



# IN VITRO ANTIOXIDANT POTENTIAL OF THE EDIBLE FLOWERS OF BOMBAX CEIBA- AN UNDERUTILIZED TROPICAL TREE

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## ABSTRACT

Free radicals are defined as chemicals with one or more unpaired electron in their outer shell. Biological free radicals are highly unstable molecules and react with organic substances like lipids, proteins and DNA and damage them. There are many beneficial compounds which protect the body against the free radicals known as antioxidants. During the present times endogenously produced antioxidants are not sufficient. To overcome the negative effects of free radicals, there is a need of some natural antioxidant compounds or exogenous supply of antioxidants is required. Thus, keeping this thing in mind the present study was conducted to evaluate the antioxidant potential of *Bombax ceiba*. It was assessed in terms of scavenging of DPPH, hydroxyl and hydrogen peroxide radicals; total antioxidant activity (TAA) and ferric reducing antioxidant potential (FRAP) activity. The current study depicts that the aqueous extract of the flowers exhibited high or significant scavenging activity against DPPH, hydroxyl and hydrogen peroxide radicals. Further, this study reveals that high amount of phenolic and flavonoid was present in the flowers of the test plant. So, it is thus clear that *Bombax ceiba* possesses good antioxidant potential and could be used for human health in future.

**Keywords:** Antioxidants, Flavonoids, Flowers, Free radicals, Phenolics,

## I. INTRODUCTION

Agriculture is the primary source of food but the diversity of species cultivated for food has narrowed down. Today, people depend on limited number of species for meeting their requirements of food, medicine, clothing, fuel, timber, and other essential purposes. There are 7000 edible plant species known to mankind, but just 30 contribute to the food needs [1]. This indicates that several plant species remain unexploited or underutilized in the hands of various human communities. The changed lifestyle and food habits neglect the role of traditional food in the health of human beings and lead towards increasing incidence of poor health and malnutrition. Earlier, it was reported that there are a number of plants whose economic potential is either underestimated or underexploited, though they have a large contribution in the diet of the rural people as they serve rich source of macro and micronutrients and are able to compensate the dietary deficiencies in human diet [2,3]. Apart from their commercial, medicinal and cultural value, underutilized plants are also considered important as they are



well adapted to specific marginal soil and climatic conditions, and can be grown with minimal external inputs [4,5]. Underutilized food ranging from leaves, flowers, fruits, seeds, roots, rhizomes and tubers have the potential to make a substantial contribution to food and nutrition security. Ikram *et al.* [6] pointed out that several underutilized plants have also drawn the attention of many researchers as a natural source of antioxidants. These, being rich sources of phytochemicals, may provide protection against many ailments. Kalt and Kushad [7] reported that protective effects of wild fruits and vegetables are attributed to the occurrence of natural antioxidants such as vitamin E, ascorbic acid,  $\alpha$ -tocopherol and  $\beta$ -carotene, which act as free radical scavengers. In recent years, the flowers of the underutilized plants are receiving a great attention due to their great nutritional value and are used in the form of salad, soups, desserts, drinks and vegetables [8,9,10]. According to Price [11], many flowers are a rich source of calcium and potassium. Apart from their edible uses, some flowers have good medicinal properties [12,13,14]. Many poor people use flowers of *B. ceiba* for edible purpose. During the months of Feb-March the flowers are collected and used as vegetable. So keeping this thing in mind, free radical scavenging and antioxidant potential of the edible flowers of *Bombax ceiba* were evaluated in the present study.

## II. MATERIAL AND METHODS

### 2.1 Preparation of extract concentration for the study

Flowers of *Bombax ceiba* were collected from the Botanical garden of Panjab University Chandigarh, India. Plant material was plucked and dried under shade. After drying, plant material was grinded and the powder was obtained for analysis. The powder (1 g) was dissolved in 100 ml of distilled water in a conical flask. The mixture was left for 12 hours at room temperature for further use. For estimation of antioxidant activity of the flowers of *B. ceiba*, various concentrations of extract were prepared by dissolving plant material in water. Five different concentrations were prepared (0.0625, 0.125, 0.25, 0.5 and 1%).

