served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer [25]. The assay was calculated as:

$$\text{Radical scavenging activity} = \frac{(\text{Control OD-Test OD})}{\text{Control OD}} \text{ x } 100\% \quad \text{- (2)}$$

3. Result and Discussion
3.1 Extractions
The extract was prepared by Maceration extraction with three different solvents with increasing polarity. Water has a high polar index, hexane has less value. Acetone has an intermediate value. i.e., 10.2, 0.1, 5.1 respectively.

3.2 Phytochemical screening
Qualitative phytochemical analysis of leaf extract of *Morinda citrifolia* showed the presence of primary metabolites like Carbohydrate, Protein and Amino acid and secondary metabolites like Alkaloids, Cardiac glycosides, Flavanoids, Phenol, Saponins, Steroids, Terpenoids and Tannins. The different fraction of extract contains different metabolites (Table. 1 and fig.1).

Table 1: Phytochemical analysis of *Morinda citrifolia*

Chemical Constituents	Acetone	Hexane	Water
Carbohydrates	+	-	+
Proteins	-	-	+
Amino acid	-	-	+
Alkaloids	-	-	+
Cardiac glycosides	-	-	+
Flavonoids	+	-	+
Phenol	+	-	+
Saponins	-	-	+
Steroids	+	-	+
Terpenoids	+	-	+
Tannin	-	-	+

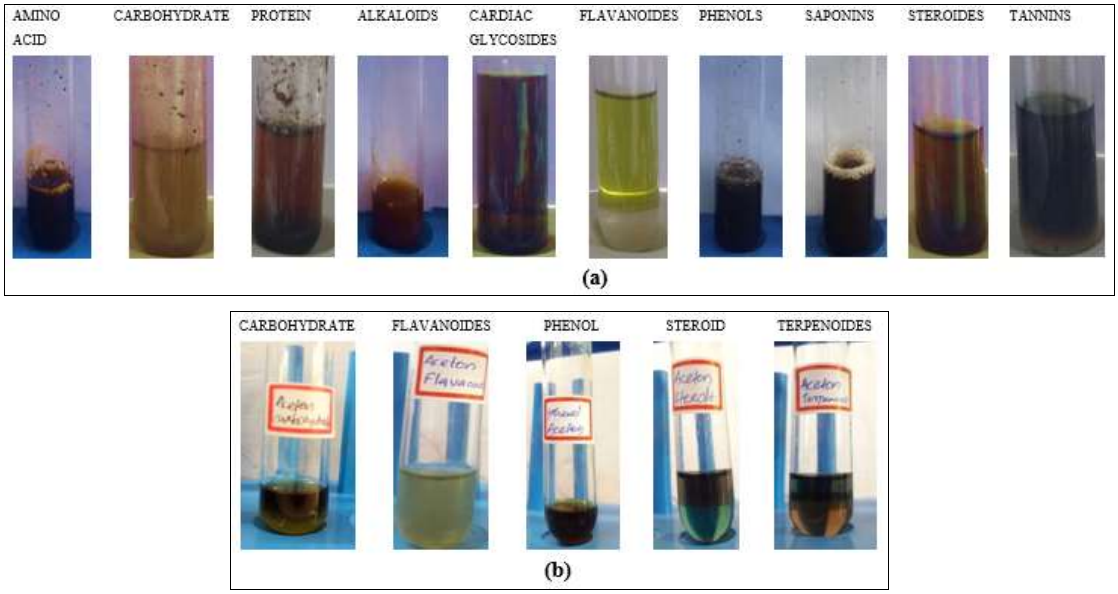

Fig 1: Phytochemical analysis of leaf extracts of *Morinda citrifolia* using (a) water and (b) acetone solvent

3.3 Antibacterial Activity

Antimicrobial activity against *Pseudomonas aeruginosa* shows a low percentage of inhibition in a small amount of extract concentrations. That is in 10µl concentration shows 10.12% of inhibition.

The highest antibacterial activity was recorded in 100µl concentration where the percentage of inhibition is 61.46%. So as the concentration of the extract increase, the antibacterial inhibition also increases. Both the microbes, antibacterial inhibition percentage is maximum in 100 ml of sample solution and it is increased with increasing concentration of sample solutions. Antimicrobial inhibition is maximum in the case of Pseudomonas aeruginosa (61.46%) than Escherichia coli (52.24%) (Fig.2).

