Potential use of *Muntingia calabura* L. extracts against human and plant pathogens

Sibi G,* R. Naveen, K. Dhananjaya, K.R. Ravikumar and H. Mallesha

R & D Centre, Robust Materials Technology Pvt. Ltd., Bengaluru – 560 072, Karnataka, India

**ABSTRACT**

Studies were carried out to determine the phytochemical and antimicrobial properties of various parts of *Muntingia calabura* L. Aqueous and methanol extracts of leaf, bark and fruits were prepared and phytochemical analysis of the parts revealed the presence of glycosides and flavonoids as the major biologically active compounds. Bacterial isolates of clinical importance and fungal phytopathogens were tested against the various extracts of *M. calabura*. Various degree of inhibition was observed with various extracts and significant antibacterial activity was recorded against *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Bacillus cereus*. Antifungal activity of the methanol extracts were seen against *Fusarium* sp and *Penicillium* sp and was mainly due to the presence of tannins. Nil antifungal activity of aqueous extract was described in the study due to the absence of bioactive compounds in the extracts. The presence of glycosides, tannins and flavonoids has influenced the antimicrobial properties of the plant especially against *M. luteus* and *P. aeruginosa*. Methanolic extracts have shown better efficacy against the test isolates than the aqueous extracts throughout the study revealing the soluble nature of the phytochemicals in the solvent. The broad antimicrobial activity suggests the use of *M. calabura* as a source of new bioactive principles for the development of drugs against human and plant pathogens.

**Keywords:** *Muntingiacalabura*, glycosides, tannins, flavonoids, antimicrobial.

**INTRODUCTION**

*Muntingia calabura* L. (Elaeocarpaceae) is a small, evergreen tree growing in tropical regions. The plant has been reported to possess antiproliferative, antioxidant, antinociceptive, cardioprotective and antipyretic effects.[1,2,3,4,5,6] A total of 42 volatile compounds has been identified in the vacuum distillation extract of ripe fruits.[7] Various parts (bark, roots and leaves) contains flavonones, flavones, flavans and biflavans which exhibited cytotoxic effects.[8,9,10,11] Determination of biologically active compounds from plants and their pharmaceutical potential for human use is necessary to challenge the illness caused by microorganisms. Due to the genetic variability of microorganisms against commonly used antibiotics, new formulations especially from plant origin are required to suppress the resistant strains. The present study aims to determine the phytochemical properties of *M. calabura* and their influence on its antimicrobial activity human and phytopathogens.

**MATERIALS AND METHODS**

Plant collection and extraction

Various parts of the plant (leaves, root, bark) were collected from its natural habitat in Bengaluru, Karnataka state, India and air dried at room temperature for 72 hours. The dried parts were ground into powder, sieved (60 mesh) and extracted with water and methanol at room temperature for 72 hrs and 48 hrs respectively. After extraction, it was filtered by using Whatman No. 1 filter paper and concentrated to dryness under reduced pressure in a rotary evaporator and stored in sterile vials at 4°C until used.
**Phytochemical studies**

Concentrated crude extracts were dissolved in the same solvent to determine the major phytochemicals by following the methods described earlier.[12,13]

**Test for glycosides**

5 ml of each extract was added with 2 ml of glacial acetic acid which was followed by the addition of 1 drop of ferric chloride solution and 1 ml of conc. sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

**Test for flavonoids**

1 ml of the extract was taken in the test tube and ammonia solution was added (1:5) followed by the addition of conc. sulphuric acid. Appearance of yellow color and its disappearance on standing indicates the positive test for flavonoids.

**Test for phlobatannins**

1% of HCl was added to the extract (1 ml) and boiled in hot water bath. Formation of red precipitate indicates the presence of phlobatannins.

**Test for saponins**

1 ml of the extract was taken in a test tube and distilled water (2 ml) was added to it. The test tube was then kept in boiling water bath for boiling and was shaken vigorously. Existence of froth formation persisted for next one hour confirms the presence of saponins.

**Test for tannins**

1 ml of the extract was added with 5 ml of distilled water and kept for boiling in hot water bath. After boiling, sample was cooled down and to this 0.1% ferric chloride solution was added. Appearance of brownish green or blue black coloration confirms the presence of tannins.

**Test for terpenoids**

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of conc. sulfuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpenoids.

**Antibacterial assay**

Concentrated extracts were diluted in 20% DMSO to determine the antimicrobial activity of the plant with DMSO as the control. The agar well diffusion method was used to study the antibacterial activity of *M. calabura* extracts. Bacterial isolates viz., *Bacillus cereus*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Serratia marcescens* were tested against the extracts. Each bacterial culture was diluted as 1:100 with fresh sterile nutrient broth and inoculated (2.5 × 10³ CFU/ml) on sterile Mueller Hinton agar (MHA) plates by swabbing. 50 µl of the extracts were loaded in each well (6 mm) of Mueller Hinton agar plates and the plates were allowed to stand for 45 minutes for the diffusion of the extracts prior to incubation. Diameter of the zone of inhibition was measured by using HiMedia antibiotic scale after 24 hours of incubation.

**Antifungal assay**

Aliquot of 100 µl spores suspension (1 × 10⁸ spores/ml) of *Aspergillus oryzae*, *Fusarium sp* and *Penicillium sp* were inoculated by swabbing on the surface of media plates. Wells of 6 mm in diameter were performed in the PDA media, and each well was filled with the crude extract. Dimethyl sulfoxide (DMSO) was used as control and the cultured plates were incubated at 25ºC for 3–5 days. The radius for the zone of inhibition was measured in two directions at right angles to each other. Experiments were carried out with three replicates per treatment and each treatment was repeated at least twice.