## III. FREE RADICAL SCAVENGING ACTIVITY

### 3.1 DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging assay

The method of DPPH radical scavenging activity was determined as per Bozin *et al.* [15] with slight modifications. Briefly, 200  $\mu$ l of aqueous extract were taken to which 3 ml of DPPH (0.1 mM in methanolic solution) was added. A decrease in absorbance of DPPH solution indicates increased DPPH radical scavenging activity. *Rosmarinus officinalis* were used as the positive control. It was calculated in percent by using the following formula:

$$\% \text{ DPPH radical scavenging activity} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A means absorbance / optical density

### 3.2 Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of the test plants was determined as per the method of Yu *et al.* [16] with slight modifications. The assay mixture comprised of 0.02 ml of ferrous chloride (0.02 M), 0.5 ml of 1, 10-Phenanthroline (0.04 M), 1 ml of phosphate buffer (0.2 M, pH 7.2) and 1 ml of sample. After 5 minute of



incubating at room temperature, absorbance was read at 560 nm using UV-1800 double beam spectrophotometer. *Rosmarinus officinalis* were used as the positive control. The percent scavenging of hydroxyl radical was calculated using the following formula:

$$\% \text{ of } \cdot\text{OH} \text{ radical scavenging activity} = [(A_{\text{sample}} - A_{\text{control}}) / (A_{\text{blank}} - A_{\text{control}})] \times 100$$

Where A means absorbance / optical density

### 3.3 Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) scavenging assay

It was estimated as per the method of Ruch *et al.* [17]. A solution of 40 mM H<sub>2</sub>O<sub>2</sub> was prepared in 0.1 M phosphate buffer (pH 7.4). The absorbance of the solution was read at 230 nm after 10 min against a blank containing phosphate buffer without H<sub>2</sub>O<sub>2</sub>. *Rosmarinus officinalis* were used as the positive control. The percent scavenging of H<sub>2</sub>O<sub>2</sub> was calculated by using the formula:

$$\% \text{ scavenging of H}_2\text{O}_2 \text{ radical} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

## IV. ANTIOXIDANT POTENTIAL

### 4.1 Total Antioxidant Activity

The antioxidant capacity of extracts was evaluated by the phosphomolybdenum method as given by Prieto *et al.* [18]. To 0.1 ml of sample solution (water extracts), added reagent of solution (6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The reaction mixture was incubated at 95 ° C for 90 minutes. The samples were cooled at room temperature and the absorbance of solution was measured at 695 nm using Shimadzu UV-1800 double beam spectrophotometer. *Rosmarinus officinalis* were used as the positive control. Total antioxidant capacity was estimated in percent by using the following formula:

$$\% \text{ Total antioxidant activity} = [(A_{\text{sample}} - A_{\text{control}}) / (A_{\text{sample}})] \times 100$$

Where A means absorbance / optical density

### 4.2 Ferric reducing antioxidant power (FRAP) assay

For determination of FRAP, the method of Oyaizu [19] was followed with slight modifications. Samples (different concentrations of water extract; 0.2 ml) were mixed with 0.6 ml of phosphate buffer (0.2 M, pH 6.6) and 0.6ml of potassium ferricyanide (1% w/v). The mixture was kept for 10 min and the absorbance was read at 700 nm using Shimadzu UV-1800 double beam spectrophotometer against a blank containing ferric chloride and distilled water. Increased absorbance of the reaction mixture indicates increased reducing activity. *Rosmarinus officinalis* were used as the positive control. The activity was estimated in terms of percent inhibition by using the following formula

$$\% \text{ inhibition} = [(A_{\text{sample}} - A_{\text{control}}) / A_{\text{sample}}] \times 100$$

Where A means absorbance / optical density



## V. PHYTOCHEMICAL ANALYSIS

### 5.1 Total phenolic content (TPC)

TPC was determined by the method of Swain and Hills [20] using Folin- Ciocalteu reagent (FCR). The extract equivalent to 0.125 ml was mixed into 0.875 ml of distilled water and 0.5 ml of FCR was added and the contents were shaken thoroughly. After 3 minute, 1 ml of 7.5% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was added and the mixtures were allowed to stand for 30 min to 1 h. The intensity of the blue color so developed was read at 760 nm using Shimadzu UV-1800 double beam spectrophotometer against a standard of gallic acid ( $50 \mu\text{g ml}^{-1}$ ). The concentration of the phenolic compounds was expressed as mg gallic acid equivalents per gram ( $\text{mg GAE g}^{-1}$ ) of the plant tissue.

