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Research Article

# ANTIOXIDANT AND ANTIHEMOLYTIC ACTIVITIES OF BOMBAX CEIBA PENTANDRA SPIKE AND FRUIT EXTRACTS

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**ABSTRACT**

*Bombax ceiba pentandra* belongs to the family *Malvaceae*, is being exploited largely for its wide therapeutic applications in various tribal communities around the world. Present study investigated antioxidant and antihemolytic activities of spike and young fruit extracts of *Ceiba pentandra*. Aqueous, methanol, chloroform and ethyl acetate extracts of spikes and fruits were used for the study. Antioxidant competence of the four extracts was assessed by DPPH and TBARS method. Antihemolytic assay was determined using erythrocytes model and the extent of membrane damage was determined by quantifying Malondialdehyde. Among all the four extracts studied, methanolic extract exhibited significant antioxidant and antihemolytic activities.

**Keywords:** Bombax ceiba pentandra, Antioxidant, Antihemolytic, Erythrocytes, Malondialdehyde.

## INTRODUCTION

Free radicals have been implicated in the etiology of several degenerative disorders including cancer, diabetes, rheumatoid arthritis, atherosclerosis, liver cirrhosis, Alzheimer's disease and other neurodegenerative disorders[1]. Antioxidants, the compounds that can scavenge free radicals play a significant role as they prevent damage of cell proteins, lipids, carbohydrates, nucleic acids as well as biomembranes caused by reactive oxygen species[2-3].

Hemolysis is caused due to the destruction of erythrocytes membrane causing the release of hemoglobin and other internal components into the surrounding fluid. Due to the preponderance of polyunsaturated fatty acids in the erythrocyte membranes, they are highly susceptible to oxidative damage whose consequences are lipid peroxidation and hemolysis.

Plants are known to be the potential sources of natural antioxidants. With the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very interesting as they may serve as promising sources with the novel mechanism of action[4-6]. In an effort to screen for the active compounds a widely used medicinal plant *Bombax ceiba pentandra*, commonly known as red silk cotton tree has been selected.

In various tribal communities around the world, the stem bark of the tree is being used for relieving fever, asthma and edema. Spike and young fruits of the tree are used in the treatment of snake bite as well as in inflammatory diseases[7]. B. Ceiba pentandra leaves extract showed antipyretic and antihypertensive activities[8]. Toxicological studies proved that B. ceiba pentandra has very low toxicity profile in all the tested animals and it is safe for oral medication[9]. We here in report the proximate analysis for various components, antioxidant as well as antihemolytic activities of spike and young fruit extracts of B. ceiba pentandra.

## MATERIALS AND METHODS

### Plant material

Bombax ceiba pentandra young fruits and spikes were collected from herbal garden maintained by JSS College, Mysore, Karnataka, India in the month of January and authenticated from the Department of Botany, University of Mysore, Karnataka, India.

### Preparation of plant material

Fresh spikes and young fruits were collected and dried at room temperature. The dried samples were powdered separately. 100gm each of the samples were extracted separately with different solvents starting with non polar to polar solvents in the order of hexane, ethyl acetate, methanol and water. The crude residues were obtained by removing the solvents in rotary evaporator and each of the extracts were resuspended in the respective solvents for further study.

### Proximate analysis of the solvent extracts

The proximate composition of spikes and fruits extracts were carried out to determine the content of ascorbic acid, tannins, saponins, glycosides, proteins, total phenols, flavanoids as well as alkaloids. The protein content was estimated by Bradford method[10]. Total phenolic content was estimated using Folin–Ciocalteue reagent[11]. Flavanoids were estimated following the method of Woiskey and Salatino[12]. Ascorbic acid content was estimated using DNPH reagent[13]. Qualitative analysis of tannins, alkaloids, Saponins and glycosides were performed for the different extracts[14].

### Determination of Antioxidant activity

### DPPH radical scavenging assay

1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity was determined as per the method of Habila[15]. 0.1mL of the solvent extracts were taken in different test tubes and volume in each of the test tube was made up to 100µL using methanol. 3mL of 0.1mM DPPH in methanol was added to each of the test tube and the mixture was shaken vigorously and allowed to stand for 20 minutes. Absorbance of the solutions were measured at 517ηm using spectrophotometer (Shimadzu UV-2550). Ascorbic acid (0.1mg/mL) was used as control for the assay.

