Evaluation of Antioxidant and Antimicrobial Activity of Ethanolic Extract of Medicinal Plants

*Baccaurea Ramiflora* and *Microcos Paniculata*

Suvendu Saha¹*, T. Shivaraj Gouda², Arunabha Mallik³

¹Associate Professor, Department of Pharmacology, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad-500043, Telangana, India.
²Department of Pharmacology, NET Pharmacy College, Raichur, India.
³Professor, Department of Pharmacology, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad-500043, Telangana, India.

*Correspondence Author:

Suvendu Saha
E-mail: suvendu.belonia@gmail.com
Mobile. No. 9533400909
Mailing Address: Suvendu Saha, Plot no. 140, Road no.8, Manjeera Nagar Colony, Old Alwal, Hyderabad, Pin-500010

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**Abstract**

**Objective:** The present study was undertaken to investigate the antioxidant and antimicrobial effect of ethanolic leaf extract of *Baccaurea ramiflora* and *Microcos paniculata*.

**Methods:** DPPH radical scavenging activity, Nitric oxide scavenging activity, Super oxide anion radical scavenging activity, Reducing power assay were used to assess antioxidant efficacy. Zone of Inhibition determination by Agar well diffusion assay was used to assess antimicrobial activity.

**Results:** The *Baccaurea ramiflora* and *Microcos paniculata* leaves extracted with different solvents such as petroleum ether, chloroform, ethanol and water among that in leaves ethanolic extract produce 10.78, 10.38 percentage yield respectively. Both the extracts subjected for phytochemical investigation revealed the presence of alkaloids, glycosides, tannins, saponins, proteins and flavonoids. Ethanolic extract of *Baccaurea ramiflora* showed maximum inhibition zone diameter was obtained in *Salmonella typhi* (Gram-negative bacteria) with diameter 29 mm and 25 mm respectively at 200mg/ml and 100mg/ml. Similarly, Ethanolic extract of *Microcos paniculata* showed minimum inhibition zone diameter compare to *Baccaurea ramiflora* was obtained in *Salmonella typhi* (Gram-negative bacteria) with diameter 23 mm and 19 mm respectively at 200mg/ml and 100mg/ml. Ethanolic extract of *Baccaurea ramiflora* showed maximum inhibition zone diameter was obtained in *Aspergillus fumigates* with diameter 25 mm and 22 mm respectively at 200mg/ml and 100mg/ml. Similarly, Ethanolic extract of *Microcos paniculata* showed minimum inhibition zone diameter compare to *Baccaurea ramiflora* was obtained in *Aspergillus fumigates* with diameter 21 mm and 19 mm respectively at 200mg/ml and 100mg/ml.

**Conclusion:** The current findings point to *Baccaurea ramiflora* and *Microcos paniculata* antioxidant and antimicrobial properties. However future studies should be designed to isolate the active constituents responsible for the specified effect.

**Keywords**

*Baccaurea ramiflora*, *Microcos paniculata*, antioxidant, antimicrobial, scavenging, *Salmonella typhi*, *Aspergillus fumigates*
INTRODUCTION

*Baccaurea ramiflora* (family: Euphorbiaceae) is native to Southeast Asia region and is found distributed in the sub-Himalayan tract, mainly from Nepal to Sikkim, Darjeeling hills, Arunachal Pradesh, Tripura, Assam, Bhutan, Burma, Peninsular Malaysia, Tibet and Andaman islands. It is an evergreen tree reaching a height of about 5-10 m. The leaf is simple, alternately arranged, with petiole.[1] *Microcos paniculata* (family: Euphorbiaceae) is a shrub that is abundant in secondary forests and also grown as hedges. Microcos is tall semi-deciduous tree, sometimes shrubby. Leaves 10-15 cm long, elliptic-oblong, acuminate, entire or slightly and irregularly toothed.[2,3]

Many studies have established that *Baccaurea ramiflora* and *Microcos paniculata* leaves extract have potent ulcer protective, and hepatoprotective properties. Since no work was reported relating to antioxidant and antimicrobial effect of *Baccaurea ramiflora* and *Microcos paniculata* plant leaves, the present study was designed to investigate the antioxidant and antimicrobial effect of the ethanolic extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves.