**RESULTS**

Table 1 summarizes the phytochemical analysis of the plant extracts. The results revealed that glycosides and tannins were the major phytochemicals present in *M. calabura* followed by flavonoids and terpenoids. Among the various parts, leaves contain more number of phytochemicals followed by fruits and bark. Absence of saponins in all the parts was observed.

Results presented in Table 2 shows the in vitro antimicrobial activities of aqueous and methanolic extracts of *M. calabura*. Aqueous leaf extract has exhibited inhibitory activity against *M. luteus* and *P. aeruginosa* and bark extract with the addition of *B. cereus*. Aqueous fruit extract

<table>
<thead>
<tr>
<th>Phytochemical analysis of <em>M. calabura</em>.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytochemicals</td>
</tr>
<tr>
<td>Glycosides</td>
</tr>
<tr>
<td>Flavonoids</td>
</tr>
<tr>
<td>Phlobatannins</td>
</tr>
<tr>
<td>Saponins</td>
</tr>
<tr>
<td>Tannins</td>
</tr>
<tr>
<td>Terpenoids</td>
</tr>
</tbody>
</table>
was failed to control the growth of test isolates except *M. luteus*. Methanolic extracts of the plant were able to control the growth of *B. cereus* and *M. luteus*. Significant antifungal activity of methanolic extracts was recorded against *Fusarium* and *Penicillium* sp. A significant inhibitory effect on *M. luteus* was exhibited by both aqueous and methanolic extracts throughout the study. Highest inhibitory activity was seen with aqueous leaf extract. However, same degree of action was observed in the presence of methanolic leaf and fruit extracts.

**DISCUSSION**

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs which has necessitated the search for new antimicrobials from alternative sources and natural compounds are a source of numerous therapeutic agents. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action are also very likely to differ. A wide range of medicinal plants parts are used as raw drugs as they possess varied medicinal properties. Bioactive substances from plant origin could be employed in the formulation of antimicrobial agents for the treatment of various bacterial and mycotic infections.[14]

The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *B. cereus* (food infections), *K. pneumoniae, P. aeruginosa, S. marcescens* (neonatal and nosocomial infections), *Micrococcus* (bloodstream infections), *P. vulgaris* (urinary tract infections) are the major human pathogens. Fungal isolates of this study are mainly plant pathogens and their metabolites leads to human illness. Finding out the antimicrobial activity of *M. calabura* is an indication that the plant can be a source of bioactive substances with broad spectrum activity. Solubility of phytoconstituents varies with different solvents.[15] Aqueous leaf extract exhibited antibacterial activity but failed to control the growth of fungal isolates whereas, methanolic extracts showed both antibacterial and antifungal activity revealing the soluble nature of the phytoconstituents in that particular solvent. Interactions with membrane sterols lead to the antimicrobial activity of saponins and its absence in the extracts resulted in poor antifungal activity. Tannins are toxic to bacteria and filamentous fungi and their antimicrobial activity is influenced by its inhibition of microbial enzymes, cell envelope transport proteins, oxidative phosphorylation and complex with polysaccharides.[16,17] Presence of tannins in *M. calabura* extracts supports the medicinal use of this plant. Glycosides are potential antimicrobial compounds and the presence of this bioactive molecule was seen in leaf, bark and fruit extract of the plant.[18,19] Flavonoids have ability to inhibit spore germination of plant pathogens and proven for use against human fungal pathogens.[20] It has been reported that the total phenolic and flavonoid content in fruit extracts of *M. calabura* makes it valuable source of antioxidants.[21] In the present study, the presence of flavonoids in leaf and fruit extracts could have attributed the antifungal activity of the plant.

The results revealed that different parts of same plant species have different antimicrobial effects and the degree of effectiveness may vary with the part of the plant used even though it was prepared under same conditions. A strong antibacterial activity of the plant was observed against *M. luteus*, causative agent of bloodstream infections followed by *P. aeruginosa* responsible for nosocomial infections. Significant activity was observed with *B. cereus*. Effective antistaphylococcal activity of methanolic extract of this plant was reported earlier.[22] Antifungal activity of the extract was observed with *Fusarium* and *Penicillium* which was mainly due to the presence of tannins.

Preliminary phytochemical analysis concluded the presence of glycosides, tannins and flavonoids that might have contribute to the antimicrobial effect of *M. calabura*. These biologically active compounds interfered with the

**Table 2. Antimicrobial activity of Muntingia calabura (zone of inhibition in mm).**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Aqueous Leaf</th>
<th>Aqueous Bark</th>
<th>Aqueous Fruit</th>
<th>Methanol Leaf</th>
<th>Methanol Bark</th>
<th>Methanol Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>–</td>
<td>16</td>
<td>–</td>
<td>15</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>20</td>
<td>18</td>
<td>17</td>
<td>25</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>14</td>
<td>10</td>
<td>–</td>
<td>20</td>
<td>–</td>
<td>15</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>21</td>
<td>–</td>
</tr>
<tr>
<td><em>Aspergillus oryzae</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>13</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Fusarium sp</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>30</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td><em>Penicillium sp</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>26</td>
<td>20</td>
<td>–</td>
</tr>
</tbody>
</table>
growth of test isolates in various ways resulting in suppression of growth and proving their efficacy against the pathogens. Structural identification and purification of active principles in M. calabura responsible for antimicrobial activities would help in developing drugs for the therapeutic use in human beings.

REFERENCES