Antioxidative, Thrombolytic and Cytotoxic Potentials of Jatropha pandurifolia Stem bark and Syzygium reticulatum Leaf

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ABSTRACT: The study attempted to illustrate the antioxidant, thrombolytic and cytotoxic properties of two different medicinal plants, Jatropha pandurifolia and Syzygium reticulatum. In vitro antioxidative activity was tested through qualitative and quantitative DPPH scavenging assay which revealed the radical scavenging activity. Methanolic stem bark extract of Jatropha pandurifolia (MSJP) and ethyl acetate leaf extract of Syzygium reticulatum (EALSR) showed moderate clot rupture activity, the value of which were 61.30 ± 2.35% (**P<0.01) and 63.81 ± 1.92% (**P<0.01) accordingly, whereas streptokinase showed 73.6 ± 0.76% clot lysis capability. IC₅₀ value was 6.82 ± 0.99 µg/mL for butylated hydroxy toluene (BHT) wherein MSJP and EALSR showed 8.1± 1.44 (***P<0.001); and 10.34±2.12 (***P<0.01) µg/mL accordingly. In Brine shrimp cytotoxicity, MSJP, EALSR and vincristine sulphate exhibited mild activity with LC₅₀, the value of which were 5.73±2.85 (*P<0.05), 5.12±1.57 (*P<0.05) and 0.52 ± 0.18 µg/mL respectively. The results proved the presence of many bioactive compounds showing thrombolytic, antioxidative and cytotoxic activities.

Key words: Jatropha pandurifolia, Syzygium reticulatum, thrombolytic, antioxidative and cytotoxicity

INTRODUCTION

Medicinal plants utilized in conventional medication in different ailments through the course of advancement around the world are fascinating research subjects being a vast precursor of structurally multifaceted and versatile bioactive molecules. Thrombus or blood clot prevents blood perfusion by obstructing blood vessels causing a deficiency in blood flow and oxygen in target tissues that develop necrosis. The function of a thrombolytic drug is to dissolve thrombin in acutely occluded coronary arteries, pulmonary embolism (PE), atrial fibrillation, deep-vein thrombosis and mechanical prosthetic heart valves¹ thereby to ensure blood supply to myocardium to limit ischemia and to develop prognosis with minimum blood loss.² It is well noted that available thrombolytic agents in the market like streptokinase, urokinase, alteplase, anistreplase,³ tissue-type plasminogen activator offer some limitations such as partial fibrin specificity, side effects, having large dose and in some cases coupled bleeding tendency.⁴ Persistent research will offer novel insights and uphold evolution toward the devolution of the supreme thrombolytic therapy.⁵ There has been some evidence to recommend that free radicals and some reactive nitrogen species prompt and amplify oxidation that may proceed to cell death mechanisms like apoptosis and necrosis⁸ associated with other disorders including arthritis, Alzheimer’s disease, atherosclerosis, diabetes and cancer.⁹,10 Several reports suggest that antioxidant could decline oxidation as well as increase survival times.¹¹,¹² The brine shrimp lethality bioassay is

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simple, rapid (24 h), robust, inexpensive and easy technique. The bioassay is linked to have a good cytotoxic activity in carcinogenic tissue and represents some pesticidal activity. Brine shrimp lethality bioassay is based on the mortality activity of test compounds on a brine shrimp organism and was first proposed by Michael et al., and further modified later. 

_Jatropha pandurifolia_ or _J. integerrima_ Jacq. is an erect tiny, ornamental evergreen shrub having star-shaped red, pink, or vermilion blooms. In Asia, Latin America, and Africa it is also known as peregrine or spicy jatropha. For era different parts of this plant are utilized by folks. Phytochemicals namely alkaloid like jatrophine, jatrophan and curcin, and diterpene like jatrophone, jatrophantsone A–B were identified from this plant. Furthermore, sitosterol, glycoside, lignin, tannin, saponin fatty acids (palmitic, oleic, and linoleic acids), carotenoid, flavonoid etc were also reported. Pharmacological activities such as antinflammatory, antituberculosis, antiplasmodial, cytotoxicity, anticancer, antimicrobial, antifungal antioxidant potentials are revealed from different parts of the plant. Leaves, stem, bark, roots, and oil have long been used for a variety of diseases as styptic agent, emetic agent, antidote, purgative, moreover in warts, toothaches, ringworm, gastric emptying, rheumatic pain, gum bleeding, and skin diseases.