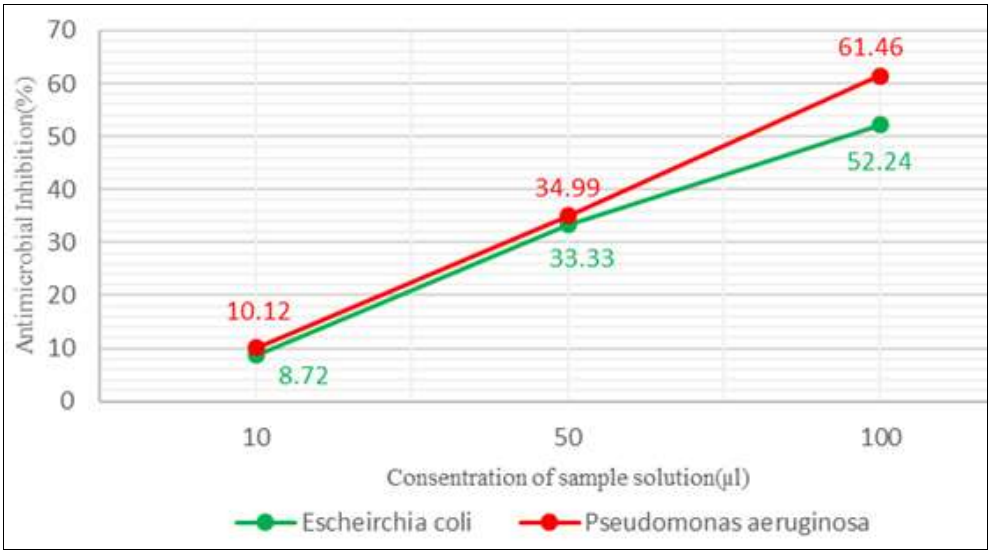

Fig 2: Antibacterial activity of aqueous extract of *Morinda citrifolia* in different concentration against *Escherichia coli* and *Pseudomonas aeruginosa*

In both antimicrobial activities, the optical density of the solution was decreased from the increasing concentration of the sample solution. In *Pseudomonas aeruginosa*, final test OD (optical density) of the sample solution was 0.506, 0.366, and 0.217 respectively, for 10ml, 50ml and 100ml sample solution and in *Escherichia coli* 0.816, 0.596 & 0.427 respectively for 10ml, 50ml and 100ml sample solution. Final test OD is the difference between the OD of the extract and OD of the test. Control OD value of *Escherichia coli* in Table. 5 are 0.894 and *Pseudomonas aeruginosa* are 0.563. The common control is the mixture of nutrient broth and culture.

3.4 Antioxidant Assay

All the three extract were not used for the antioxidant assay. Only water extract is taken. The result obtained through DPPH Assay is given in the table.2.

Table 2: DPPH Assay

Concentration(µl)	Wave length	OD of Test	OD of control	% of Inhibition
50	518nm	0.096	0.190	49.47
100	518nm	0.075	0.190	57.36
200	518nm	0.059	0.190	68.94

The antioxidant activity of plant *Morinda citrifolia* assessed using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH)[25].In the assay the inhibition for aqueous extract was detected by 518nm in a spectrophotometer. Methanol served as the blank and a tube without the extracts served as the positive control. The given result reveals that the radical scavenging activity in percentage of inhibition of water extract of *Morinda citrifolia* increases from low to high concentrations (50µl -200µl).

4. Conclusion

The phytoconstituents in the extract of *Morinda citrifolia* indicates the possibility of using leaves as a source of remedies for many bacterial diseases and also used for antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. In the case of both organisms, the antibacterial activity of the aqueous extract of *Morinda citrifolia* is maximum in 100µl. The antibacterial activity of leaf extract against *Pseudomonas aeruginosa* was higher when compared with *Escherichia coli*. Aqueous extract of *Morinda citrifolia* showed antioxidant activity in DPPH assay. Higher concentration of extract show more radical scavenging activity. So as the concentration of the extract increase, the antioxidant activity also increases. As a result of this investigation, it can be concluded that water is good for phytochemical screening. Because many important secondary metabolites presented with the aqueous extract showed good antibacterial and antioxidant activity. The study provides an alternative eco-friendly approach for bacterial disease control by utilizing this plant and also emphasizes the ethno-botanical significance of the plant. Since *Morinda citrifolia* leaf extract showed good antioxidant activity, it can be further investigated for the isolation of active components.

5. Acknowledgements

The authors thankto UniBiosys Biotech Research Lab, Kalamassery, Cochin, Kerala for providing the necessary facilities for undertaking this research project and all M.Sc. students2017-2019 batch, Department of Botany, M E S Asmabi College, Thrissur, Kerala for their support.

