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Research Article

Assessment of the In-vitro antioxidant activity of the alcoholic extract/fractions of *Bombax malabaricum* DC.

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ABSTRACT

Various parts of *Bombax malabaricum* are used in traditional Indian medicine for the treatment of inflammatory diseases, diarrhea, fever, acute swelling and diuretic. Current research on *Bombax malabaricum* DC is aimed at finding of natural plants derived from antioxidants that provide sustenance through additional components or synergistic training as the plant's cellular reinforcements are necessary to prevent the movement of interfaced free radical problems. The antioxidant activity in *Bombax malabaricum* DC can be detected by alcoholic extract fraction using unique laboratory models. These include free radical activity of 1,1-Diphenyl-2-picryl-hydrazil (DPPH) assay, ABTS assay, total antioxidant capacity, and o-phenanthroline assay/Iron chelating assay. The plant contains a large number of phenolic and flavonoid compounds. The plant shows great antioxidant activity. The thought was taught to investigate the use of the *Bombax malabaricum*

Keywords: *Bombax malabaricum*, Antioxidant, DPPH, ABTS, O-phenanthroline.

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INTRODUCTION:

Responsive oxygen species (ROS) such as Superoxide (O_2^-), hydrogenradicals (OH^-), and hydrogen peroxide (H_2O_{2}-) are considered as significant elements causing numerous disorders like cardiovascular, diabetes, swelling, malignant growth and neurogenerative diseases[1,2]. ROS are corrupted to non-receptive structures by Enzymatic and Non-Enzymatic resistance components. Free radicals respond with known biological molecule and harm protein, breakdown of DNA strands and starts peroxidation of different particles. Antioxidant agents go about as a noteworthy guard against radical-interceded poisonous quality, by securing the harms brought about by free radicals[3]. Antioxidative segments of common source have pulled in unique intrigue since they can shield human body from free radicals. Enzymatic cancer prevention agents incorporate essential catalysts like Superoxide Dismutase, Catalase, Glutathione peroxidase and auxiliary proteins incorporate Glutathione reductase etc[4].

Non-Enzymatic antioxidant agents incorporate either water-solvent (Vitamin C and phenolic agents) or lipid-dissolvable (vitamin E and carotenoids) agents which go about as protection against oxidative stress[5,6]. Anyway antioxidant agent like Phenols, Flavonoids scavenge these free radicals and shield the framework from the Oxidative mechanism.

The plants of antiquity are used for traditional treatment therapies and the literature explains the use of different parts of the plant in different dosage forms for diseases of different origins, including the infectious nature [7,8]. Products of natural plant origin are known for their strong medicinal activities. These plants are the sources of medicines from the many years. All plants contain active chemical constituents and groups of secondary plant metabolites such as alkaloids, steroids, glycosides etc.[9,10]. The Indian subcontinent is blessed by the nature and strength of traditional local healers, helping society maximize the impact of nature[7]. Many antioxidant compounds from plant sources that occur naturally as free radicals or oxygen and active nitrogen. Flavonoids are the most important group of natural antioxidants. Its biological effects are mainly related to the cleaning of free radicals or ionic minerals. Metal ions participate directly in the formation of interactive species [11].

Bombax malabaricum DC belongs from family Bombacaceae, commonly known as salmali. It is widely distributed throughout India, in the forest up to about 1500 meters high, since it was raised on the farm and found as rice in Malaysia.

In India, it is distributed from Rajasthan, the southern wing of sarakallu and in the adjacent area of chittoor district, Andhra Pradesh. The leaves are large, scattered, saturated, digitates, leaflets, insoles, 3-7 entire. The flowers are red, many, appear, when the tree is stripped of the stems arranged largely in beautiful bundles of 9-12 each and an inner package of 15. Fruit capsule, exposing 5 leather or woody valves. The seeds are smooth, black or gray, set in a long white wool. Gray or brown bark covered with hardness, sharpness and conical prickles. It is clear that the gums are brownish in color and gradually become dark brown. Various parts of the *Bombax malabaricum* such as roots, leaves, seed, stem bark, flowers, fruits and gums are documented to have healing properties in ethnographic investigations conducted by ethnologists and in traditional medicine systems such as Ayurveda[12,13].

