Phytochemical and Antimicrobial Activity of Ethanol Extract of *Madhuca longifolia* Flowers

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**Abstract**

The advent of multidrug resistance among pathogenic bacteria is imperiling the worth of antibiotics, which have previously transformed medical sciences. Further, commercial antibiotic drugs caused side effects to body. However, herbal remedies often do not produce any side effects, and safe to be use. Hence the aim of the present study was to perform phytochemical study, and evaluate antimicrobial activity of ethanol extract of *Madhuca longifolia* against various bacterial and fungal species. The ethanol extract of flower of *Madhuca longifolia* were prepared and screened for phytochemical study. The different concentration of ethanol extract (50 μg/ml, 100 μg/ml, 150 μg/ml, 200 μg/ml and 250 μg/ml) was evaluated for antimicrobial activity against bacteria and fungi by disc diffusion method. The phytochemical study indicates the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins and polyphenol in ethanol extract. The extract demonstrated the mild to moderate antibacterial and antifungal activity. The maximum antibacterial activity of extract was found on *Staphylococcus aureus*, while the highest antifungal activity was found on *Aspergillus niger*. The present study was concluded that the antimicrobial activity of *Madhuca longifolia* might be due to presence of flavonoids and polyphenol component.

**Keywords:** *Madhuca longifolia*, Antimicrobial activity, Phytochemical

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1 Introduction

Nowadays, one key problem in human health is the less effectiveness of commercial antibiotics against several pathogenic bacteria isolates. The gram positive and gram negative bacteria considered as one of the world’s most important infectious agents causing disease outbreaks related to food consumption, badly treated wounds and hospital-associated infections. Its epidemiological relevance is mainly due to their ability of becoming highly resistant to common antimicrobials such as tetracycline, vancomycin, penicillin G, and methicillin and to a less degree to oxacillin, lincomycin, clindamycin, erythromycin, streptomycin, cefoxitin, kanamycin, chloramphenicol, and gentamicin.

The medicinal plant incorporating different types of chemical constituents and can protect humans against diseases. Many plant extracts have been shown to inhibit the growth of microorganisms. These extracts consist of chemicals and are usually considered to play a role in defense reactions of plants against infections by pathogenic microorganisms.

*Madhuca longifolia* belongs to family sapotaceae, and termed as universal panacea of ayurvedic medicine. The tree is valued for its flowers, fruits, seeds and timber. The various parts *Madhuca longifolia* has shown efficacy in the treatment of epilepsy, diabetes, inflammation, bronchitis, ulcer and other diseases. The oil extracted from *Madhuca longifolia* seed is used as biofuel, edible fats and has shown good antioxidant properties. The flowers are well known for its reducing sugar content and have been used as cooling agent, astringent, demulcent and clinical study proves its activity in increasing the sperm count.

Leaves of *Madhuca longifolia* are used in Cushing's disease and bronchitis and have antioxidant properties. The barks reported the treatment for itching, swelling, snake poisoning and diabetes. It consists of terpenoids, proteins, starch, anthraquinone glycosides, phenolic compounds, mucilage,
cardiac glycosides, tannins and saponins. The present study was proposed to conduct phytochemical and antimicrobial activity of ethanol extract of Madhuca longifolia flowers.

2 Materials and Methods

2.1 Collection and authentication

The flower parts of Madhuca longifolia were collected from the old trees of medicinal garden of Mahakal Institute of Pharmaceutical Studies, Ujjain (M.P.), India. The identification of the trees specimens was confirmed by the use of local floras and standard references. The plant was also authenticated by Mrs. Chitralekha Kadel, Professor and Head, Department of Botany, Vikram University, Ujjain, Madhya Pradesh, India.

2.2 Preparation of extracts

The flowers were washed with tap water and then with distilled water. The flowers were dried in shade. Flowers were comminuted to coarsely powder and extracted with ethanol using soxhlet apparatus. The extracts were concentrated under reduced pressure using rotary evaporator.