MATERIALS AND METHODS

Plant material and extraction: [4,5,6]
The leaves of *Baccaurea ramiflora* and *Microcos paniculata* belonging to family Euphorbiaceae were collected from the forest of Tripura, India during May – July and authenticated (ID No. is BOT/HEB/AC23072011 and BOT/HEB/AC23072512) by Dr. B. K. Datta, Professor of Botany, Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura, India. After collection of the plants, the leaves of both the plants were rinsed thoroughly in tap water and dried in shade for about 20 days under controlled temperature (25 ± 2 °C). Then the crude material was powdered, passed through a 40 mesh sieve and stored in a well closed container for further usage. Coarsely powdered and dried leaves were successively soxhlated using petroleum ether, chloroform, ethanolic and water for 72h. The extracts were filtered and the solvents were evaporated to dryness under reduced pressure in a rotary evaporator at 40 °C to 45 °C. The leaves extract was subjected to phytochemical evaluation.

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on petroleum ether, chloroform, ethanolic and aqueous extracts of *Baccaurea ramiflora* and *Microcos paniculata* for qualitative identification of phytomarkers present.

ANTIOXIDANT ACTIVITY

Anti-oxidant activity was carried out by four models such as DPPH radical scavenging, Nitric oxide scavenging activity, Superoxide scavenging activity and reducing power assay.

DPPH radical scavenging activity: [7,8,9]

2,2-diphenyl-picryl-1-picryl-hydrazyl radical (DPPH) scavenging activity was measured according to the method of Blois. Ethanol extract of the samples at various concentrations (100, 200 μg/mL,) was added separately to each 5mL of 0.1mM ethanolic solution of DPPH and allowed to stand for 30 mins. After a 30 mins of reaction at room temperature, the absorbance of the solution was measured at 517 nm. Ascorbic acid was used as standard. The free radical scavenging activity of each fraction was determined by comparing its absorbance with that of a blank solution (no sample). The ability to scavenge the DPPH radical was calculated using the following equation:

\[
\text{DPPH scavenging activity (\%) } = \frac{(A_0 - A_1)}{A_0} \times 100
\]

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

Nitric oxide scavenging activity: [10]

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions, which can be estimated using Griess Illovosy reaction 10. Scavengers of NO compete with oxygen, leading to reduced production of NO and a pink coloured chromophore is formed. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Percentage inhibition was calculated as:

\[
\text{NO scavenging activity (\%) } = \frac{(A_0 - A_1)}{A_0} \times 100
\]

Where A0 is the absorbance of the control, and A1 is the absorbance of the sample.

Superoxide anion radical scavenging activity: [11]

Superoxide anion radical scavenging activity Superoxide dismutase (SOD) is a metalloenzyme that catalyze the dismutation of superoxide radical...
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into hydrogen peroxide (H$_2$O$_2$) and molecular oxygen (O$_2$) and consequently provide an important defense mechanism against superoxide radical toxicity. The principle involved in this assay is the conversion of Nitroblue Tetrazolium (NBT) into NBT diformazan via superoxide radical. SOD utilizes the highly water-soluble tetrazolium salt and that produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with O$_2$ is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD.

1ml of NBT solution, 1ml of NADH solution, 0.1ml of plant extract (10mg in 0.1ml DMSO and 0.9ml PO4 buffer) and 0.1ml of PMS solution were added together and incubated at 25°C for 5 min. After 5 min the absorbance was read at 560 nm.

**Reducing power assay: [12,13]**

Reducing power was determined by the method prescribed by Oyaizu et al. The sample in 1ml of methanol at various concentrations was mixed with a phosphate buffer (5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5 ml, 1%), and the mixture was incubated at 50°C for 20 min. Next, 5ml of trichloroacetic acid (10%) were added to the reaction mixture, which was then centrifuged at 3000 RPM for 10 min. The upper layer of the solution (5 ml) was mixed with distilled water (5ml) and ferric chloride (1 ml, 1%), and the absorbance was measured at 700 nm. A stronger absorbance will indicate increased reducing power.