_Syzygium reticulatum_ (also known as Eugenia reticulata Wight) belongs to Myrtaceae family. This pant is widely distributed in Bangladesh (Sylhet), India (Assam, Meghalaya) and Myanmar. This is a medium-sized evergreen tree. Leaves are ovate-lanceolate with thick coriaceous nervation on both surfaces. It has drupe like fruit holding covered seeds. Fruits and flowering period is from March to June. Wood is used for handles of tools and for making arches.

For _J. pandurifolia_, a few biological profiling studies were examined, but no report for _S. reticulatum_ has yet been discovered. This study aims to examine the antioxidant, thrombolytic and cytotoxic properties of various parts of the two plants.

**MATERIALS AND METHODS**

**Collection and identification of the plant.** In August 2019, _J. pandurifolia_ stem barks were collected from botanical garden, University of Dhaka, Bangladesh and _Syzygium reticulatum_ was collected in 2022 from Lawachara, Sylhet. The plant components were recognized by a taxonomist (Ms. Nasrin Aktar), Bangladesh National Herbarium, where a voucher specimen was deposited for future reference (DACB Accession No. JP: 54445, SR: 65585)

**Preparation of plant samples.** The plant parts of both species were cleaned properly to remove dirt, shed dried and the air-dried parts were then crushed into coarse powder and the powder was preserved in a labeled airtight container with an identification label for further investigation. The powdered stem-barks of _J. pandurifolia_ (3.0 kg) and leaves of _S. reticulatum_ (2.0 kg) were soaked into ethyl acetate over the period of 15 days, filtered through a cotton plug followed by Whatman filter paper and finally concentrated using a Buchii rotary evaporator under minimal pressure.

**Solvents and reagents.** The solvents and reagents used in this investigation were analytical or laboratory grade. The organic solvents utilized in the tests were methanol, ethyl acetate and DMSO, which were distilled before use. Additionally, DPPH, streptokinase, butylated hydroxytoluene (BHT), vincristine sulphate and saline water were needed for the experiments.

**a. Thrombolytic activity**

5 mL of blood were taken in different pre weighed sterile vials each containing 1 mL of blood. Tubes were incubated at 37°C for 45 minutes, allowed to be clotted and weighed again. After clot formation, the clear portion was removed carefully. Clot weight was measured subtracting the previous two weights. 100 μL test samples (1 mg/100 μL water) were added separately to each clot containing vial. 100μL streptokinase (30,000 I.U) and 100 μL...
distilled water were used as positive control and negative control, respectively. After incubation at 37°C for 90 minutes clot lysis was observed. The released clear plasma was removed and vials were again weighed to calculate the weight difference. The following calculation was used to measure clot lysis percentage.

\[
\% \text{ of thrombolytic activity} = \left( \frac{\text{wt. of clot after treatment}}{\text{wt. of clot before treatment}} \right) \times 100
\]

b. Antioxidant activity

**Qualitative antioxidant activity test.** The plant extract was diluted in a suitable solvent and spotted on a TLC plate. A suitable mobile phase was prepared with different polarities. n-Hexane:ethyl acetate (2:1) as a non-polar solvent, CHCl₃:CH₂OH (5:1) as a medium polar solvent and CHCl₃:CH₂OH:H₂O (40:10:1) as a polar solvent were used. 0.02% w/v DPPH in methanol was used as a detector of antioxidative components.

**DPPH free radical scavenging assay.** 2 mL of the different concentrations (500, 250, 125, 62.5, 31.25, 15.63, 7.83, 3.91, 1.96 and 0.98 µg/mL) of the test samples were mixed with 2 mL of DPPH solution (20 µg/mL methanol), incubated (30 mins, 25°C in dark place) for afterward UV absorbance measurement at 517 nm. IC₅₀ values were calculated from the regression equation by plotting concentrations vs percentage of scavenging the free radicals. Butylated hydroxyl toluene (BHT) and methanolic DPPH solution were used as positive control and negative control, accordingly.

\[
I\% = 1 - \left\{ \frac{A_0}{A_1} \right\} \times 100
\]

Where A₀ is the absorbance of the control, and A₁ is the absorbance of test samples or standard. The experiment was repeated in triplicate at each concentration.

