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RESEARCH ARTICLE

Determining the Antioxidant Activity of *Bombax ceiba* Flower Extracts

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ABSTRACT

The present study was aimed to investigate the antioxidant activity of extracts of dried flower powder of *Bombax ceiba* which is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. *Bombax ceiba* is an important medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani. It has wide range of medicinal and pharmacological application like *In-vitro* Anti-inflammatory, Anti-diabetic, Antiobesity, Hypotensive, Antioxidant, Antiangiogenic, Antimicrobial, Cytotoxicity, Aphrodisiac and Antipyretic. Antioxidant study was performed on hydroalcoholic extract of shade dried flowers. Modern phytochemical screening of the flower has shown the presence of phenolic compounds, fatty acids, flavonoids, tannins and glycosides. Extracted flowers were evaluated for their antioxidant activity. The present study revealed that the *Bombax ceiba* different extracts of flower a plot was obtained of the percentage inhibition of IRT against concentration of the sample solutions was prepared and the IC_{50} values of the extracts were determined from the calibration curve. The methanol extract of the flowers showed high antioxidant activity with an IC_{50} of 1.827±2 mg/ml. However, the hexane and chloroform exhibited less antioxidant activity.

KEYWORDS

Bombax ceiba, antioxidant activity, IC_{50}, Antiobesity, Antiangiogenic

INTRODUCTION

Medicinal plants represent a rich source of antibiotic, antifungal, antiseptic and analgesic qualities[1]. They are used medicinary in different countries[2,3]. ***Bombax ceiba*** (Linn) Family (Bombacaceae), commonly known as salmali. It is widely distributed throughout India, in forest up to an elevation of about 1500m, also raised in plantation. In India, it is distributed from Rajasthan, and south ward into sarakallu and adjacent area of chittoor district, Andhra Pradesh. The leaves are large, spreading, glabrous, digitate, leaflets, lanceolate, 3-7 entire.

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This tree produces large crimson coloured Flowers, which are ornithophilous, the flowers have a hard perianth with stiff filaments and a well protected ovary when the tree is bare of stems many arranged in fine bundles of 9-12 each and an inner bundle of 15. Fruit capsule, dehiscing by 5 leathery or woody valves. Seed smooth, black or gray embedded in long white wool. Bark gray or brown covered with hard, sharp, conical prickles. Gum is light brown in colour resembling the galls, and gradually becomes opaque a dark brown. The various part of *B. ceiba* such as roots, leaves, seed, stem bark, flower, fruit and gum are documented to possess medicinal properties in ethnobotanical surveys conducted by ethno botanist and in traditional system of medicine such as ayurvedic. The

young leaves, petioles and seed cake (with very little or no gossypol) are used as excellent cattle feed. The immature calyx known as Semargulla is consumed as a vegetable in Uttar Pradesh, in addition to the flowers and fleshy calyx. The plant is literature survey to possess beneficial effect as astringent, cooling, stimulant, diuretic, aphorodisiac, demulcent, dysentery and tonic. It is also beneficial to in asthma, expectorant, diarrhoea, wound, leucorrhoea, anaemia, splenomegaly and tuberculosis. A literature review of plant to be possessed some important pharmacological activity such as, anti-inflammatory and hepatoprotective, anticancer and anti-HIV activity, anti-helicobacter pylori activity, antiangiogenic activity, analgesic and antioxidant activity, inhibitory effects on tube-like formation of human umbilical venous cells**,** hypotensive, hypoglycemic activity, cholinesterase and antimicrobial activity. The present review articles of plant are to be discussed folk, ayurvedic uses, and pharmacological and phytochemistry studies conducted on *B. ceiba* and also pinpoints unexplored potential of it.