**ANTIMICROBIAL ACTIVITY**

**Culture media:**

Nutrient agar was used for bacteria and Potato Dextrose Agar for fungi.

**Standard drugs used for antimicrobial assay:**

Ciprofloxacin and Amphotericin-B were used as reference antibiotics against bacteria and fungi, respectively.

**Microorganisms used:**

*Bacillus subtilis* (Gram-positive), *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), *Salmonella typhi* (Gram-negative) *Aspergillus fumigates*.

**Preparation of inocula:**

For the preparation of the inocula 24h culture was emulsified in 3 ml sterile saline following the McFarland turbidity to obtain a concentration of 10$^8$cells/ml. The suspension was standardized by adjusting the optical density to 0.1 at 600 nm (ELICO SL-244 spectrophotometer). One hundred microlitres (100 µl) of cell suspension with approximately 10$^6$-10$^8$ bacteria per millilitre was placed in petridishes and dispersed over agar.

**Zone of Inhibition determination by Agar well diffusion assay: [14,15]**

Antimicrobial activities of the crude extracts were first screened for their zone of inhibition by the agar well-diffusion method. Briefly, crude extracts were prepared concentration of 100 mg/ml and 20 mg/ml with dimethyl sulphoxide (DMSO) as solvent. The Mueller Hinton Agar (MHA) medium (Hi Media) was prepared and sterilized at 121°C 15 lp/sq for 20 min in the autoclave. Thirty milliliters of this sterilized agar medium (MHA) were poured into each 9 cm sterile petridishes under aseptic conditions and allowed to settle.

In the following, a well was prepared in the plates with the help of a sterile stainless steel-borer (6 mm diameter) two holes per plates were made into the set agar containing the bacterial culture. Each well 100 µl of the plant extracts at the various concentration. For each bacterial strain controls were maintained where pure solvents, instead of extract as a negative control. Plant extracts and reference drug (Ciprofloxacin 1mg/ml) were allowed to diffuse for 1 hr into the plates and then incubated at 37°C for 18h in inverted position. The results were recorded by measuring the zone of growth inhibition (mm) surrounding the wells. Each assay was performed in triplicates and repeated twice.

**ANTIFUNGAL ACTIVITY [16]**

All the fungal species was cultured in Potato Dextrose broth for 48h at 27°C and Sabouraud Dextrose Agar (SDA) was employed for the agar well diffusion experiments. Fungal suspensions were adjusted to10$^7$cells/ml as explained above. The zone of Inhibition was determined after incubation for 48h at 27°C. All tests were performed in triplicates and repeated twice.

**RESULTS**

**Plant Material and Extraction:**

The *Baccaurea ramiflora* and *Microcos paniculata* leaves extracted with different solvents such as petroleum ether, chloroform, ethanol and water among
that in leaves ethanolic extract produce 10.78, 10.38 percentage yield respectively. These details of the results are summarized in table 1: and 2:

**Qualitative Phytochemical Analysis of Extracts:**

All the extracts subjected for phytochemical investigation revealed the presence of alkaloids, glycosides, tannins, saponins, proteins and flavonoids.


**ANTIOXIDANT ACTIVITY**

The scavenging activity is shown in the Table 5: .Ethanolic extracts of *Baccaurea ramiflora* and *Microcos paniculata* showed different inhibitory concentration values for different activities. Ascorbic acid was taken as standard.