### 5.2 Total flavonoid content (TFC)

The TFC was determined as per the method given by Meda *et al.* [21]. Approximately 1 ml of 2% of Aluminum trichloride ( $\text{AlCl}_3$  dissolved in methanol) was added into 1ml of water extracts. After 10 minute the absorbance was read at 415 nm on Shimadzu UV-1800 double beam spectrophotometer. Quercetin was used as a standard. The amount of flavonoid was expressed as mg of quercetin equivalents per gram ( $\text{mg QE g}^{-1}$ ) plant tissue.

### 5.3 Statistical analysis

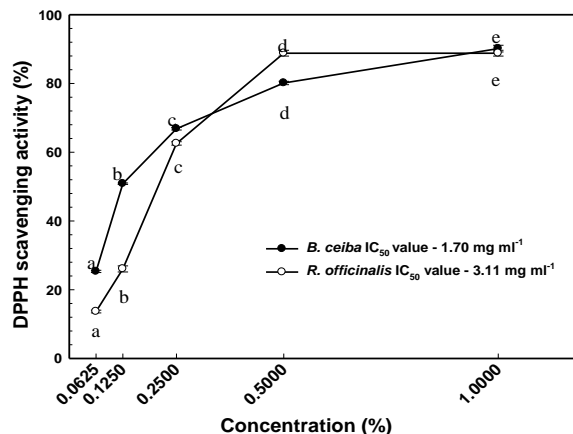
The final values are presented as mean  $\pm$  standard errors. The significance values was checked and analyzed by one-way ANOVA followed by the comparison of mean values using post-hoc Tukey's test at  $P \leq 0.05$ . Minimum five replicated were maintained.

## VI. RESULTS AND DISCUSSION

### 6.1 DPPH radical scavenging activity

The present study was focused on the determining the antioxidant / radical scavenging properties of the flower extract of *B. ceiba* and compared with the *R. officinalis* (used as positive control). The DPPH radical scavenging activity of both the extracts is presented in Fig.1. In general, there was an increase in scavenging activity with increasing concentration of the extracts. The increase in radical scavenging activity was statistically significant at all the test concentrations (Fig. 1). At higher concentration of 1%, the scavenging activity was found to be the maximum in the flower extract of in *B. ceiba* (~ 88%) and was comparable to the *R. officinalis* used as positive control. The  $\text{IC}_{50}$  value however of *B. ceiba* was  $1.70 \text{ mg ml}^{-1}$ , whereas, in case of the positive control, *R. officinalis*, the  $\text{IC}_{50}$  value was  $3.11 \text{ mg ml}^{-1}$ . These observations indicate that the flower extract of *B. ceiba* exhibited better scavenging ability than that of positive control. There are numerous reports in literature where various wild and underutilized plants show similar type of radical scavenging activity [22,23,24].

**Fig. 1. DPPH radical scavenging activity of the flowers of *B. ceiba* in comparison to *R. officinalis*.**

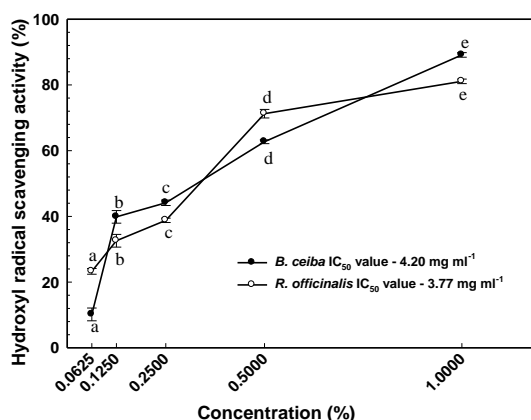


Data presented as mean±standard error. Different alphabets along each curve represent significant difference at  $P \leq 0.05$  applying Tukey's test.

