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In-vitro Anti-Inflammatory Evaluation of Crude *Bombax ceiba* Extracts

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KA and JAJS designed the study. Author KA develop the protocol and wrote the first and second drafts of the manuscript. Authors KA and JAJS performed the experimental works. Authors TVA, RA and SK involved in the collection of literature, interpret the results and performed the statistical analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The present work was decided to evaluate the *in-vitro* anti-inflammatory activity of crude extracts of *Bombax ceiba* in order to characterize the role of this extract in affecting the inflammatory process.
Study Design: Extraction of *B. ceiba* bark, phytochemical screening, and evaluation of *in-vitro* anti-inflammatory activity.
Methodology: Petroleum ether, ethanol and aqueous extracts of *B. ceiba* barks were prepared by maceration technique and subjected to preliminary phytochemical tests. The *in-vitro* anti-inflammatory activity of all extracts (1000 mcg/ml) was assessed by HRBC membrane stabilization method.
Results: Ethanol extract showed significant (p<0.001) response followed by aqueous extract (p<0.01) when compared with standard, diclofenac potassium (50 mcg/ml).
Conclusion: The study suggests that the extracts possess enough potential to reduce inflammation by *in-vitro* and directs the importance of further research and development of novel anti-inflammatory agents.

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Keywords: In-vitro anti-inflammatory; Bombax ceiba; extracts; HRBC membrane stabilization.

1. INTRODUCTION

Plants have contributed lot of medicinal compounds being used today to treat diseases like cancer, hormonal imbalances, jaundice, diabetes, inflammation etc. Medicinal plants are very commonly available in abundance especially in the tropics. They are the vital sources of wide variety of chemicals from which novel anti-inflammatory agents can be discovered [1].

Bombax ceiba is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. It is one of the important medicinal plants in tropical and subtropical India and also occurs in Sri Lanka, Pakistan, Bangladesh, Myanmar, Malaysia, Java, Sumatra and Northern Australia. It has number of traditional uses and its medicinal usage has been reported in the Indian traditional systems of medicine such as Ayurveda, Siddha and Unani [2]. The various parts of *B. ceiba* have been reported for hypotensive and hypoglycaemic [3] antiangiogenic [4], analgesic [5], antiulcer [6], antioxidant [7], hepatoprotective [2] and antimicrobial [8] activities. Also it was used for the treatment of sexual debility [9], bleeding wounds [10] and vaginal infections [11]. Since there is no scientific report for anti-inflammatory effect of *B. ceiba* bark extract, the present study was an attempt to evaluate the anti-inflammatory activity.

2. EXPERIMENTAL DETAILS

2.1 Plant Material

The barks of *Bombax ceiba* Linn were collected from Jalan Tapah, Bidor, Perak, Malaysia in the month of July 2012. It was authenticated by Dr. J. Anbu Jeba Sunilson, Pharmacognosist, KPJ University College, Nilai, Negeri Sembilan, Malaysia. A voucher specimen (KPJUC/HA/B001) was deposited in the departmental herbarium.

2.2 Preparation of Extract

The collected barks were shade-dried at room temperature. The dried barks were size reduced to coarse powder (1500 g) and divided into three equal portions. Then the powdered *B. ceiba* was macerated with petroleum ether (pet. ether), alcohol and distilled water separately for six days [12]. The extracts of *B. ceiba* barks were collected separately and filtered using Whatman filter paper. All the extracts were concentrated and the excessive solvents were evaporated using rotary vacuum evaporator under vacuum. The colour, consistency and percentage yield were tabulated in Table 1. All the extracts were kept in desiccators until further use.

2.3 Preliminary Phytochemical Analysis

The preliminary phytochemical studies on the extracts of *B. ceiba* bark were conducted as per the standard procedures [13, 14] to find out the presence of various phytoconstituents and depicted in Table 2.

2.4 Screening of *In-vitro* Anti-Inflammatory Activity

In-vitro anti-inflammatory activity of extracts of *B. ceiba* was assessed by Human Red Blood Corpuscles (HRBC) membrane stabilizing method [15, 16] with slight modifications. The blood was collected from healthy human volunteer who had not taken any anti-inflammatory drugs for 2 weeks prior to the experiment and transferred to the heparinized centrifuge tubes and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension in normal saline was made. Diclofenac potassium (50 mcg/ml) was used as standard. The reaction mixture (4-5 ml) consisted 2 ml of hypotonic saline (0.25% w/v NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4), 1 ml of test solution (1000 mcg/ml) in normal saline and 0.5 ml of 10% HRBC in normal saline. For control, 1 ml of isotonic saline was used instead of test solution. The mixtures were incubated at 56ºC for 30 min. and cooled at running tap water, centrifuge at 3000 rpm for 20 min. The absorbance of supernatant was read at 560 nm using visible Spectrophotometer. The experiment was performed in triplicates. The control represents 100% lyses. The results were depicted in Table 3. The Percentage membrane stabilization was calculated (Padmanabhan and Jangle, 2012) using the following formula.