**ANTIMICROBIAL ACTIVITY**

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**Table 1:** Extractive values of *Baccaurea ramiflora* leaves

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Solvent</th>
<th>Colour &amp; consistency</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pet. ether</td>
<td>Dark green Sticky</td>
<td>5.52%</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform</td>
<td>Dark Brown Solid</td>
<td>3.74%</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>Very Dark Brown Sticky</td>
<td>10.78%</td>
</tr>
<tr>
<td>4</td>
<td>Aqueous</td>
<td>Brown Solid</td>
<td>8.54%</td>
</tr>
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</table>

**Table 2:** Extractive values of *Microcos paniculata* leaves

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Solvent</th>
<th>Colour &amp; consistency</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pet. ether</td>
<td>Dark green Sticky</td>
<td>3.57%</td>
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<td>2</td>
<td>Chloroform</td>
<td>Brown Sticky</td>
<td>4.52%</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol</td>
<td>Dark Brown Sticky</td>
<td>10.38%</td>
</tr>
<tr>
<td>4</td>
<td>Aqueous</td>
<td>Brown Solid</td>
<td>9.27%</td>
</tr>
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</table>

**Table 3:** Phytochemical constituents of *Baccaurea ramiflora* leaves

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Alk</th>
<th>Carb</th>
<th>Gly</th>
<th>Tan</th>
<th>Phytos</th>
<th>Flav</th>
<th>Sapo</th>
<th>Pro</th>
<th>Muci</th>
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</thead>
<tbody>
<tr>
<td>Pet. ether</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Ethanol</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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</tbody>
</table>

**Table 4:** Phytochemical constituents of *Microcos paniculata* leaves

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Alk</th>
<th>Carb</th>
<th>Gly</th>
<th>Tan</th>
<th>Phytos</th>
<th>Flav</th>
<th>Sapo</th>
<th>Pro</th>
<th>Muci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pet. ether</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Anti-bacterial activity

The antibacterial activity of both the experimental plants was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standard, viz., Ciprofloxacin (1.0 mg/disc). The results revealed that ethanolic extracts of both the plants are potent antibacterial activity against all the microorganisms studied. Ethanolic extract of Baccaurea ramiflora showed maximum inhibition zone diameter was obtained in Salmonella typhi (Gram-negative bacteria) with diameter 29 mm and 25 mm respectively at 200mg/ml and 100mg/ml. Similarly, Ethanolic extract of Microcos paniculata showed minimum inhibition zone diameter compare to Baccaurea ramiflora was obtained in Salmonella typhi (Gram-negative bacteria) with diameter 23 mm and 19 mm respectively at 200mg/ml and 100mg/ml. More specifically, ethanolic extract of Baccaurea ramiflora represented higher susceptibility to all bacterial strains compare to Microcos paniculata.

Table 5: Effect of ethanolic extract of Baccaurea ramiflora and Microcos paniculata on scavenging activity

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Scavenging methods</th>
<th>B. ramiflora IC₅₀ (mg/ml)</th>
<th>M. paniculata IC₅₀ (mg/ml)</th>
<th>Standard (Ascorbic acid) IC₅₀ (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>DPPH scavenging activity</td>
<td>27.82 ±2.370</td>
<td>26.47 ±0.949</td>
<td>10.63 ±1.282</td>
</tr>
<tr>
<td>02</td>
<td>Nitric oxide scavenging activity</td>
<td>40.55 ±1.498</td>
<td>37.30 ±2.042</td>
<td>7.210 ±0.8577</td>
</tr>
<tr>
<td>03</td>
<td>Super oxide scavenging activity</td>
<td>35.19 ±2.055</td>
<td>42.43 ±0.840</td>
<td>14.39 ±0.9484</td>
</tr>
<tr>
<td>04</td>
<td>Reducing power method</td>
<td>37.79 ±2.839</td>
<td>44.78 ±1.82</td>
<td>15.26 ±1.994</td>
</tr>
</tbody>
</table>

Table 6: Effect of anti-bacterial activity of ethanolic extract of Baccaurea ramiflora and Microcos paniculata

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Compounds</th>
<th>Anti bacterial activity (Gram positive) Zone of Inhibition (mm)</th>
<th>Anti bacterial activity (Gram Negative) Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bacillus subtilis ATCC 6633</td>
<td>Staphylococcus aureus ATCC 29737</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 mg/ml</td>
<td>100 mg/ml</td>
</tr>
<tr>
<td>1.</td>
<td>EEBR</td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>2.</td>
<td>EEMP</td>
<td>19</td>
<td>16</td>
</tr>
<tr>
<td>3.</td>
<td>Control</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>4.</td>
<td>Ciprofloxacin 1 mg/ml</td>
<td>40</td>
<td>34</td>
</tr>
</tbody>
</table>
DISCUSSION