6. References

1. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World journal of agricultural sciences 2008;4(5):839-843.
2. Nagesh KS, Shanthamma C. Antibacterial activity of Curculigo orchioides rhizome extract on pathogenic bacteria. African Journal of Microbiology Research

2009;3:05-09.
3. Atef NM, Shanab SM, Negm SI, Abbas YA. Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. Bulletin of the National Research Centre 2019;43(144):1-11.
4. Hernández-Cortez C, Palma-Martínez I, Gonzalez-Avila LU, Guerrero-Mandujano A, Solís RC, Castro-Escarpulli G. Food Poisoning Caused by Bacteria (Food Toxins). Chapter 3: Poisoning - From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis 2017, 40. Doi: 10.5772/intechopen.69953.
5. Elisha IL, Botha FS, McGaw LJ, Eloff JN. The antibacterial activity of extracts of nine plant species with good activity against Escherichia coli against five other bacteria and cytotoxicity of extracts. BMC Complementary and Alternative Medicine 2017;17(133):1-10.
6. Pallavali RR, Degati VL, Lomada D, Reddy MC, Durbaka VRP. Isolation and *in vitro* evaluation of bacteriophages against MDR bacterial isolates from septic wound infections. PLoS ONE 2017;12(7):179-245.
7. Bassetti M, Vena A, Croxatto A, Righi E, Guery B. How to manage *Pseudomonas aeruginosa* infections. Drugs Context 2018;7:212-227.
8. Kalimuthu K, Vijayakumar S, Senthilkumar R. Antimicrobial Activity of the Biodiesel Plant, Jatropha Curcas L. International Journal of Pharma and Bio Sciences 2010;1(3):1-5.
9. Chokki M, Cudălbeanu M, Zongo C, Dah-Nouvlessounon D, Ghinea IO, Furdui B *et al*. Exploring Antioxidant and Enzymes (A-Amylase and B-Glucosidase) Inhibitory Activity of *Morinda lucida* and *Momordica charantia* leaves from Benin. Foods 2020;9(434):1-26.
10. Frezza C, De Vita D, Spinaci G, Sarandrea M, Venditti A, Bianco A. Secondary metabolites of tiliatomentosa moench inflorescences collected in central Italy: Chemotaxonomy relevance and phytochemical rationale of traditional use. Natural Product Research 2019;12:1-8.
11. Vanderplanck M, Glauser G. Integration of non-targeted metabolomics and automated determination of elemental compositions for comprehensive alkaloid profiling in plants. Phytochemistry 2018;154:1-9.
12. Pertuit D, Mitaine-Offer AC, Miyamoto T, Tanaka C, Delaude C, Lacaille-Dubois MA. Terpenoid glycosides from the root's barks of *Eriocoelum microspermum* radlk. Ex Engl. Phytochemistry 2018;152:182-190.
13. Sofowora A. Medicinal plants and traditional medicine in Africa. Ibadan. Nigeria: Spectrum Books Ltd 1993.
14. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK *et al*. *Morinda citrifolia* (Noni): a literature review and recent advances in Noni research. Acta pharmacologica Sinica 2002;23(12):1127-1141.
15. Nerurkar Pratibha, Phoebe Hwang, Erik Saksa. Anti-Diabetic Potential of Noni: The Yin and the Yang. Molecules 2015;20(10):17684-17719. https://doi.org/10.3390/molecules201017684
16. Chan-Blanco Y, Vaillant F, Perez A, Reynes M, Brillouet J, Brat P. The noni fruit (*Morinda citrifolia* L.): A review of agricultural research, nutritional and therapeutic properties. Journal of food composition and analysis 2006;19:645-654. Doi: 10.1016/j.jfca.2005.10.001.
17. Ahmad VU, Bano S. Isolation of β-sitosterol and ursolic acid from *Morinda citrifolia* Linn, Journal of the Chemical Society of Pakistan 1980;2:71.
18. Saludes JP, Garson MJ, Franzblau SG, Aguinaldo AM. Antitubercular constituents from the hexane fraction of Morinda citrifolia Linn. (Rubiaceae), Phytotherapy Research 2002;16:683-685.
19. Takashima J, Ikeda Y, Komiyama K, Hayashi M, Kishida A, Ohsaki A. New constituents from the leaves of *Morinda citrifolia*, Chemical and Pharmaceutical Bulletin 2007;55:343-345.
20. Sang S, Cheng X, Zhu N, Stark RE, Badmaev V, Ghai G *et al*. Flavonol glycosides and novel iridoid glycoside from the leaves of *Morinda citrifolia*, Journal of Agriculture and Food Chemistry 2001;49:4478-4481.
21. West BJ. Phytochemical: A Global perspective of their Role in Nutrition 2007.
22. Kokat CK. Pharmacognosy 16th Edn, NialiPrakasham, Mumbai India 2001.
23. Senthilmurugan GB, Vasanthe B, Suresh K. Screening and antibacterial activity analysis of some important medicinal plants, International Journal of Innovation and Applied Studies 2013;2(2):146-152.
24. Harborne JB. Phytochemical Method: Modern method Guidelines of plants analysis (Bandung: ITB) Indonesia 1987.
25. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology 1995;28(1):25-30.