## 6.2 Scavenging of hydroxyl radicals

The ·OH radical scavenging activity of the test plant increased significantly with increasing concentration of the flower extracts. However, the activity was found to be more in the flower extract of *B. ceiba* as compared to the extracts of *R. officinalis* at 1% (Fig. 2). In *B. ceiba*, the ·OH radical scavenging activity ranged from ~21% to ~88% at the concentration range of 0.0625 to 1%. The IC<sub>50</sub> values of the extracts were also calculated. It was 4.20 mg ml<sup>-1</sup> in *B. ceiba* and 3.77 mg ml<sup>-1</sup> in *R. officinalis* used as the positive control. The hydroxyl radical is one of the most reactive radicals that cause serve damage to the biomolecules [25]. The observations made in the current study point that the flowers of *B. ceiba* possessed superior activity for scavenging hydroxyl radicals. The present observations that underutilized plants possess ·OH radical scavenging activity are supported by some other studies on the ·OH radical scavenging activity of *Averrhoa carambola* [26] and *Aegle marmelos* [27].

**Fig. 2. Hydroxyl radical scavenging activity of flowers of *B. ceiba* in comparison to *R. officinalis*.**

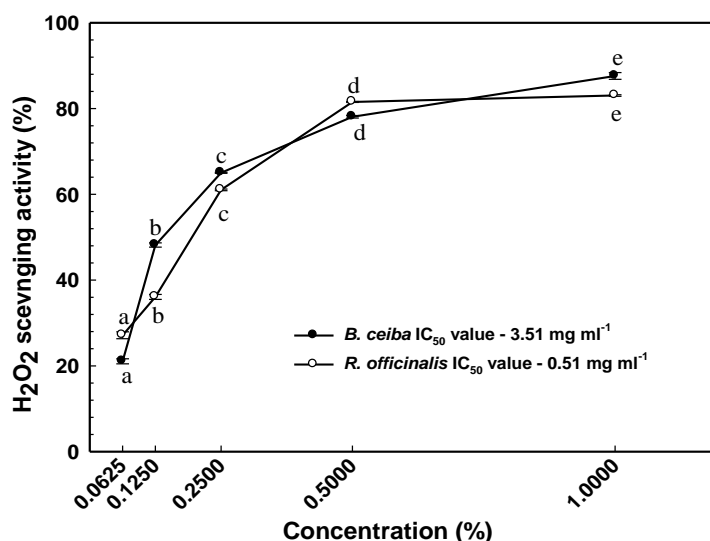


Data presented as mean±standard error. Different alphabets along each curve represent significant difference at  $P \leq 0.05$  applying Tukey's test.

### 6.3 Hydrogen peroxide radical scavenging activity

Hydrogen peroxide as already mentioned, itself is not toxic but it enhances the production of hydroxyl radicals. However, an antioxidant can interfere in the formation of hydroxyl radicals by directly reacting with  $H_2O_2$  [28]. So, there is a need of some natural antioxidants that can inhibit the reaction by directly reacting with  $H_2O_2$ . In the present study, it was observed that flowers of the test plant possessed good scavenging activity against  $H_2O_2$  radical in a dose-dependent manner (Fig. 3). The increase at each concentration was statistically significant. The study indicates that the scavenging activity was more in the extract of *B. ceiba* compared to the *R. officinalis* (positive control) at 1% concentration (Fig. 3). The  $H_2O_2$  radical scavenging activity in *B. ceiba* varied from ~22 to ~88% for concentrations ranging from 0.0625% to 1%. Further, the  $IC_{50}$  value of the flower extract of *B. ceiba* was calculated to be  $3.51 \text{ mg ml}^{-1}$ , whereas that of *R. officinalis* was  $0.51 \text{ mg ml}^{-1}$ . It is thus clear that flowers of *B. ceiba* plants possess good potential for the scavenging of  $H_2O_2$ . Other studies have also reported that that underutilized plants possess good scavenging potential for  $H_2O_2$  radical [29,30].

**Fig. 3.  $H_2O_2$  radical scavenging activity of flowers of *B. ceiba* in comparison to *R. officinalis*.**



Data presented as mean±standard error. Different alphabets along each curve represent significant difference at  $P \leq 0.05$  applying Tukey's test.

