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ORIGINAL ARTICLE

Phytochemical Screening of Qualitative and Quantitative Analysis of *In vitro* Antioxidant Activities in Methanolic Extract of *Bombax Ceiba* (Flower)

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ABSTRACT

The present study was carried out to evaluate the in vitro antioxidant activities in methanolic extract of Bombax ceiba (flower). Followed by Qualitative analysis of phytoconstituents antioxidant activity of the methanolic extract was studied by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, Reducing power activity, Superoxide scavenging activity,, hydrogen peroxide scavenging activity and nitric oxide scavenging activity. The data of Qualitative analysis, on phytochemicals is in the order of methanol extract > ethanol extract >chloroform≥water extract were obtained. Presence of Alkaloids, Cardiac Glycosides, Carbohydrates, Flavonoids, Phenols, Proteins, Saponins, Tannins, Terpenoids, Quinones were recorded on methanol extract. Highest free radical scavenging activity of DPPH assay, Reducing power scavenging activity, superoxide radical scavenging activity, hydrogen peroxide scavenging activity and nitric oxide radical scavenging activity, was found to be.87.47±5.98µg/ml, 75.53±5.13/ml, 83.32±5.79µg/ml, 0.69±0.07µg/ml,86.28±5.87µg/ml, at highest concentration of methanol extract of 80µg/ml respectively in this study. The present study concluded the in vitro antioxidant activity of Bombax ceiba which might be due to the presence of the phenolic and flavonoid compounds.

KEYWORDS: *Bombax ceiba (flower), Qualitative analysis, Quantitative analysis and Phytochemical, In vitro antioxidant, scavenging activity.*

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INTRODUCTION

Since the ancient times, nature has been a huge source of medicinal agents. All over the world plants have served as the richest source of raw materials for traditional as well as modern medicine. The medicinal value of plants is mainly due to the presence of some chemical substances known as phytochemicals. They are basically plant metabolites, are synthesized in all part of plant body by itself and have some definite physiological action onanimals [1].The medicinal plant significant part of our medication system. Most of the medicines is made up of the herbal plant. It is also named as a medicinal plant. The herbal plant was used in our traditional medicine system for long ago. Countries in Asia and Africa 80% user used traditional medicinal system, which includes herbal or medicinal plant. [2]. It has many pharmacological activities like in-vitro Anti-inflammatory, Anti-diabetic, Anti-obesity, Hypotensive, Antioxidant, Antiangiogenic, Antimicrobial, Cytotoxicity, Aphrodisiac, Haemostatic, Astringent Diuretic, Cardiotonic, Demulcent, Anti-dysentric, anti-diahorreal, and Antipyretic effects [3].

The *Bombax ceiba* flower extract is commonly used for the treatment of diarrhoea, fever, chronic inflammation, catarrhal affection and ulceration of the bladder and kidney in traditional systems of medicine [4].It is universally known fact that, plants are the potential source of natural antioxidants. Free radicals implicated in the etiology of several degenerative disorders including cancer, diabetes, rheumatoid arthritis, atherosclerosis, liver cirrhosis, Alzheimer's disease and other neurodegenerative

disorders [5]. Many antioxidant compounds from naturally occurring plant sources have been identified as free
radical or active oxygen and nitrogen scavengers. The flavonoids are the most important group among the natural antioxidants. Their biological effects are attributed principally to free radical scavenging or metal ion chelation.Metal ions are directly involved in the generation of reactive species[6].The ethnomedicinal activity of *Bombax ceiba*L. Plant part Traditional medicinal uses, Thorn Used in the different formulation to treat Acne, Androecium Used for Food purpose, Petals for Skin and Cosmetics, Leaf for treatment of Diarrhoea, Larvicidal Activity, Root for the treatment of Piles, Bark Used for Wound Flower: The flowers show bitter action and also shows acrid cooling, dry, astringent to the bowels, anti-inflammatory action [7].*Bombax ceiba* has many significant medicinal values. The tree is a powerful, fast-growing lightdemander. It thrives especially in valleys, on sandy loams that are deep and in regions with annual rainfall of 50 to 460cm [8]. All parts of the *Bombax ceiba*, are known as to be different medicinal properties which is proved by the ethno botanists in many surveys and in the traditional medicine such as Ayurvedic. The bark has hard-sharp conicles and grey-brown or silver-grey colored. The leaves are broad, spreading, glabrous, lanceolate with having leaflets. The plant seeds are shiny, black or brown, embodied in wool viz long and white, irregularly shaped obovoid, oily and shiny with thick, silky hair. Gum of tree is light brown to translucent locally known as "KATIRA"[9].