c. Brine shrimp lethality bioassay

The brine shrimp lethality bioassay was used to predict the cytotoxic activity of pure compounds in 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.83, 3.91, 1.96 and 0.98µg/ml using serial dilution technique. The solutions of different concentrations were then added to 10 live nauplii in 5 mL simulated seawater into different vials. The vials were examined after 24 h using a magnifying glass and the number of existing live nauplii in each vial was counted. The percentage of mortality was calculated and plotted against LogC. Then lethal concentrations (LC₅₀) were calculated to measure cytotoxic activity. Saline water and vincristine sulphate (VS) were used as negative control and positive control, accordingly.

\[
\% \text{ mortality} = \left( \frac{\text{no. of dead nauplii}}{\text{initial total no. of live nauplii}} \right) \times 100
\]

RESULTS AND DISCUSSION

a. Thrombolytic activity. In this study MSJP and EALSR showed moderate clot rupture activity valued as 61.30 ± 2.35% (**P<0.01) and 63.81 ± 1.92% (**P<0.01) accordingly wherein standard Streptokinase showed 73.6 ± 0.76% thrombus lysis capabilities. In this study, clot lysis activity of the crudes was evaluated as a part of revelation of cardioprotective drugs which was observed as the highest percentage of clot lysis capability. It is noted that numerous secondary metabolites such as flavonoids, alkaloids, tannins, saponins show thrombolytic properties as a natural reservoir. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>% of clot lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSJP</td>
<td>61.30± 2.35</td>
</tr>
<tr>
<td>EALSR</td>
<td>63.81± 1.92</td>
</tr>
<tr>
<td>Streptokinase (STK)</td>
<td>73.6 ± 0.76</td>
</tr>
<tr>
<td>Negative control (water)</td>
<td>5.03± 0.70</td>
</tr>
</tbody>
</table>

(Values are expressed as mean ± SD, n = 3; Significance level among different groups at P ≤0.05 (*P<0.05; **P<0.01, ***P<0.001); Test groups were compared to standard group; MSJP: Methanolic stem bark extract of J. pandurifolia; EALSR: Ethyl acetate leaf extract of S. reticulatum)

b. Antioxidant activity. In the qualitative antioxidant activity test, on TLC plates dense yellow spots were observed under UV spectroscopy which indicates the abundance of antioxidative compounds.
Reactive oxygen species (ROS) and other oxidants have been linked to a number of ailments and diseases, according to a large body of research. Free radical reactions have a well-established function in disease pathophysiology and are known to contribute to a wide range of both acute and chronic human illnesses, including diabetes, atherosclerosis, aging, immunosuppression, and neurodegeneration. The body's natural antioxidant capacity and ROS levels were out of balance, which led to the requirement of dietary and/or pharmacological supplementation, especially during a disease assault. Commonly superoxide dismutase, catalase enzymes or glutathione transferase role models having antioxidant activity in most medicinal plants in addition to phytochemicals like polyphenolic, proanthocyanidins, flavonoids, tannins, tocopherols etc.

Table 2. Antioxidant activity of MSJP and EALSR.

<table>
<thead>
<tr>
<th>Conc. (µg/ ml)</th>
<th>% of Scavenging</th>
<th>Free radical scavenging activity (IC₅₀ in µg/ ml)</th>
<th>Butylated hydroxy toluene (BHT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSJP</td>
<td>EALSR</td>
<td>MSJP</td>
<td>EALSR</td>
</tr>
<tr>
<td>1000</td>
<td>75.67</td>
<td>82.45</td>
<td>8.1***±1.44</td>
</tr>
<tr>
<td>500</td>
<td>71.18</td>
<td>69.85</td>
<td>100</td>
</tr>
<tr>
<td>250</td>
<td>68.6</td>
<td>82.39</td>
<td>12.5</td>
</tr>
<tr>
<td>125</td>
<td>65.97</td>
<td>83.28</td>
<td>6.25</td>
</tr>
<tr>
<td>62.5</td>
<td>61.37</td>
<td>79.1</td>
<td>5.82</td>
</tr>
<tr>
<td>31.25</td>
<td>58.69</td>
<td>75.43</td>
<td>3.125</td>
</tr>
<tr>
<td>15.63</td>
<td>55.82</td>
<td>90.3</td>
<td>1.5625</td>
</tr>
<tr>
<td>7.813</td>
<td>53.28</td>
<td>60.3</td>
<td>0.78125</td>
</tr>
<tr>
<td>3.91</td>
<td>49.4</td>
<td>48.51</td>
<td>41.8</td>
</tr>
<tr>
<td>1.96</td>
<td>30.93</td>
<td>41.8</td>
<td>38.88</td>
</tr>
<tr>
<td>0.98</td>
<td>14.01</td>
<td>38.88</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, n = 3; Significance level among different groups at P ≤0.05 (*P<0.05; **P<0.01, ***P<0.001); Test groups were compared to standard group; MSJP: Methanolic stem bark extract of J. pandurifolia; EALSR: Ethyl acetate leaf extract of S. reticulatum.