2.3 Phytochemical screening

The extract was screened for qualitative phytochemical tests. The tests were done according to the standard procedures described into literature by Kokate to detect the following bioactive compounds: alkaloids, carbohydrates, glycosides, flavonoids, tannins and phenolic compounds, steroids, protein, fat and oil test.

2.4 Antimicrobial activity

2.4.1 Preparation of test solution

The test solution of ethanol extract of Madhuca longifolia flowers were prepared in 5 successive dilutions namely 50 μg/ml, 100 μg/ml, 150 μg/ml, 200 μg/ml and 250 μg/ml, and were subjected to antibacterial and antifungal activities.

2.4.2 Microorganisms used

The bacterial strain Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa were used for antibacterial activity. The Aspergillus oryzae and Aspergillus niger were used for the antifungal activity.

The standard drug Ciprofloxacin (5μg/ml) and Clotrimazole (10 μg/ml) were used as antibacterial and antifungal activity, respectively.

2.4.3 Determination of antibacterial activity

The antimicrobial activity of the extracts was evaluated by disc diffusion method. The paper discs containing different concentration of extracts (50 μg/ml, 100 μg/ml, 150 μg/ml, 200 μg/ml and 250 μg/ml) were placed individually on the surface of the petri plates, containing 20 ml of respective media seeded with 0.1 ml of previously prepared microbial suspensions individually (10 CFU/ml). Standard antibiotic Ciprofloxacin (20 μg/disc) obtained from Hi-media, Mumbai, was used as positive controls.

The discs containing ethanol served as negative controls. The assessment of antibacterial activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 24 h at 37°C and the diameter of the inhibition zones was recorded with the help of antibiotic zone reader.

2.4.4 Determination of antifungal activity

The extracts were tested for antifungal activity using modified disc diffusion method. Sabouraud agar was used to culture the fungi (Aspergillus oryzae and Aspergillus niger). The paper discs containing different concentration of extracts (50 μg/ml, 100 μg/ml, 150 μg/ml, 200 μg/ml and 250 μg/ml) were placed individually on the surface of the petri plates, containing 20 ml of respective media seeded with 0.1 ml of previously prepared microbial suspensions individually (10 CFU/ml). Standard antibiotic Clotrimazole (10 μg/disc) obtained from Hi-media, Mumbai, was used as positive controls.

The discs containing ethanol served as negative controls. The assessment of antifungal activity was based on measurement of inhibition zones formed around the discs. The plates were incubated for 72 h at 37 °C and the diameter of the inhibition zones was recorded with the help of antibiotic zone reader.

2.5 Statistical analysis

Each of the extracts was tested in triplicate, and the average values were obtained from three repeated experiments.

3 Results and Discussions

3.1 Phytochemical screening

Preliminary phytochemical investigations of the ethanol extract of Madhuca longifolia flowers revealed the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins and polyphenol. The details are presented in table 1. The maximum numbers of chemical constituents were present in the ethanol extract.

It is scientifically documented that the flavonoids and polyphenols are effective against microorganism, and cure different types of skin diseases. The findings of phytochemical screening exhibited the presence of flavonoids and polyphenol in extract, and can imparts antimicrobial activity against bacterial and fungal strains. Looking on above statement the ethanol extract of Madhuca longifolia flowers were screened for the antimicrobial activity.

3.2 Antimicrobial activity

Ethanol extracts of Madhuca longifolia flowers were screened for antimicrobial activities against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,
Aspergillus oryzae and Aspergillus niger at dose level ranging from 50 μg/ml to 250 μg/ml. The finding of antimicrobial activity are displayed in Table 2.