### Nitric oxide radical scavenging assay

Nitric oxide radical scavenging assay was conducted as per the method of Marcocci[16]. 0.1mL of the plant extracts were added to the reaction mixture containing 2.5mL of sodium nitroprusside (10mM), 0.5mL of phosphate buffered saline (pH-7.4). The reaction mixture was incubated at room temperature for 150 minutes and 1mL of Griess reagent was added to all the test tubes. The reaction mixture was allowed to stand for 30 minutes at room temperature and the absorbance of the chromophore formed was read at 546ηm. BHT (0.1mg/mL) was used as positive control for the assay.

### Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the extracts was estimated as per the method of Shang[17]. 0.1mL each of the plant extract was added to the reaction mixture containing 0.1mL of Deoxyribose (3mM), 0.5mL of $FeCl_3$(0.1mM), 0.5mL of EDTA (0.1mM), 0.5mL of Ascorbic acid (0.1mM), 0.5mL of $H_2O_2$ (1mM) and 0.8mL of Phosphate buffer (20mM pH 7.4). The reaction mixture was incubated at 37°C for 1hour. Then 1mL of Thiobarbutiric acid (TBA) as well as 1mL of 2.8% Trichloro acetic acid (TCA) were added and incubated at 100°C for 20 minutes. Thiobarbutiric acid reactive

substances formed were measured after cooling the mixture and measuring the absorbance at 532ηm.

### Superoxide radical scavenging assay

The assay was done following the method of Khanna[18]. 0.1mL each of the plant extract was added to the reaction mixture containing 50mM phosphate buffer (pH-7.6), 20µg/ml riboflavin, 12mM EDTA and 0.1mM NBT. The reaction was initiated by illuminating the reaction mixture for 5 minutes and the absorbance was measured at 590ηm. Inhibition of blue formazone formation was considered for scavenging activity. Quercitin (0.1mg/mL) was used as positive control.

### *In vitro* Antihemolytic activity

The inhibition of human erythrocyte hemolysis by the plant extract was evaluated according to the procedure described by Ebrahimzadeh[19]. In this experiment, venous blood samples were collected from healthy, well nourished human adults into glass centrifuge tube containing a known amount of anticoagulant. The tube was centrifuged at 1500rpm for 10 minutes and supernatant was discarded. The resulting pellet was washed carefully with phosphate buffered saline (0.1M) to get buff colored cells called packed RBCs.

To study $H_2O_2$ induced oxidative damage 0.1mL of 1mM $H_2O_2$, 0.1mL of 0.1mM $FeCl_3$ and 100µL of plant extracts were incubated for 15minutes. Then 500µL of packed RBC was added and incubated at 37°C for 1 hr. Ascorbic acid (0.1mg/mL) was used as positive control. Thereafter the content of the tube was centrifuged at 1500rpm for 10 minutes and absorbance was measured at 540ηm using spectrophotometer.

Further the extent of erythrocyte damage due to lipid peroxidation of cell membrane was measured in terms of Malondialdehyde content.

### Lipid peroxidation

Malondialdehyde content was estimated according to the methods described by Hodges[20]. To 100µL of the sample, 0.5mL of 20% TCA, 0.5ml of 0.5% Butylated hydroxy toluene (BHT) and 1.0ml of 0.65% TBA were added. Then the test tubes were kept in boiling water bath for 25 minutes, cooled to room temperature and centrifuged at 12,000rpm for 10 minutes. Absorbance of the supernatant was read at 532ηm.

### Thin Layer Chromatography (TLC)

The solvent system was standardized for the separation of active principle from the methanol extracts of spike and fruit that exhibited significant antihemolytic property. The solvent system used was – Ethyl acetate: hexane: methanol: acetic acid::2:2:1:0.5. Isolated fractions were once again subjected to 2D – TLC and preparatory TLC for further purification of the active principle.

Scrapped matter was isolated, redissolved in methanol and supernatant was collected. UV absorption spectrum was taken for the TLC fraction and $\lambda_{max}$ was determined to check the purity of the isolated active principle scanning from 200 to 700ηm. Flavanoids and total phenols were estimated from the TLC fractions to determine their presence and concentration.

## RESULTS

### Proximate analysis

Table 1, Shows the results of proximate analysis of B. ceiba pentandra spike and fruit extracts. Considerable amount of protein and ascorbic acid were reported in the aqueous extract of the fruits as compared to that of spikes. Higher levels of flavanoids and polyphenols were present in the methanol extract of fruits and spikes in comparison to hexane, ethyl acetate and aqueous extracts.

