Revealing the Cytotoxic Potential of Medicinal Folklore: Bombax Ceiba L.

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REVEALING THE CYTOTOXIC POTENTIAL OF MEDICINAL FOLKLORE:

BOMBAX CEIBA L.

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ABSTRACT

Current study was intended to investigate the cytotoxic potential of Bombax ceiba L. by executing a wide range of in vitro assays. Brine shrimp lethality assay showed that stem wood’s chloroform (CH) extract was highly toxic (LC₅₀ 42.41 ± 3.40 µg/ml) followed by acetone : ethyl acetate (AC:EA) extract with LC₅₀ value of 72.92 ± 4.41 µg/ml. n-Hexane (NH) extract of root wood part caused 70.95 ± 1.77% inhibition of THP-1 cell line at 20 µg/ml concentration (IC₅₀ 15 ± 0.56 µg/ml). NH and CH extracts of stem wood part showed promising results in protein kinase inhibitory assay with 21 ± 1.2 and 29 ± 1 mm bald ZOI (MIC= 50 µg/disc) respectively. The present findings revealed that CH and NH extracts of stem wood as well as root wood part as an enriched source of phytochemicals possessing cytotoxic potential.

Key Words: Bombax ceiba L., Cytotoxic, Folklore.

INTRODUCTION

Plants are nature’s gifts that are being widely to cure various disorders either in the form of conventional preparations or as pure active compounds (Auddy et al., 2003). People are getting attracted towards herbal products after intimating the side effects of synthetic drugs (Verma et al., 2008). Cancer has become the second major cause of death in the human beings after cardiovascular diseases. (Ismail et al., 2012). Although, allopathic medicine system has been significantly progressed, the alternative system of medicine is still being practiced in developing countries. Because, these traditional medicinal plants contain a reservoir of secondary metabolites possessing cytotoxic potential (Thomford et al., 2015). However, credibility for therapeutic potential of traditionally used plants; demands scientifically valid data. Therefore, current research is intended to investigate the therapeutic significance of folklore plant abundantly found in Pakistan i.e. Bombax ceiba L. Bombax” (Greek word; silkworm) “ceiba” (silk cotton tree) is a medicinal plant locally called “Sumbal” in Pakistan (Family Bombacaceae). It is a fast growing, strong tree which attains about 40 m of height (Gupta, 2012; Sint et al., 2013). In December, it sheds all leaves and in January it blossoms into bright crimson flowers. It grows best in valleys and in regions where rainfall is about 60 to 450 cm
annually (Rameshwar, 2014). The *B. ceiba* L. is a tropical and subtropical tree native to South and East Asia, Indian subcontinent, western Africa and Northern Australia. It is naturally distributed in Myanmar, Pakistan, India, Taiwan, China, Moluccas, Philippine, Java, Borneo, Sulawesi, Lesser Sunda Island, and New Guinea (Sint et al., 2013). Undertaken study aims to evaluate the phytochemical profile and antioxidant potential of *Bombax ceiba* L.

**MATERIAL AND METHODS**

**Preparation of Extracts**

Plant was collected and sorted to remove unwanted substances, rinsed with tap water and shade dried. The dried parts were pulverized separately by commercial miller to coarse powder. The sonication aided maceration technique was employed for extraction by using fourteen different solvents either alone or 1:1 combination. The accurately weighed plant powder (50 g) was soaked in 200 ml solvent using Erlenmeyer flask at room temperature for 72 hrs with frequent agitation on ultrasonic bath (temperature 25°C, frequency 25 kHz). After 3 days, plant material was strained by muslin cloth and filtered. Finally, filtrates were concentrated (at room temperature) and dried in vacuum oven (Mermant, Germany). The crude extracts were then stored at -20°C. The different solvents employed for extraction process included; n-hexane (NH), chloroform (CH), ethyl acetate (EA), chloroform : methanol (CH:M), chloroform : ethanol (CH:E), acetone : ethyl acetate (AC:EA), methanol : ethyl acetate (M:EA), ethanol : ethyl acetate (E:EA), acetone (AC), methanol (M), ethanol (E), acetone : distilled water (AC:W), methanol : distilled water (M:W) and distilled water (W).

**Cytotoxicity assays**

**Brine shrimp lethality assay**

Already reported protocol was adopted to assess preliminary toxicity of test extracts (Nasir et al. 2017).

**Antiproliferative activity against THP-1 human leukemia cell line**

The *in vitro* antiproliferative effect of the test samples was measured by adopting the formerly revealed protocol (Ahmed et al., 2017).

**Protein kinase inhibition assay**

The protein kinase inhibitory potential of test samples was detected by utilizing the strain of *Streptomyces 85E* (Nasir et al. 2017). The results were evaluated by measuring the bald and clear zones of inhibition (mm).

**RESULTS AND DISCUSSION**

It is estimated that the worldwide mortality rate due to cancer will cross 10 million by 2020 (Soliman et al., 2013). Therefore, it is highly imperative to discover new bioactive leads with greater safety and efficacy. The brine shrimp lethality assay is a robust method for the assessment of toxicity of natural products (Hamidi et al., 2014). The results showed that stem wood’s CH extract was found to be highly toxic (LC₅₀ 42.41 ± 3.40 µg/ml) followed by AC:EA extract with LC₅₀ value of 72.92 ± 4.41 µg/ml. However moderate toxicity was found in root wood’s CH extract with an LC₅₀ value
of 140.6 ± 5.10 µg/ml followed by NH extract (LC₅₀ 170.6 ± 5.76 µg/ml). The other extracts showed either minimal toxicity (with LC₅₀ values > 200 µg/ml) or no significant activity. LC₅₀ of Doxorubicin (positive control) was 3.48 ± 0.35 µg/ml. Potent activity was found in non-polar (NH) and moderately polar (AC:EA) extracts which can provide a reservoir of bioactive leads possessing anticancer potential. Islam et al. (2011) revealed the cytotoxic potential of aqueous extract of Bombax ceiba L. but current study employed a wide range of solvents to extract the phytoconstituednts of different polarities. Therefore during undertaken study, multiple extracts of Bombax ceiba L. were subjected for evaluation of cytotoxic potential.

