Potential Ex-vivo Anti-inflammatory, Cardioprotective Properties and Phytochemical Properties of Leaves of Mussaenda Roxburghii Hook

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Abstract: Background: mussaenda roxburghii hook. Belongs to family rubiaceae, is a perennial shrub moist area of valley and grows in the foothills. Objective: the present investigation was carried out to phytochemicals and pharmacological activity such anti-inflammatory, cardioprotective properties of methanolic crude extract & its fractions such as dcem extract (fraction 1), ethyl acetate extract (fraction2). Materials and methods: residual methanolic extract (fraction 3) of mussaenda roxburghii leaves which were obtained by solvent-solvent extraction process from crude methanolic extract of mussaenda roxburghii. Anti-inflammatory activity was measured by observing the mean inhibition of protein denaturation. Cardioprotective activity was evaluated by thrombolytic assay. Phytochemical screenings have done by using usual procedures. Results: an ex-vivo anti-inflammatory test demonstrated that; mean inhibition of protein denaturation of 1000/kg of crude methanolic extract of leave of m. Roxburghii was 17.399%. Cardioprotective properties of different extracts of m. Roxburghii was demonstrated which revealed that after treatment of clot with mother methanolic extract, dcm extract (fraction 1), ethyl acetate extract (fraction 2), residual methanolic extract (fraction3) of leaves and methanolic extract of root % clot lysis was 30.56%,27.61%,46.35%,26.02%,49.90 % respectively. Different tests with crude extracts showed that different types of alkaloids, carbohydrates, tannins were present in the leaves of m. Roxburghii. Conclusion: in these investigations demonstrates that different extracts of m. Roxburghii has significant pharmacological activities.

Keywords: Ex-vivo Anti-inflammatory, Cardioprotective Properties, Phytochemicals Investigation, Mean Inhibition of Protein Denaturation

1. Introduction

The goals of using plants as sources of therapeutic agents are to isolate bioactive compounds for direct use as drugs. Eg: taxol, digitoxin, morphine, digoxin, vinblastine & vincristine as well as reserpine, produce bioactive compounds of novel or known structures as lead compounds for semisynthesis to produce patentable entities of higher activity and/or lower toxicity.e.g: taxotere, teniposide etc [1]. Mussaenda roxburghii hook. Belongs to rubiaceae; is a perennial shrub grows in the foothills and moist areas of valley. Roots are traditionally used in treatment of cuts, jaundice, wounds, boils and skin diseases etc [2,3]. Leaves are used in the ailments of bone fracture [4]. The paste obtained from leaf of this plant is applied to treat boils [5]. Previous phytochemical investigation led to isolation of a new iridoid, shanzhiol which showed mild antibacterial activity against both staphylococcus aureus and escherichia coli with a mic of 100 µg/ml by the broth dilution method [6]. As part of our ongoing research with medicinal plant of bangladesh [9,10] the present study has been undertaken to evaluate the preliminary cytotoxicity
and antimicrobial activity of m. Roxburghii as well as to find out logical evidence for its folk uses and for discovery of new drug candidates.

2. Materials and Methods

2.1. Plant Materials

The leaves of mussaenda roxburghii were collected from chittagong, bangladesh, in november 2013. A voucher specimen for this plant has been maintained in bangladesh national herbarium, dhaka, bangladesh (accession no. Mk137. Ctg uh). The sun dried and powdered (500 gm) leaves of p. Cereifera was macerated in 2.5 l of methanol for 7 days and then filtered through a cotton plug followed by whatman filter paper number 1. All extracts were prepared molisch’s reagent + mixed thoroughly + all owed 2

2.4. Qualitative Sreening of Phytochemicals

One gram of the methanol extract of mussaenda roxburghii was dissolved in 100 ml of methanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents [13-15]. A small portion of the dry extract was used for the phytochemical tests for compounds which includes carbohydrate, tannins, flavonoids, alkaloids, saponins, steroids, resins, and glycose in accordance with the methods of with little modifications. All test procedure for chemical groups described [16]. We identified several compound in table-9.

(i). Test for Carbohydrate

(a). Molisch’s Test

2ml of an aqueous extract of the plant material in a test tube have to take + then have to add 2 drops of freshly prepared molisch’s reagent + mixed thoroughly + allowed 2 ml of conc. Sulphuric acid to flow down the side of the inclined test tube. So that acid forms a layer beneath the aqueous solution. A red or reddish violet ring will form at the junction of the 2 layers. On standing or shaking a dark purple solution will form. After diluted with 5 ml of water a dull violet precipitate will form.

(b). Benedict Test

0.5 ml of an aqueous extracts of the plant material + added 5 ml of benedicts solution + boil for 5 minutes and allow cooling spontaneously. A red precipitate of cuprous oxide is formed in the presence of a reducing sugar.