Antioxidant compounds play vital role in protecting plants against destructive chemical compounds including free radicals and reactive oxygen species (ROS) that are continuously produced by the cell metabolism and their concentration increases under stress conditions[4] Free radicals, in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS), are an integral part of normal physiology. Free radicals or reactive oxygen species, such as superoxide anion ($O^{-2\cdot}$), hydroxyl radical ($OH^{\cdot}$) and peroxyl radical ($ROO^{\cdot}$), are particularly reactive and are known to reduce the concentration of molecular oxygen in the cell[5] They damage the macromolecules such as nucleic acids, proteins and membrane lipid and consequently trigger a series of aging-related problems[6]. Free radical-mediated oxidative stress is believed to be the primary cause of many disorders, such as cardiovascular diseases, brain dysfunction, cataract, diabetes mellitus, arthritis, cancer and ageing. Thus there is the need of antioxidant of natural origin because they can protect the human body from the diseases caused by free radicals[7,8]. In the treatment of these diseases, antioxidant therapy has gained utmost importance in the recent years[9]. Antioxidants are able to scavenge or deactivate free radicals before they attack plant cells. Flavonoids and polyphenols exist widely in plants and are considered as important dietary antioxidants, which are responsible for the prevention of oxidative damage in mammalian system[10,11].

MATERIAL AND METHODS

Flower Material

The flowers of *Bombax ceiba* were collected from nearby villages of Paderu, Visakhapatnam and brought to the laboratory. Then the flowers were rinsed twice with distilled water and air dried on a clean sheet for one week at room temperature. It was made into small pieces using sharp sterile scissors and powdered using sterile mortal and pestle.

Extraction: The dried flowers were coarsely powdered. 50 gm powder of flowers was subjected to successive solvent extract. Hexane, chloroform and methanol using a Soxhlet apparatus at 65°C. After 24 hours it was filtered through 8 layers of muslin cloth and centrifuged at 5000xg for 15min. The supernatant was collected and the solvent was evaporated to make the final volume one- fourth of the original volume and it was stored at 40^{o}C in air tight bottles for further studies.

Total Phenolic Compound Analysis

The total phenolics were determined using the Folin Cio-calteau reagent as reported by Javanmardi et al[12]. The total phenolic compounds play several important functions in plants. They represent a striking example of metabolic plasticity enabling plants to adapt to changing biotic and abiotic environments. These compounds also provide to plant products, i.e. colour, taste, technological properties and putative health promoting benefits. Most plant phenolic natural compounds are derived from trans-cinnamic acid, formed by deamination of L-Phenylalanine by L-Phenylalanine ammonia-lyase. The hydroxyl (-OH) groups of phenolic

compounds reduce the phosphomolybdic acid to molybdenum blue in the presence of an alkaline medium (present in Folin's reagent). The blue coloured complex was then spectrophotometrically measured at 760 nm.

In this method 100µl of the each sample, 2ml of diluted Folin Cio-calteau reagent and 2ml of 7.5% (W/W) sodium carbonate was added and incubated at 45°C for 15mins. The absorbencies were taken by using spectrophotometer at 765nm. The results were Expressed as mg of gallic acid equivalent per/mg weight.

Ferric Ion Reducing or Antioxidant Power Assay (FRAP)

Total antioxidant power of the sample was assayed by the method of Benzie IFF and J.J Strain[13]. At low PH the reduction of a ferric tri pyridyl triazene [Fe III-TPTZ] complex to the ferrous form, which has an intense blue colour, can be monitored by measuring the change in absorbance at 593nm. The reaction is non – specific, in that any half reaction that has a lower redox potential, under reaction conditions, then that of the Ferric /ferrous half reaction will drive the ferric (Fe III) to ferrous (Fe II) reaction. The change in absorbance, therefore, is directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. In this method 3ml of FRAP working reagent was taken in a test tube then 100µl of plant extract was added, this is vortex mixed and the absorbance was read at 593nm against a reagent blank at a predetermined time after sample- reagent mixture. The results were Expressed as mg of gallic acid equivalent per/mg weight. or FRAP units.

Iron (III) to Iron (II) Reducing Activity (or) Reducing Power Assay

The ability of the extracts to reduce iron (iii) was assessed by the method of Oyaizu, M.[14]. Antioxidants in the plants may disrupt the Fe^{3+} to Fe^{2+} transformation by competing with O_2^- and, thereby causing a decrease in the formation of hydroxyl radicals. The antioxidants present in the sample reduced the oxidant probe and the respective product interacted with some colouring agents to form a colured complex. In this method, 0.1ml of plant extract dissolved in distilled water was mixed with 1ml of phosphate buffer (0.2M, PH 6.6) and add 1ml of Fecl3 and add 1ml of TCA and add 200µl of potassium hexacynoferrate and the absorbance was recorded at 700nm. The antioxidants reduced the Fe^{3+} to Fe^{2+}. This ion then conjugated with the ferricyanide ion to form a Prussian blue coloured product, which is spectrophotometrically measured at 700 nm. The change in optical density is directly related to the total reducing power of the electron donating antioxidants available in the reaction mixture.