MATERIAL AND METHODS

Chemicals

All chemicals were procured from Ponmani Scientific Chemicals Suppliers, Tiruchirappalli, Tamilnadu, India and were of analytical grade.

Sample

In this study, *Bombax ceiba*,was collected from a thanjavur, Tamilnadu, India.

Samples preparation

The (*Bombax ceiba*) plant Materials was cleaned and shade dried until the water molecules evaporated and the dried plant materials (petals of flower) was taken and grinded into coarse powder. The powder samples were stored in a clean glassware container until needed for analysis with proper labeling.

Sample extraction

The *Bombax ceiba* flower extract was prepared by Soxhlet extraction method. The 20 g of powder was uniformly packed into a thimble and extracted with 250 ml of different solvents separately. Solvents used for petroleum ether, chloroform, ethyl acetate, acetone methanol, ethanol and water as per polarity. The process continues for 24 hours or till the solvent in siphon the extraction was colorless. The extract was taken in a beaker and kept in hot plate and heated at 30-40 ºC till the solvent got evaporated. The dried extract was kept in refrigerator at 4 ºC for used in phytochemical analysis.

Evaluation of in vitro antioxidant activity

Petroleum ether, chloroform, ethyl acetate, acetone methanol, ethanol and water extract of *Bombax ceiba* flower were tested for in vitro antioxidant activity using standard procedures.

Determination of DPPH scavenging activity.

The 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance was measured at 517nm in spectrophotometer method. The lower absorbance of the reaction mixture was indicated higher free-radical scavenging activity.

Radical scavenging activity (%) = 100 —A_C – A_S/A_C

Where $A_{C=}$ control is the absorbance and $A_{S=}$ sample is the absorbance of reaction mixture in the presence of sample(10).

Reducing power scavenging activity

The assay based reduction of Mo (VI)–Mo(V) by the extract subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 ml extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95◦C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid. The scavenging activity was calculated according to the following equation: % Inhibition.

$$\% \text{ of Inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract(11).

Superoxide scavenging activity

The experiments of superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 µM) solution, 0.75 ml of NADH (936 µM) solution 0.3 ml of different concentrations of the extract. The reaction was calculated by adding 0.75 ml of PMS (120 µM) to the mixture. After the 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity is calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0\text{-}A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract(12).

Hydrogen peroxide scavenging activity

To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of FeCl2 (2mM) was added. After the 30 s, 0.1 ml ferrozine (5mM) was added. The ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe2+-Ferrozine complex was measured at 562 nm.

The chelating activity of the extract for Fe2+ was calculated as:

$$\text{Chelating rate (\%)} = (A0 - A1) / A0 \times 100$$

Where A0 was the absorbance of the control (blank, without extract) and A1 was the absorbance in the presence of the extract (13).

Nitric oxide scavenging activity

The various concentrations was mixed with 0.75 ml of phosphate buffer (0.2M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate (K3Fe(CN)6) (1%, w/v), followed by incubating at 50oC in a water bath for 20 min. The reaction was stopped by 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 800g for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride (FeCl3) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured by reducing power. Higher absorbance of the reaction mixture indicated greater reducing power (14).

Statistical analysis

All data are represented as mean + standard deviation. Differences between treated versus time-matched control animals were evaluated by analysis of variance using ANOVA test followed by a Student t-test. All p-values<.05 were considered to be statistically significant. No adjustment was made for multiple testing.