(c). Fehling’s Test

2 ml of an aqueous extract of the plant material + add 1 ml of a mixture of equal volume of fehling’s solutions a& b. Boil for a few minutes. A red or brick red precipitate is formed if a reducing sugar is present.
**(ii). Test for Alkaloids**

<table>
<thead>
<tr>
<th>Test name</th>
<th>Experimentation</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Mayer’s test</td>
<td>1 ml of filtrate + few drops of mayer’s reagent.</td>
<td>White or creamy white precipitate indicates the presence of alkaloids.</td>
</tr>
<tr>
<td>2) Wagner’s test</td>
<td>1 ml of filtrate + few drops of wagner’s reagent.</td>
<td>Brown or deep brown ppt indicates the presence of alkaloids.</td>
</tr>
<tr>
<td>3) Hager’s test</td>
<td>1 ml of filtrate + few drops of hager’s reagent.</td>
<td>Yellow crystalline precipitate indicates the presence of alkaloids.</td>
</tr>
<tr>
<td>4) Tannic acid test</td>
<td>1 ml of filtrate + few drops of tannic acid reagent.</td>
<td>Dirty white or black precipitate indicates the presence of alkaloids.</td>
</tr>
</tbody>
</table>

**Test name**
- Dichromate test
- Potassium acetate test
- Lead chloride test
- Salkowski's test

**Observation**
- A greenish colour will form at the junction of 2 layers, which turns blue on standing, indicates presence of steroids.
- A red color is produced in the chloroform layer if steroids are present.

**General test**
- To add 0.05 ml of sulphuric acid.
- Cool & have of the plant material in 5 to 10 ml of water for 3-5 minutes.
- Add 2 ml of a chloroform extract of the plant material + 1 ml of conc. Sulphuric acid.
- A blue, blue blackgreen or blue green colour or precipitation is produced in the presence of tannins. On addition of a few ml of dilute sulphuric acid the colour disappears followed by the formation of a yellowish brown ppt.

**Testing of Alkaloids**
- **Salkowski’s test**
  - A small amount of extract dissolved in small amount of water + few drops of aqueous sodium hydroxide solution.
  - Presence of yellow colour indicates the presence of glycosides.

**Test for Glycosides**

<table>
<thead>
<tr>
<th>Test name</th>
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<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>General test</td>
<td>A small amount of extract dissolved in small amount of water + few drops of aqueous sodium hydroxide solution.</td>
<td>Presence of yellow colour indicates the presence of glycosides.</td>
</tr>
</tbody>
</table>

**Test name**
- Libermann-burchard’s test
- Salkowski’s test

**Observation**
- A greenish colour will form at the junction of 2 layers, which turns blue on standing, indicates presence of steroids.
- A red color is produced in the chloroform layer if steroids are present.

**Test for Steroids**

<table>
<thead>
<tr>
<th>Test name</th>
<th>Experimentation</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferric chloride test</td>
<td>5 ml of alcoholic extract solution + 1 ml 5% ferric chloride solution.</td>
<td>A blue, blue blackgreen or blue green colour or precipitation is produced in the presence of tannins. On addition of a few ml of dilute sulphuric acid the colour disappears followed by the formation of a yellowish brown ppt.</td>
</tr>
<tr>
<td>Lead acetate test</td>
<td>5 ml of an aqueous extract of the plant material + few drops of a 1% solution of lead acetate.</td>
<td>A yellow or red ppt is formed.</td>
</tr>
<tr>
<td>Potassium dichromate test</td>
<td>5 ml of alcoholic extract of plant material + 1 ml 10% potassium dichromate solution.</td>
<td>Yellow orange ppt indicates the presence of tannins.</td>
</tr>
</tbody>
</table>

**Test for Tannins**

<table>
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<tr>
<th>Test name</th>
<th>Experimentation</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using gentle heat, to dissolve a small amount of chloroformic or ethanolic extract of the plant material in 5 to 10 ml of acetic anhydride. Cool &amp; have to add 0.05 ml of sulphuric acid.</td>
<td>If resin are present, a bright purplish red colour,rapidly changing to violet,is produced.</td>
<td></td>
</tr>
</tbody>
</table>

**Test for Resins**

<table>
<thead>
<tr>
<th>Test name</th>
<th>Experimentation</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. Hel and alcoholic test</td>
<td>Small amount of alcoholic extract of the plant material + few drops of conc. Hel.</td>
<td>Immediate development of red color indicates the presence of flavonoids.</td>
</tr>
</tbody>
</table>

**Test name**
- Salkowski’s test
- Libermann-Burchard’s test
- Test for flavonoids
- Test for saponins

**Observation**
- Yellow orange ppt indicates the presence of alkaloids.
- A yellow or red ppt is formed.
- Dirty white or black precipitate indicates the presence of alkaloids.
- Production of a persistent frothing (which remains stable on heating) indicates the presence of saponins.