In literature survey the reports are indicating that the leaves of both Baccaurea ramiflora and Microcos paniculata containing polyphenolic compounds like tannins and flavonoids. These polyphenolic compounds are known to have anti-oxidant property and anti-oxidants are having organo-protective role against various experimentally induced organ damage with an intention of verifying the claims of a native practitioner and correlate the results with the earlier reports that leaves are being selected for the study.

In the present study the effect of both Baccaurea ramiflora and Microcos paniculata leaves was evaluated for antioxidant and anti-microbial activity by using different experimental models.

In the initial step of project work various extracts of coarsely powdered and dried leaves have been prepared by successive extraction procedure and thus obtained extracts were subjected to preliminary phytochemical screening. Preliminary phytochemical
tests have indicated the presence of tannins, triterpene and flavonoids in ethanolic and aqueous extracts. Since ethanolic and aqueous extracts revealed the presence of related phytochemical constituents like flavonoids and tannins and these flavonoids and tannins are reported to have anti-oxidant property. The ethanolic extract is selected for further study.

The ethanolic extracts were subjected for screening.
Antioxidant activity by using following models.
1. DPPH radical scavenging activity
2. Nitric oxide scavenging activity
3. Super oxide scavenging activity
4. Reducing power assay

Ethanolic extract of *Baccaurea ramiflora* and *Microcos paniculata* exerted more inhibition at 200 μg/ml than that of standard.

The ethanolic extracts were subjected for screening anti-microbial activity by using one model.

Zone of Inhibition determination by Agar well diffusion assay.

The antibacterial activity of both the experimental plants was evaluated according to their zone of inhibition against various pathogens and the results (zone of inhibition) were compared with the activity of the standard. The results revealed that ethanolic extracts of both the plants are potent antibacterial activity against all the microorganisms studied. Ethanolic extract of *Baccaurea ramiflora* showed maximum inhibition zone diameter was obtained in *Salmonella typhi* (Gram-negative bacteria). Similarly, Ethanolic extract of *Microcos paniculata* showed minimum inhibition zone diameter compare to *Baccaurea ramiflora* was obtained in *Aspergillus fumigates*. More specifically, ethanolic extract of *Baccaurea ramiflora* represented higher susceptibility to fungal strain compare to *Microcos paniculata*.

However to understand the exact mechanism of action of all the activities, future study should be designed to isolate the active constituents responsible for the specified effect.

**CONCLUSION**

In the present investigation, the leaves extract of both *Baccaurea ramiflora* and *Microcos paniculata* was subjected to preliminary phytochemical and pharmacological investigation. The preliminary phytochemical studies showed the presence of alkaloids, glycosides, tannins, saponins, proteins and flavonoids in the leaves part of both the plants. The ethanolic extracts of leaves contain flavonoids and tannins, selected for the pharmacological investigation. Ethanolic extracts of leaves were found safe at a dose of 2000 mg/kg. The leaves part of both the plants also exhibited antioxidant activity. The leaves part of both the plants showed significant inhibitory concentration in a dose dependent manner for all the scavenging methods. Antimicrobial activity was shown by the leaves part of both the plants against all the microorganisms studied. The antimicrobial activity was signified by maximum inhibition zone diameter obtained in different microorganisms. So we can conclude that the leaves part of both the plants showed antimicrobial property. Literature survey reveals that
**Baccaurea ramiflora** and **Microcos paniculata** processes anti-oxidant property. This anti-oxidant property of ethanolic extracts may be due to the presence of tannins and polyphenolic compounds. Further studies are required to establish the phytoconstituents responsible for the antioxidant and antimicrobial activity.

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**Conflict of interest**

There are no conflicting interests, as the authors have stated.

**References**