### 6.4 Ferric ion reducing antioxidant power (FRAP) activity

The antioxidant activity of the test plant was also determined through their reduction power expressed as the reduction of ferric ions ( $Fe^{3+}$ ) to ferrous ions ( $Fe^{2+}$ ). In the current study, the aqueous extract of *B. ceiba* flowers exhibited significant FRAP (Table 1). The activity of *B. ceiba* extract ranged from ~21 to 86% at the concentration range of 0.0625 to 1%. The  $IC_{50}$  value was calculated to be  $2.15 \text{ mg ml}^{-1}$  in *B. ceiba* and it was

0.64 mg ml<sup>-1</sup> in the positive control of *R. officinalis* (Table 1). Several studies also support the fact that underutilized plants possess an ability to reduce ferric ions to ferrous ions based on FRAP assay [31,32,33].

**Table1. FRAP of flowers of *Bombax ceiba* in comparison to *R. officinalis*.**

Concentration (%)	<i>B. ceiba</i>	<i>R. officinalis</i>
0.0625	21.76±1.84a	23.01± 0.40a
0.125	46.03± 0.65b	64.56± 0.66b
0.25	72.77± 0.09c	82.71± 0.69c
0.5	79.15± 0.67d	89.36± 0.51d
1	85.68± 1.31e	90.78± 0.71e
IC <sub>50</sub> values	2.15 mg ml <sup>-1</sup>	0.64 mg ml <sup>-1</sup>

Values are mean±SE. Different alphabets within a column represent significant difference at  $P \leq 0.05$  applying Tukey's test.

### 6.5 Total antioxidant activity (TAA)

TAA is widely used method to detect antioxidants in plants. In the present study, TAA of *B. ceiba* flowers ranged from ~22 to 87% at 0.0625-1% of extract concentration (Table 2). TAA activity increased significantly ( $P \leq 0.05$ ) at all the concentrations. The IC<sub>50</sub> value of the flower extract of *B. ceiba* was calculated to be 0.79 mg ml<sup>-1</sup> compared to 0.98 mg ml<sup>-1</sup> of the *R. officinalis* used as positive control (Table 2). These observations indicate that the flowers of *B. ceiba* possessed better TAA than that of positive control. Some other studies have also reported that underutilized plants possess good antioxidant activity [34,35,36].

**Table2. TAA of flowers of *Bombax ceiba* in comparison to *R. officinalis*.**

Concentration (%)	<i>B. ceiba</i>	<i>R. officinalis</i>
0.0625	22.55±0.53a	37.21±0.78a
0.125	58.02±0.30b	47.77±0.36b
0.25	76.82±0.92c	77.83±0.59c
0.5	83.47±0.32d	86.48±0.81d
1	86.67±0.13e	92.23±0.71e
IC <sub>50</sub> values	0.79 mg ml <sup>-1</sup>	0.98 mg ml <sup>-1</sup>

Values are mean±SE. Different alphabets within a column represent significant difference at  $P \leq 0.05$  applying Tukey's test.

### 6.6 Total Phenolic content (TPC) and flavonoid content (TFC)

TPC was evaluated in the flower extracts of *B. ceiba* (Table 3). TPC was measured to be ~29 mg GAE g<sup>-1</sup> in *B. ceiba* and it was ~22 mg GAE g<sup>-1</sup> in positive control. The TFC also varied among different extracts. In case of *B. ceiba*, the content was ~84 mg QE g<sup>-1</sup>, while in positive control of *R. officinalis* it was ~ 65 mg QE g<sup>-1</sup> (Table 3). The selected underutilized plants identified in the present study were found to be rich in phytochemicals like phenolics and flavonoids. In general, the phenolics and flavonoids are the secondary metabolites and widespread among plants. The higher antioxidant and radical scavenging properties of test underutilized plant can be attributed to presence of phenolics and flavonoids in their edible parts.

**Table 3. Total phenolic and flavonoid content of the flower extracts of *B. ceiba* and *R. officinalis*.**

Plant name	Phenolic content (mg GAE g <sup>-1</sup> )	Flavonoid content (mg QE g <sup>-1</sup> )
<i>B. ceiba</i>	28.57 ± 0.56	84.10 ± 0.90
<i>R. officinalis</i>	21.94 ± 0.61	65.94 ± 0.71

## VII. CONCLUSIONS

In the present study, the amount of phenolics and flavonoids was higher in flowers of *B. ceiba* compared to the positive control. Its scavenging ability, particularly for DPPH, hydroxyl and hydrogen peroxide was also higher than that of positive control. It is thus concluded that flowers of *B. ceiba* can be used as a good source of antioxidants.

## VIII. ACKNOWLEDGEMENT

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