DPPH Radical Scavenging Assay

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the nonradical form DPPH-H[15].

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). To 4 ml of DPPH radical solution, add 100µl of the extract and the reaction mixture is vortex and allow to stand at room temperature for 30 min. The absorbance is read at 517nm by using UV-Vis spectrophotometer. Compare with the 75% ethanol which acts as control solution. The percentage of DPPH radical scavenging activity is expressed as

DPPH % = 1- Test sample absorbance/ Blank sample absorbanceX100

RESULTS AND DISCUSSION

In this study the antioxidant activities of the extracts were evaluated by using the TPP, FRAP, IRT and DPPH assay. The results of the study indicate that methanol extract of *Bombax ceiba* flower possess the significant antioxidant activity. From the result a plot was obtained of the percentage inhibition of IRT against concentration of the sample solutions was prepared and the IC_{50} values of the extracts were determined from the calibration curve. The

methanol extract of the flowers showed high antioxidant activity with an IC_{50} of 1.827±2 mg/ml. However, the hexane and chloroform exhibited less antioxidant activity. The total phenolic contents of the extracts were also determined using the Folin- Ciocalteu reagent and expressed in terms of gallic acid equivalent (mg/g dry mass).The hexane extract of the flowers had a total phenolic content of 0.819±2 mg/g extract, whereas chloroform and methanol extracts had a total phenolic content of 0.324±2 and 0.321± 2 mg/g extract. The decrease in absorbance of DPPH radical caused by antioxidants, because of the reaction between antioxidant molecule and radical progresses, results in the scavenging of the radical by hydrogen donation[16].

Table 1: TPP, FRAP, IRT and DPPH activity of *Bombax ceiba* different flower extracts

Name of the extract	Absorbance at 750 nm	Absorbance at 593 nm	Absorbance at 700 nm	Absorbance at 517 nm
	TPP	FRAP	IRT	DPPH
Hexane	0.819	0.132	1.653	0.303
Chloroform	0.324	0.174	1.427	0.307
Methanol	0.321	0.491	1.827	0.324

From the obtained values it is observed that methanol fraction shows maximum DPPH radical scavenging activity (0.324± 2) than hexane and chloroform fraction. FRAP assay measures the ability to reduce ferric tripyridyltriazine (Fe3+-TPTZ) to a ferrous form (Fe2+-TPTZ) while DPPH assay was used on the capability to donate a hydrogen radical or an electron to DPPH. Also, the total phenolics present in the sample or their reducing capacity were determined by FC assay. The methanol extract of the flower showed maximum FRAP activity (0.491±2) value than hexane and chloroform.

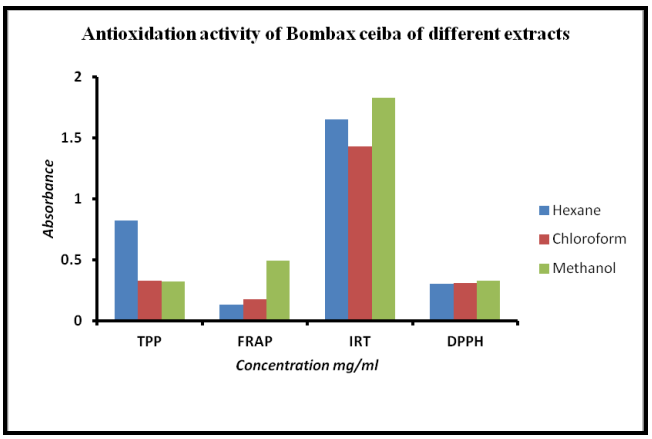

Figure 1: Antioxidant activity of *Bombax ceiba* of different extracts

CONCLUSION

Based on the results, it can be concluded that methanolic extracts of the studied medicinal plants had different level of antioxidant potential. ther, isolation and identification of active components and evaluation of possible synergism among them for their antioxidant activity can be done.

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