### Antioxidant activity

Table 2A, refers to DPPH radical scavenging assay. DPPH, a nitrogen centered free radical was reduced in the presence of different solvent extracts of fruit and spike. Ascorbic acid in water, used as positive control showed 92.91% scavenging activity which is followed by methanol extract of fruit showing 82.95% activity. Methanol extract of spike as well as aqueous extract of fruits exhibited considerable scavenging activity of 76.25% and 70.01% respectively.

Table 2B, refers to Nitric oxide radical scavenging activity. Nitric oxide generated from sodium nitroprusside interacts with oxygen to form nitric ions whose concentration was estimated using Griess reagent. Methanol extract of fruits and spike showed maximum scavenging activity of 90.04% and 84.48% respectively. BHT in water showed a scavenging activity of 92.4%.

Table 2C, refers to Hydroxyl radical scavenging assay. Hydroxyl radicals were generated by the Fenton reaction and the TBA reacting substance was estimated photo metrically. Aqueous and methanol extracts of fruits showed higher scavenging activities of 80.42% and 80.14% when compared to other extracts.

Table 2D, refers to Superoxide radical scavenging activity. The superoxide radical generated from Riboflavin and NBT in the presence of light were scavenged by the different solvent extracts of spike and fruits. With respect to the positive control Ascorbic acid (82.7%), methanol extract of spike (77.83%) and fruit (75.40%) exhibited considerable scavenging activities.

Table 3, explains the antihemolytic activity of various extracts of spike and fruits. The lysis of erythrocytes in the presence of $H_2O_2$, $FeCl_3$ in the absence of plant extract was considered as 100% hemolytic activity. Among all the extracts, methanolic extracts of fruit and spike exhibited considerable antihemolytic activity and lesser amount of MDA. In presence of aqueous, ethyl acetate, chloroform and hexane extracts, erythrocytes were partially hemolysed.

Partially purified bioactive molecules from TLC fraction of methanol extract of spike and fruit established high antihemolytic activity and therefore lesser MDA content.

Further, table 4 refers to the concentration of flavanoids as well as polyphenols in the partially purified methanol extract of fruit and spike. UV spectra of the TLC fraction (Fig 1 and 2) indicates maximum absorption at 226 and 280ηm confirming the presence of phenolics and flavanoids. Also quantitative estimation revealed higher concentration of phenols as well as flavanoids in the methanol extract of fruit and considerable amount of flavanoids in the methanol extract of spike.

**Table 1: Proximate analysis of different solvent extracts of B. ceiba pentandra fruits and spike**

| Solvent extract | Proteins | Phenols | Flavanoids | Tannins | Alkaloids | Saponins | Ascorbic acid | Glycosides |
|---|---|---|---|---|---|---|---|---|
| Aqueous extract (spike) | ++ | ++ | ++ | + | + | + | + | + |
| Aqueous extract (fruit) | ++ | ++ | ++ | + | + | + | + | + |
| Methanol extract (spike) | - | +++ | +++ | ++ | - | - | - | - |
| Methanol extract (fruit) | - | +++ | +++ | ++ | - | - | - | - |
| Ethyl acetate extract (spike) | - | + | + | - | - | - | - | - |

| Ethyl acetate extract (Fruit) | - | + | + | - | - | - | - | - |
|---|---|---|---|---|---|---|---|---|
| Chloroform extract (spike) | - | + | - | - | - | - | - | - |
| Chloroform extract (fruit) | - | + | - | - | - | - | - | - |
| Hexane extract (spike) | - | - | - | - | - | - | - | - |
| Hexane extract (fruit) | - | - | - | - | - | - | - | - |

-: absent, +: low concentration, ++: Moderate concentration, +++: High concentration

**Table 2A: DPPH radical scavenging assay**

| Sample | Aqueous extract | Methanol extract | Ethyl acetate extract | Chloroform extract | Hexane extract |
|---|---|---|---|---|---|
| Ascorbic acid (Positive control) | 92.91+0.120[a] | 82.27+0.087[a] | 86.03+0.067[a] | 86.43+0.092[a] | 84.01+0.071[a] |
| Spike | 67.26+0.038[a] | 76.25+0.056[a] | 46.72+0.045[a] | 64.5+0.098[a] | 38.87+0.075 |
| Fruit | 70.01+0.090[a] | 82.95+0.105[a] | 54.13+0.087[a] | 67.21+0.069[a] | 68.03+0.064[a] |

Values are means + SEM; n=5, significant at [a]$p<0.0001$ as compared to Ascorbic acid (positive control)