2.5. Statistical Analysis

The experimental results were expressed as the mean ± standard deviation (sd). Statistical significance of the mean mortality at each concentration was analyzed using one-way analysis of variance (anova) and compared using duncan’s multiple range tests. Values of p≤0.05 were taken to be statistically significant.

3. Results and Discussion

Denaturation of proteins is a well-documented cause of inflammation. Salicylic acid, phenylbutazone, flufenamic acid (anti-inflammatory drugs) etc, have shown dose dependent ability to thermally induced protein denaturation[6]. Ability of extracts to inhibit protein denaturation was studied to indentify mechanism of the anti-inflammatory activity. Both the extracts were effective in inhibiting heat induced albumin denaturation at different concentrations as shown in figure-1. In the present study for the in-vitro anti-inflammatory test, the crude methanolic extracts of mussaenda roxburghii hook. Showed mean inhibition of protein denaturation 17.399 ± 0.01937. Whereas, for asa it was found to be 42.491 ± 0.00698. The ability of methanol extract of leaves of m. Roxburghii to inhibit thermal and hypotonic protein denaturation was found to be less significant than the positive acetyl salicylic acid.

Addition of 100 ul of streptokinase, a positive control (1500000 i.u) to the clots along with 90 minutes of incubation at 37 ℃, exhibit 69.35% clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (6.23%). After treatment of clot with m. Roxburghii (mother methanolic extract), m. Roxburghii (dcm extract ,fraction 1), m. Roxburghii (ethyl acetate extract, fraction 2), m. Roxburghii ( residual methanolic extract, fraction 3), m. Roxburghii (methanolic extract of leaves) 30.56%,27.6096%,46.35%,26.02%,49.90% clot lysis were observed respectively and mean of percentage of clot lysis was more than water. Results are shown in figure-2.

The result obtained in the present investigation phytochemicals screening of the methanol extract of leaves of m. Roxburghii revealed that the crude extract contained...
flavonoids, saponins, steroids, tannins and triterpenoids, terpinoids, cardiac glycosides and anthraquinones (table-1). M. Roxburghii leaves can also have various medicinal values such as anti-inflammatory, membrane stabilizing and thrombolytic activity. The presence of flavonoids exhibit significance anticancer, cardioprotective, anti-microbial activity may demonstrate gram-positive, gram-negative as well as fungi also. Among of the microorganisms are resistance to several drugs each though, this is only a preliminary study of the occurrence of certain properties of m. Roxburghii leaves an in-depth study will provide a good concerted base of all the phytochemicals and several pharmacological functions mention above.

**Table 1. Chemical group test result for Mussaenda roxburghii leaves parts.**

<table>
<thead>
<tr>
<th>Examination</th>
<th>Test performed</th>
<th>Result for M. roxburghii leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Carbohydrate</td>
<td>1) Molisch’s test (test for Gum) (-) ve</td>
<td>2) Benedict test (test for Reducing sugar) (+) ve</td>
</tr>
<tr>
<td>2) Alkaloids</td>
<td>1) Mayers test (+) ve</td>
<td>2) Wagner’s test (+) ve</td>
</tr>
<tr>
<td>3) Tannins</td>
<td>1) Ferric chloride test (+) ve</td>
<td>2) Lead acetate (-) ve</td>
</tr>
<tr>
<td>4) Resins</td>
<td>1) Ferric chloride test (+) ve</td>
<td>3) Tannic acid test (-) ve</td>
</tr>
<tr>
<td>5) Flavonoids</td>
<td>1) Concentrated HCl &amp; Alcoholic test. (-) ve</td>
<td>2) Lead acetate (-) ve</td>
</tr>
<tr>
<td>6) Saponins</td>
<td>1) General test (-) ve</td>
<td>3) Potassium dichromate test (+) ve</td>
</tr>
<tr>
<td>7) Glycosides</td>
<td>1) General test (-) ve</td>
<td>2) Salkowski’s test (-) ve</td>
</tr>
<tr>
<td>8) Steroids</td>
<td>1) Liebermann Barchards test (-) ve</td>
<td>2) Salkowski’s test (-) ve</td>
</tr>
</tbody>
</table>

(+ = present and (-) = absence)

**Figure 1.** Mean inhibition of protein denaturation (%MIPD) and Mean protein denaturation (%MPD) VS treatment groups. Here, ASA = Acetyl salicylic acid, MEMR = Methanolic extract of Mussaenda roxburghii.
Figure 2. % clotlysis of different extracts of Mussaenda roxburghii (Leaves).

Here, MMEMR= Mother methanolic extract of *M. roxburghii*, DCM F1 MR= DCM extract (fraction 1) of *M. roxburghii*, EA F2 MR= Ethyl acetate extract (fraction2) of *M. roxburghii*, RME F3 MR= Residual methanolic extract (fraction3) of *M. roxburghii*.

4. Conclusion

In the present study, we have found that most of the biologically active phytochemicals were present in the methanolic extract of *M. Roxburghii* leaves. This is only a preliminary study and to make final comment the extract should thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

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References