The present study expressed half maximal inhibitory concentration i.e IC₅₀ : 6.82± 0.99 µg/mL for butylated hydroxy toluene (BHT) wherein MSJP and EALSR exerted strong to moderate DPPH scavenging property with IC₅₀: 8.1± 1.44 (**P<0.001); and 10.34±2.12 (***P<0.01) µg/mL accordingly reporting noteworthy scavenging activity indicating the abundance of relevant phytochemicals. The results are presented in Table 2.

c. Cytotoxic activity. Brine shrimp lethality bioassay is a fast and quick method used to calculate the potential of any cytotoxic compound that could be lethal to living organism. The bioassay has a strong association with both pesticidal activity and cytotoxic activity in various human solid tumors. MSJP and EALSR exhibited mild lethality activity with LC₅₀ value 5.73±2.85(*P<0.05) and 5.12±1.57 (*P<0.05) µg/ mL, respectively whereas vincristine sulphate showed LC₅₀: 0.52 ± 0.18 (µg/mL). The findings demonstrate that both plants' crude extracts contain a large number of phytochemicals with cytotoxic activity. The results are projected in Table 3.
Table 3. Cytotoxic activity of MSJP & EALSR.

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Log C</th>
<th>% of mortality</th>
<th>LC₅₀ (µg/ml)</th>
<th>Vincristine Sulphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSJP</td>
<td>EALSR</td>
<td>MSJP</td>
<td>EALSR</td>
</tr>
<tr>
<td>1000</td>
<td>3</td>
<td>100</td>
<td>5.73±2.85</td>
<td>5.12±1.57</td>
</tr>
<tr>
<td>500</td>
<td>2.699</td>
<td>90</td>
<td>20</td>
<td>1.602</td>
</tr>
<tr>
<td>250</td>
<td>2.398</td>
<td>80</td>
<td>10</td>
<td>1.000</td>
</tr>
<tr>
<td>125</td>
<td>2.097</td>
<td>60</td>
<td>5</td>
<td>0.699</td>
</tr>
<tr>
<td>62.5</td>
<td>1.796</td>
<td>50</td>
<td>2.50</td>
<td>0.398</td>
</tr>
<tr>
<td>31.25</td>
<td>1.495</td>
<td>40</td>
<td>1.25</td>
<td>0.097</td>
</tr>
<tr>
<td>15.63</td>
<td>1.194</td>
<td>30</td>
<td>0.63</td>
<td>−0.201</td>
</tr>
<tr>
<td>7.813</td>
<td>0.893</td>
<td>30</td>
<td>0.31</td>
<td>−0.509</td>
</tr>
<tr>
<td>3.91</td>
<td>0.591</td>
<td>20</td>
<td>0.16</td>
<td>−0.796</td>
</tr>
<tr>
<td>1.96</td>
<td>0.292</td>
<td>20</td>
<td>0.078</td>
<td>−1.108</td>
</tr>
<tr>
<td>0.98</td>
<td>-0.01</td>
<td>10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Values are expressed as mean ± SD, n = 3. Significance level among different groups at P ≤0.05 (*P<0.05; **P<0.01, ***P<0.001); Test groups were compared to standard group; MSJP: Methanolic stem bark extract of J. pandurifolia; EALSR: Ethyl acetate leaf extract of S. retilulatum)

CONCLUSION

The outcomes recovered in this research confirmed the conventional curative uses of these plants in different ailments for treating different diseases. The thrombolytic, antioxidant and cytotoxic activities were unveiled that would be noteworthy analysis of concealed ethno-pharmacological effectiveness to ascertain numerous persuasive bioactive molecules.

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REFERENCES