RESULT AND DISCUSSION

The qualitative phytochemical screening in methanolic, ethanolic and chloroform extract of *Bombax ceiba* flower extract was given presented in table1. totally 13 Different phytochemical like alkaloids, glycosides, saponins, tannins, terpenoids, reducing sugars, phenolic compounds, flavanoids, protein and carbohydrates were identified and the results were given in Table.1.among the 4 solvent methanolic extract shows presence of all the phytochemical followed by ethanolic extract (n=10).

Table 1. Preliminary phytochemical analysis of *Bombax ceiba* (flower)

S. NO	Phytochemicals Solvent Extracts	Water	Ethanol	Methanol	Chloroform
1	Alkaloids	++	++	++	++
2	Cardiac Glycosides	--	++	++	--
3	Carbohydrates	++	++	++	++
4	Flavonoids	--	++	++	--
5	Phenols	--	++	++	--
6	Phlobatannin	--	--	+	--
7	Proteins	++	++	++	--
8	Saponins	++	++	++	++
9	Sterols	--	--	+	--
10	Tannins	++	++	++	++
11	Terpenoids	--	++	++	--
12	Quinones	++	++	++	--
13	Oxalates	--	---	+	--

(+) Presence; (-) Absence; (++) High concentration

The above data showed the presence of Alkaloids, Saponins, Tannins was recorded in all four type of extract. Flavonoids and Phenols were identified only on ethanol and methanol extraction. The DPPH scavenging effect increased with the increasing concentrations of *Bombax ceiba* powder extract as compared to standard ascorbic acid and highest DPPH scavenging activity of *Bombax ceiba* was observed as 87.47±5.98%inhibition at 80 µg/ml concentration which indicates the DPPH scavenging effect of *Bombax ceiba* as compared to ascorbic acid (Table 2).

Table 2- % of DPPH scavenging activity of *Bombax ceiba* flower extract at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Bombax ceiba flower extract	24.23±1.57	50.16±4.47	65.29±4.48	87.47±5.98	47.58
Standard (Ascorbic acid)	27.9±2.07	63.28±4.98	89.99±7.18	99.37±7.98	36.08

Values were expressed as Mean ± SD for triplicates

Reducing power scavenging activity

The Reducing power effect increased with the increasing concentrations of *Bombax ceiba* powder extract as compared to standard ascorbic acid and highest Reducing power activity of *Bombax ceiba* was observed as 75.53±5.13% inhibition at 80 µg /ml concentration which indicates the Reducing power effective of *Bombax ceiba* as compared to ascorbic acid[Table 3].

Table 3- % of Reducing power scavenging activity of *Bombax ceiba* flower extract at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Bombax ceiba flower extract	23.37±1.39	38.00±2.49	50.18±3.39	75.53±5.13	59.18
Standard (Ascorbic acid)	25.39± 1.87	54.24± 4.13	76.59± 5.82	89.38± 6.98	45.49

Values were expressed as Mean ± SD for triplicates

Superoxide scavenging activity

The superoxide scavenging effect increased with the increasing concentrations of *Bombax ceiba* powder extract as compared to standard ascorbic acid and highest superoxide scavenging effect of *Bombax ceiba* was observed as 83.32±5.79.% inhibition at 80µg /ml concentration which indicates the superoxide scavenging effective of *Bombax ceiba* as compared to ascorbic acid[**Table 4]**.

Table 4- % of Superoxide scavenging activity of *Bombax ceiba* flower extract at different concentrations

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**IC_{50} (µg/ml)**
Bombax ceiba flower extract	25.12±1.67	44.58±2.93	66.86±4.49	83.32±5.79	49.39
Standard (Ascorbic acid)	33.29 ± 2.56	68.29 ± 5.18	92.58 ± 7.21	99.54 ± 7.90	33.68

Values were expressed as Mean ± SD for triplicates

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging effect increased with the increasing concentrations of *Bombax ceiba* powder extract as compared to standard ascorbic acid and highest hydrogen peroxide scavenging effect of *Bombax ceiba* was observed as 0.79±0.08.% inhibition at 80 µg /ml concentration which indicates the superoxide scavenging effective of *Bombax ceiba* as compared to ascorbic acid[Table 5].