**Table 2B: Nitric oxide radical scavenging assay**

| Sample | Aqueous extract | Methanol extract | Ethyl acetate extract | Chloroform extract | Hexane extract |
|---|---|---|---|---|---|
| BHT (Positive control) | 92.4+0.164[a] | 88.27+0.096[a] | 86.04+0.088[a] | 87.08+0.396[a] | 82.21+0.152[a] |
| Spike | 77.14+0.111[a] | 84.48+0.095[a] | 58.55+0.110[a] | 63.98+0.091[a] | 48.44+0.094[a] |
| Fruit | 69.31+0.085[a] | 90.04+0.059[a] | 67.14+0.068[a] | 81.51+0.127[a] | 65.52+0.129[a] |

Values are means + SEM; n=5, significant at [a]$p<0.0001$ as compared to BHT (positive control)

**Table 2C: Hydroxyl radical scavenging assay**

| Sample | Aqueous extract | Methanol extract | Ethyl acetate extract | Chloroform extract | Hexane extract |
|---|---|---|---|---|---|
| Quercitin (Positive control) | 82.22+0.090[a] | 82.92+0.104[a] | 74.53+0.126[a] | 77.87+0.124[a] | 71.00+0.057[a] |
| Spike | 69.21+0.075[a] | 72.05+0.056[a] | 36.12+0.092 | 47.98+0.095[a] | 53.21+0.065[a] |
| Fruit | 80.42+0.111[a] | 80.14+0.068[a] | 65.05+0.071[a] | 68.25+0.083[a] | 69.56+0.103[a] |

Values are means + SEM; n=5, significant at [a]$p<0.0001$ as compared to Quercitin (positive control)

**Table 2D: Superoxide radical scavenging assay**

| Sample | Aqueous extract | Methanol extract | Ethyl acetate extract | Chloroform extract | Hexane extract |
|---|---|---|---|---|---|
| Ascorbic acid (Positive control) | 80.46+0.116[a] | 82.70+0.098[a] | 72.92+0.069[a] | 75.54+0.130[a] | 73.50+0.125[a] |
| Spike | 70.53+0.098[a] | 77.83+0.147[a] | 69.65+0.114[a] | 50.23+0.141[a] | 42.71+0.095[a] |
| Fruit | 64.46+0.045[a] | 75.40+0.094[a] | 52.71+0.086[a] | 60.23+0.087[a] | 49.62+0.127[a] |

Values are means + SEM; n=5, significant at [a]$p<0.0001$ as compared to Ascorbic acid (positive control)

**Table 3: Antihemolytic activity of B. ceiba pentandra spike and fruit extracts**

| Sample | % Hemolysis | | Malondialdehyde (µgm/ml) | |
|---|---|---|---|---|
| | Spike | Fruit | Spike | Fruit |
| $H_2O_2$+ RBC(Negative control) | 100 | 100 | 2.330 | 2.330 |
| Ascorbic acid + RBC(Positive control) | 6.38 | 6.38 | 0.148 | 0.148 |
| $H_2O_2$+ Ascorbic acid + RBC | 10.72 | 10.72 | 0.249 | 0.249 |
| Aqueous extract + RBC | 11.56 | 10.83 | 0.269 | 0.252 |
| Methanol extract + RBC | 10.29 | 9.27 | 0.239 | 0.215 |
| Ethylacetate extract + RBC | 13.34 | 11.76 | 0.310 | 0.274 |
| Chloroform extract + RBC | 12.13 | 10.96 | 0.282 | 0.255 |
| Hexane extract + RBC | 15.54 | 13.13 | 0.362 | 0.305 |
| $H_2O_2$ + Aqueous extract + RBC | 37.45 | 32.76 | 0.872 | 0.763 |
| $H_2O_2$ + Methanol extract + RBC | 19.14 | 14.57 | 0.786 | 0.456 |
| $H_2O_2$ + Ethylacetate extract + RBC | 44.71 | 39.69 | 1.041 | 0.924 |
| $H_2O_2$ + Chloroform extract + RBC | 72.32 | 70.87 | 1.685 | 1.651 |
| $H_2O_2$ + Hexane extract + RBC | 69.18 | 61.43 | 1.611 | 1.431 |
| $H_2O_2$ + TLC fraction of Methanol extract + RBC | 11.51 | 9.21 | 0.268 | 0.214 |

**Table 4: Polyphenol and Flavanoid contents from TLC fraction of spike and fruit extracts**

| Sample | Polyphenols | Flavanoids |
|---|---|---|
| Spike | 149.5+0.491 | 68.3+0.338 |
| Fruit | 186.4+0.774 | 96.8+0.291 |

