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

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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 37°C overnight with shaking at 180 rpm. The contents of the flask were filtered. The filtrate was evaporated in a water bath at 50°C. The weight of residues was recorded. The resulting extracts were stored at 4°C 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 370°C 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:

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

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 discoloration of the purple colour was measured at 518nm in a spectrophotometer [25]. The assay was calculated as:

\[
\text{Radical scavenging activity} = \left( \frac{\text{Control OD-Test OD}}{\text{Control OD}} \right) \times 100\% \quad - (2)
\]

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>
<thead>
<tr>
<th>Chemical Constituents</th>
<th>Acetone</th>
<th>Hexane</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Amino acid</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 1: Phytochemical analysis of Morinda citrifolia

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

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3.3 Antimicrobial 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%.

![Fig 2: Antibacterial activity of aqueous extract of Morinda citrifolia in different concentration against Escherichia coli and Pseudomonas aeruginosa](http://www.florajournal.com)

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>
<thead>
<tr>
<th>Concentration(µl)</th>
<th>Wave length</th>
<th>OD of Test</th>
<th>OD of control</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>518nm</td>
<td>0.096</td>
<td>0.190</td>
<td>49.47</td>
</tr>
<tr>
<td>100</td>
<td>518nm</td>
<td>0.075</td>
<td>0.190</td>
<td>57.36</td>
</tr>
<tr>
<td>200</td>
<td>518nm</td>
<td>0.059</td>
<td>0.190</td>
<td>68.94</td>
</tr>
</tbody>
</table>

The antioxidant activity of plant *Morinda citrifolia* assessed using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH)\(^2\). 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 thank to UniBiosys Biotech Research Lab, Kalamassery, Cochin, Kerala for providing the necessary facilities for undertaking this research project and all M.Sc. students 2017-2019 batch, Department of Botany, M E S Asmabi College, Thrissur, Kerala for their support.

6. References