Table 5- Hydrogen peroxide scavenging activity of *Bombax ceiba* flower extract at different concentrations

Parameters	20(µg/ml)	40 (µg/ml)	60(µg/ml)	80 (µg/ml)
Bombax ceiba flower extract	0.25±0.03	0.48±0.06	0.69±0.07	0.79±0.08
Standard (Ascorbic acid)	0.46± 0.06	0.79 ± 0.09	0.92± 0.09	0.99 ± 0.09

Values were expressed as Mean ± SD (Optical density) for triplicates

Nitric oxide scavenging activity

The Nitric oxide scavenging effect increased with the increasing concentrations of *Bombax ceiba* powder extract as compared to standard ascorbic acid and highest Nitric oxide scavenging effect of *Bombax ceiba* was observed as 86.28±5.87.% inhibition at 80 µg /ml concentration which indicates the superoxide scavenging effective of *Bombax ceiba* as compared to ascorbic acid [Table 6].

Table 6- % of Nitric oxide scavenging activity of *Bombax ceiba* flower extract at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Bombax ceiba flower extract	23.25±1.46	46.19±3.08	69.47±4.82	86.28±5.87	48.59
Standard (Ascorbic acid)	38.28 ± 2.88	68.26 ± 5.32	79.56± 6.32	99.69 ± 7.89	32.99

Values were expressed as Mean ± SD for triplicates

The screening process alkaloids, tannins, saponins, flavonoids and terpenoids, glycosides, phenols shows different types of results in different solvents. From the *Bombax ceiba* flower. Water extract showed the presence of carbohydrate, alkaloids, saponins and tannins. The 70% ethanol and acetone had the presence cardiac glycosides, carbohydrates, flavonoids, phenols, saponins, proteins, alkaloids and terpenoids. The methanol extract had the. Presence of cardiac glycosides, carbohydrate, alkaloids, flavonoids, phenol, tannins, saponins and terpenoids. The medicinal value of plants means definite physiological action on the human body due to presence chemical substances. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against diseases. Alkaloids protect against chronic diseases. Saponins protect against hypercholesterolemia and antibiotic properties. Steroids and triterpenoids show the analgesic for central nervous system activities (15).The result indicates that *Bombax ceiba* flower hold promises as source of pharmaceutically important phytochemicals. Flavonoids generally present in a real parts like flowers play some metabolic role and control development in living system. Tannins are known to inhibit pathogenic fungi.The flavonoids and phenolic compounds in plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti- inflammatory, anti-carcinogenic, astringent, cytotoxicity, anti- diabetic, cardiotonic, antipyretic effects etc. [16]. The content of polyphenols in the extract was calculated and expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight material) (17-18).The evaluation of flavonoid. In addition, 1 ml of 2 **%**AlCl3 methanolic solution was added to 3 ml of extract or normal and allowed to stand at room temp. For 15 min. absorption was measured at 420 nm [19].The DPPH is commonly used as a tool for evaluate the free radical scavenging activity of new compounds. The stable free radical which is reduced when it receives an electron or hydrogen atom. The hydrogen donating ability is an index of the primary antioxidants [20]. The reducing power assay, potential antioxidants reduce the Fe3+/ferricyanide complex to its ferrous form which can then be monitored spectrophotometrically at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power [21]. The superoxide anion is a weak antioxidant, but it lead to the generation of powerful and hazardous hydroxyl radicals as well as singlet oxygen both of which contribute to oxidative stress. Therefore, it is very important to study the scavenging of superoxide anion [22]. The nitric oxide itself is not a very reactive free radical, however, over production of nitric oxide is responsible for the initiating lipid peroxidation and production of the free radicals (23).

CONFLICT OF INTEREST

All authors must declare any conflict of interest or any affiliation or involvement in any organization whether it is academic, commercial, financial, personal and professionally relevant to the work under consideration to avoid the potential for bias and accept responsibility for what is said in the manuscript.

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