Table 1: Phytochemicals present in ethanol extract of **Madhuca longifolia** flowers

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s test -</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Baljet test -</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molish test +</td>
</tr>
<tr>
<td>Tannins and Phenolic compound</td>
<td>Bromine water -</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda test +</td>
</tr>
<tr>
<td>Steroid test</td>
<td>Salkowski test -</td>
</tr>
<tr>
<td>Protein</td>
<td>Ninyhydrin test -</td>
</tr>
<tr>
<td>Fat and oil test</td>
<td>Spot Test -</td>
</tr>
</tbody>
</table>

+ = Present, - = Absent

Ethanol extract of **Madhuca longifolia** flowers inhibited all the bacterial and fungal strains tested. All the doses (50 μg/ml to 250 μg/ml) showed zone of inhibition against all the bacteria and fungal, even at a dose of 50 μg/ml of extract exhibited significant zone of inhibition comparable to standard antibiotic (Ciprofloxacin) against Bacillus subtilis. For Staphylococcus aureus ethanol extract exhibited maximum inhibition (20.3 mm) which was close to standard drug. For all other bacteria 100 μg/ml concentration of the extract was sufficient to produce effective inhibition. Ethanol extract of **Madhuca longifolia** flowers also effectively inhibited growth of two fungi (A. oryzae and A. niger) comparable and even equal than that of standard antifungal agent Clotrimazole. For A. oryzae 250 μg/ml concentration produced equal inhibition to standard.

Table 2: Antimicrobial activity of ethanolic extract of **Madhuca longifolia** flowers

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>Zone of Inhibition (mm)</th>
<th>Standard drug (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>A2</td>
<td>A3</td>
</tr>
<tr>
<td>Antibacterial Activity</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>10.5</td>
<td>11.9</td>
<td>13.1</td>
</tr>
<tr>
<td>B. subtilis</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>0.61</td>
<td>0.42</td>
<td>0.68</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>8.9</td>
<td>10.1</td>
<td>12.6</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td>0.35</td>
<td>0.83</td>
<td>1.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A. oryzae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibacterial Activity</td>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>5.7</td>
<td>7.1</td>
<td>9.8</td>
<td>10.4</td>
<td>12.2</td>
<td>13.4</td>
</tr>
<tr>
<td>A. niger</td>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>1.72</td>
<td>0.61</td>
<td>1.12</td>
<td>1.23</td>
<td>0.69</td>
<td>0.51</td>
</tr>
</tbody>
</table>

A1-50 μg/ml of **Madhuca longifolia** extract
A2-100 μg/ml of **Madhuca longifolia** extract
A3-150 μg/ml of **Madhuca longifolia** extract
A4-200 μg/ml of **Madhuca longifolia** extract
A5-250 μg/ml of **Madhuca longifolia** extract
Standard drug: Ciprofloxacin/Clotrimazole

Thus ethanol extracts of **Madhuca longifolia** were found to be inhibitory against all the bacteria and fungi tested. Antibacterial and antifungal activity of ethanol extracts of **Madhuca longifolia** flowers could be attributed to the presence of biological compounds like 2-Furan methanol, 4H pyran 4-one, 2,3-dihydro 3,5-dihydroxy-6-methyl, Thiophene, 2-Furancarboxaldehyde-5-(hydroxymethyl) and 1,4-tetra decanol.

The use of medicinal plants play a vital role in covering the basic health needs in developing countries and these plants...
may offer new sources of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms.

4 Conclusions

The present study indicates that *Madhuca longifolia* flower ethanol extract have broad inhibitory activities to pathogenic microorganism and to act as potential antimicrobial agent from natural sources. In general, commercial antibiotic and antifungal drugs causes side effects on liver, kidney and gastrointestinal tract. Therefore, alternative medicine becomes popular remedy to various types of ailments. The present study reveals the existence of antimicrobial substances in *Madhuca longifolia* and further studies are required to find out the active components of medicinal properties in this valuable plant.

5 Acknowledgements

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6 Conflict of interest

Nil

7 Author’s contributions

PP and MN carried out the experimental work, while DM and AS participated in statistical analysis of data. All authors read and approved the final manuscript.

8 References