Values are means + SEM; n=5

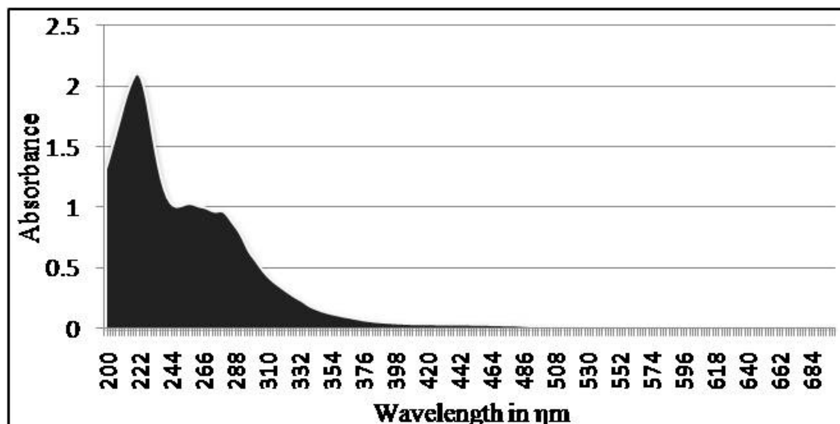


**Fig. 1: UV absorbance of TLC fraction from Spike**

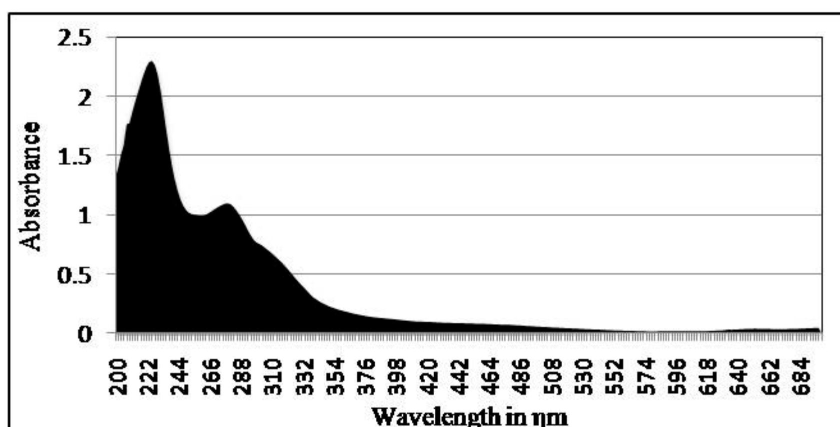


**Fig. 2: UV absorbance of TLC fraction from Fruit**

## DISCUSSION

In recent years the use of herbs in traditional medicine has gained attention as they are being proven as the promising sources of various bioactive molecules. Polyphenols are ubiquitously distributed group of plant secondary metabolites ranging from simple molecules like phenolic acids, phenyl propanoids, flavanoids to highly polymerized compounds namely lignins, melanins and tannins. Flavanoids are the most common and widely distributed subgroups[21].

In the present study proximate analysis as well as TLC fraction showed higher concentrations of phenols and flavanoids in the methanol extract of spike and fruit. The significant antioxidant activities including DPPH, Hydroxyl, Superoxide as well as Nitric oxide radical scavenging activities of methanol extract of spikes and fruits may be endorsed for the presence of higher amounts of the above mentioned plant secondary metabolites.

Many studies revealed that polyphenols exhibit a wide range pluripharmacological effects including antimicrobial, anti-inflammatory, antiallergic, hepatoprotective and anticarcinogenic actions. Many of these biological functions have been attributed to their antioxidant activity in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[22].

The effective antihemolytic activity of spike and fruit extract is because of the ability of phenolic compounds including flavanoids in neutralizing the free radicals generated by $H_2O_2$ and thereby protecting the erythrocytes membrane from destruction and lysis. Insignificant hemolysis was observed when erythrocytes were treated only with plant extracts, indicating the nontoxic nature of plant extracts. Thus the extract can be justified as harmless for the cells.

Membrane lipid peroxidation is regarded as a key factor for cell lysis. Malondialdehyde (MDA), a low molecular weight end product of lipid hydroperoxide decomposition is the most often measured parameter of membrane destruction. Lesser the cell lysis lesser will be the MDA content. Earlier reports suggested the potent antihemolytic activity of bioactive components namely Flavanoids and phenols from plant extracts[23-24]. Thus the plant secondary metabolites could be regarded as efficient scavengers of reactive oxygen species offering the cell protective function.

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