$$\% \text{ Inhibition of haemolysis} = 100 \times \frac{[\text{Absorbance of control} - \text{Absorbance of test}]}{\text{Absorbance of control}}$$

2.5 Statistical Analysis

The values were represented as mean ± S.E.M. and the data obtained from this study was subjected to one-way analysis of variance (ANOVA) followed students "t" test. The values of $^{aaa}p<0.001$, $^{aa}p<0.01$, $^{a}p<0.05$ were considered to indicate the significant levels.

3. RESULTS AND DISCUSSION

The colour, consistency and percentage yield values of the aqueous, ethanol and pet. ether extracts of *B. ceiba* were noted (Table 1). Aqueous extract of *B. ceiba* had the highest percentage yield followed by ethanol extract and pet. ether extract (Table 1). This might be due to the presence of high content of secondary metabolites which may be soluble in high polarity solvents.

Table 1. Colour, consistency and % yield of the various extracts of *Bombax ceiba* bark

Extracts	Color	Consistency	% yield
Petroleum ether	Yellowish brown	Solid	9 %
Ethanol	Light brown	Solid	16 %
Aqueous	Dark brown	Semi solid	22 %

The preliminary phytochemical investigation on *B. ceiba* extracts revealed that the presence of various secondary metabolites such as carbohydrates, flavones and flavanones, tannins and phenolic compounds, saponins, sterols, and triterpenoids in all the extracts (Table 2).

Table 2. Preliminary phytochemical studies of *Bombax ceiba* bark extracts

Chemical constituents	Pet. ether extract	Ethanol extract	Aqueous extract
Alkaloids	-	-	-
Carbohydrates and glycosides	+	+	+
Fixed oils and fats	-	-	-
Flavones and flavanones	+	+	+
Gums and mucilages	-	-	-
Tannins and phenolic compounds	-	+	+
Proteins	-	+	+
Saponins	+	-	-
Sterols	-	+	-
Triterpenoids	-	+	+

+ indicates present, - indicates absent

The *in-vitro* antti-inflammatory activity of *B. ceiba* barks extracts was assessed by HRBC membrane stabilizing method (Table 3). All the tested extracts of *B. ceiba* at the concentration of 1000 mcg/ml exhibited varying degree of anti-inflammatory activity as compare to that of Diclofenac potassium.

Table 3. *In-vitro* anti-inflammatory activity of various extracts of *B. ceiba* bark

Treatment	Absorbance	% Inhibition
Control	0.54 ± 0.42	-
Petroleum ether extract	0.42 ± 0.26^{a}	22.22
Ethanol extract	0.20 ± 0.16^{aaa}	62.96
Aqueous extract	0.29 ± 0.18^{aa}	46.30
Diclofenac potassium	0.14 ± 0.08^{aaa}	74.07

Mean ± S.E.M = Mean values ± Standard error of means of three experiments. $^{aaa}P< 0.001$, $^{aa}P< 0.01$, $^{a}P< 0.05$ *compared to control group.*

All the data (Mean±SEM) were evaluated by Student's "t" test and the probability was determined for all the extracts.

The lysosomal enzymes released during inflammation produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The non steroidal anti-inflammatory drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since HRBC membrane are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of the extracts [16].

In this present study, the results have found that ethanol extract showed highly significant ($p<0.001$) anti-inflammatory activity than aqueous extract ($p<0.01$) followed by petroleum ether extract ($p<0.05$). The findings of this study exemplified that the significant anti-inflammatory activity of *B. ceiba* extracts is due to the presence of above mentioned chemical constituents in the extracts. The earlier studies reported that the presence of tannins [17], flavanoids [18], triterpenoids [19] and phenolic compounds [20] in extracts are responsible for inflammatory activity.

4. CONCLUSION

The present *in-vitro* study is a preliminary evaluation of anti-inflammatory activity of *B. ceiba* and demonstrated that folk medicine of *B. ceiba* can be used to cure the inflammation. Further research work to analyze *in-vivo* anti-inflammatory activity of *B. ceiba* on animal models and to isolate the phytoconstituents responsible for anti-inflammatory activity are ongoing. Isolation of the respective phytoconstituents from *B. ceiba* directs to investigate the possible mechanism of action at cellular level which may become a useful approach to develop natural bioactive products. The present study suggests that *B. ceiba* would serve as a source for the discovery of novel anti-inflammatory agents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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