Among all extracts the NH extract of root wood part caused 70.95 ± 1.77% inhibition of THP-1 cell line at 20 µg/ml concentration (IC₅₀ 15 ± 0.56 µg/ml). The 5-fluorouracil and vincristine were used as standards with LC₅₀ values of 5.2 ± 0.23 and 8.10 ± 0.30 µg/ml respectively. It can be related to the brine shrimp assay where the cytotoxic activity was stronger in the non-polar extracts as compared to the polar ones. The information can be further utilized to isolate the pharmacologically active phytoconstituents. An anti-angiogenic activity on in vitro tube formation of human umbilical venous endothelial cells (HUVEC) has been reported by methanol extract of stem bark part of B. ceiba whereas no inhibition was seen against tumor cell lines such as SK-MEL-2, B16-F10 and A549 (You et al., 2003). In this assay Streptomyces 85E strain was used that closely relate with their eukaryotic counterparts thus readily identifying cytotoxic potential of samples by inhibition of the hyphae formation (due to protein kinase inhibitory activity) (Fatima et al., 2015). The protein kinase inhibitory assay also showed promising results (as given in Table 1) from stem wood’s NH and CH extracts with 21 ± 1.2 and 29 ± 1 mm bald ZOI (MIC= 50 µg/disc) respectively. Root wood’s NH and CH extracts were also found to be active at MIC 100 µg/disc. Significant bald zones were observed in different extracts of B. ceiba L. This highlights the presence of important protein kinase inhibitory compounds which can be further explored via isolation and characterization studies. Surfactin was used as a standard (ZOI 20 ± 1.02 mm at 10 /disc) while DMSO was used as a negative control. Protein kinase inhibitory potential of B. ceiba is reported for the first time which can be further exploited for the drug discovery.

**CONCLUSION**

The present findings revealed that CH and NH extracts of stem wood as well as root wood part as an enriched source of phytochemicals possessing cytotoxic potential. Bioguided isolation should be carried out to isolate the bioactive leads. Plant based green synthesis of nanoparticles is recommended as revealed by Nazli et al., (2018).
Table 1: Protein kinase inhibition potential of different parts of *B. ceiba* L. crude extracts.

<table>
<thead>
<tr>
<th>Extract name</th>
<th>Flower Bald zone (MIC)</th>
<th>Leaf Bald zone (MIC)</th>
<th>Stem bark Bald zone (MIC)</th>
<th>Stem wood Bald zone (MIC)</th>
<th>Root bark Bald zone (MIC)</th>
<th>Root wood Bald zone (MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH</td>
<td>13 ± 0.0</td>
<td>25</td>
<td>11 ± 1.1^b^ 100</td>
<td>21 ± 1.2^b^ 50</td>
<td>11 ± 1.5^b^ 100</td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>25 ± 1.0^a^</td>
<td>25</td>
<td>10 ± 1.2 100</td>
<td>29 ± 1.0^a^ 50</td>
<td>10 ± 1.3^c^ 100</td>
<td></td>
</tr>
<tr>
<td>EA</td>
<td>12 ± 1.2</td>
<td>100</td>
<td>---</td>
<td>---</td>
<td>12 ± 1.4^b^ 100</td>
<td></td>
</tr>
<tr>
<td>CH:M</td>
<td>21 ± 1.0^b^</td>
<td>50</td>
<td>---</td>
<td>15 ± 1.5^c^ 50</td>
<td>---</td>
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<tr>
<td>CH:E</td>
<td>19 ± 1.0^b^</td>
<td>25</td>
<td>---</td>
<td>21 ± 1.3^b^ 50</td>
<td>6 ± 1.2</td>
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<tr>
<td>AC:EA</td>
<td>---</td>
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<td>---</td>
<td>22 ± 1.2^b^ 50</td>
<td>7 ± 1.4</td>
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<tr>
<td>EA:M</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>17 ± 1.0^c^ 50</td>
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<tr>
<td>E:EA</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>18 ± 0.8^c^ 50</td>
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<tr>
<td>AC</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>15 ± 0.5^c^ 50</td>
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<tr>
<td>M</td>
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</tr>
<tr>
<td>E</td>
<td>11 ± 1.5</td>
<td>100</td>
<td>---</td>
<td>24 ± 1.5^b^ 50</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>AC:W</td>
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<tr>
<td>M:W</td>
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<td>W</td>
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<tr>
<td>Surfactin</td>
<td>20 ± 1.0^b^</td>
<td>20 ± 1.0^a^</td>
<td>20 ± 1.0</td>
<td>20 ± 1.0^b^</td>
<td>20 ± 1.0^a^</td>
<td></td>
</tr>
<tr>
<td>DMSO</td>
<td>---</td>
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</table>

Values are presented as mean ± Standard deviation (*n*= 3). The values with different superscript (^a-c^) letters show significantly (P < 0.05) different means. DMSO: negative control; Surfactin: positive control (20 µg/disc); -- = No activity
Jan et al.: Cytotoxic Potential of *Bombax ceiba* L.  

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