

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

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Recommended Citation

Tahsin Ullah Jan, H., Ali, S., Shabbir, A., Nasir, B., & Zahra, S. (2017). Revealing the Cytotoxic Potential of Medicinal Folklore: *Bombax Ceiba* L., *Journal of Bioresource Management*, 4 (3).

DOI: 10.35691/JBM.7102.0076

ISSN: 2309-3854 online

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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 $\mu\text{g/ml}$ followed by NH extract (LC_{50} 170.6 ± 5.76 $\mu\text{g/ml}$). The other extracts showed either minimal toxicity (with LC_{50} values > 200 $\mu\text{g/ml}$) or no significant activity. LC_{50} of Doxorubicin (positive control) was 3.48 ± 0.35 $\mu\text{g/ml}$. 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 phytoconstitutednts 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 $70.95 \pm 1.77\%$ inhibition of THP-1 cell line at 20 $\mu\text{g/ml}$ concentration (IC_{50} 15 ± 0.56 $\mu\text{g/ml}$). The 5-fluorouracil and vincristine were used as standards with LC_{50} values of 5.2 ± 0.23 and 8.10 ± 0.30 $\mu\text{g/ml}$ 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 $\mu\text{g/disc}$) respectively. Root wood's NH and CH extracts were also found to be active at MIC 100 $\mu\text{g/disc}$. 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.

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

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

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