This plant is widely used in traditional Chinese and Indian medicine for the treatment of inflammatory diseases, diarrhea, fever, chronic swelling, acute illness and as diuretic[14.15]. Earlier phytochemical researches of this plant have led to in the separation of naphthol and naphthoquinone derivatives, anthocyanins[16], flavonoids[15], several sesquiterpenoids[17], and polysaccharide[18]. Though, the viable activities or pharmacological properties of *B. malabaricum* explained, activity information antioxidant fraction of the extract is still limited. Bark utilized for astringent, diuretics, agonists, diuretics, treatment of cysts, wounds and other skin disorders. The leaves are anti-inflammatory, the roots are aphrodisiac, anti-diarrhea cure. The flowers are diuretics and laxatives and the gums are used in hemodialysis. seeds are used in gonorrhea[19]. decoction bark traditionally used abroad in the inflammatory process, and seal secondary infections, the wounded skin healing in paste form and the leaves of this plant is grounded and the mixed milk is provided for choking and inflammation. Despite the traditional use of this plant, no scientific report has focused on the biological activity of the *Bombax malabaricum*. The chemical prevention of cancer is one of the main areas investigated in the last years[20].

The consumption of many vegetables is associated with a reduced risk of degenerative diseases, which depend on oxidative stress such as atherosclerosis, tumors, diabetes and Alzheimer's disease[21,22]. Good attention is given to naturally occurring antioxidants, which can play important functions that inhibit both free radicals and oxidative chain reactions within tissues and membranes[23]. Consequently, the screening of plant materials based on potential antioxidants can have a central task to identify potential extracts or fractions to remove all the roots and initiate a chain reaction or seal catalysts for oxidizing reactions, such as some minerals[24]. From the point of view of its high antioxidant strength, it is recommended to consume high levels of many plants, so analyzing antioxidant activity in extracts and fractures is an important step before detoxifying the antioxidant and it[25]. In the present study an attempt was made to estimate the impact of antioxidants effect of extract fraction of the *Bombax malabaricum* againest by various in vitro models.

MATERIALS AND METHODS

Collection of Plant material

The plant material (*Bombax malabaricum*) was collected from smriti van, Jaipur (Jaipur, Rajasthan, India) in the month of September and authenticated by Herbarium department, University of Rajasthan, Jaipur, Rajasthan, India. The plant was deposited in the herbarium at Department of Botany (University of Rajasthan, Jaipur, Rajasthan, India).

Preparation of extract

Alcoholic extract: - The coarsely powdered (1000 g) oven-dried *Bombax malabaricum* was extracted with alcohol by using soxhlet apparatus for 20 h. After completion of extraction, the solvent was removed by distillation and concentrated. The yield obtained was 20.54 % w/v.

Fractionation of crud extract

Fractionation of alcoholic extract Completely dried ethanolic extract was suspended in distilled water and extracted successively and exhaustively with solvents of increasing polarity like petroleum ether, dichloromethane, n-butanol, ethyl acetate. Each fraction was concentrated using rotary evaporator (Rotavapor, R-210, BUCHI Laborte, Switzerland) and stored in vacuum desiccator. The percentage yield of various extracts/fractions was ethanol (BME) [26.31% w/v], petroleum ether (BMP) [2.51 % w/v], dichloromethane (BMD) [8.14 % w/v] and n-butanol (BMB) [22.81 % w/v]

Aqueous extract

1000 g of the coarsely powdered root of *Bombax malabaricum* was extracted by chloroform water (1:99) by cold maceration process for 7 days. After completion of extraction, the marc was filtered through muslin cloth and concentrated. The yield of BMA was obtained 21.56 %.

Qualitative determination of the chemical constituents

Presence of alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, phlobatannins, tannins and terpenoids in the extract and various fractions was confirmed individually by following standard procedures.

Test for alkaloids

Mixture of methanol extract of *Bombax malabaricum* extract/fraction and its various derived fractions (0.4 g) in 8 ml of 1% HCl was warmed on water bath. After filtration 2 ml filtrate from the extract and each fraction was allowed to react with few drops of potassium mercuric iodide and with potassium bismuth, separately. Turbidity or precipitation formation was considered as a confirmation for presence of alkaloids.

Test for saponins

The criterion of oil emulsion formation of saponins was used for the screening of saponins. Briefly, extract and various fractions (20 mg) suspended in 20 ml of distilled water and boiled for 5 min. In 10 ml of the above filtrate 5 ml of distilled water was added and mixed well to develop the froth. Development of emulsion after mixing the froth with olive oil confirmed the existence of saponins.

Test for terpenoids

Briefly, 2 ml of chloroform was mixed with 5 ml (1 mg/ml) of each sample in a test tube then 3 ml of concentrated H_2SO_4 was added to develop the color. Exhibition of reddish-brown coloration at the interface confirmed the presence of terpenoids.

Test for anthraquinones

To a volume of 6 ml of 1% HCl, 200 mg of each sample was added separately and boiled. Benzene (5 ml) was mixed with the filtrate and after separation of benzene layer 2 ml of 10% ammonia solution was lowered. Development of pink, violet or red color in the ammoniacal phase indicated the existence of anthraquinones[26].

Cardiac glycosides determination

An aliquot of 5 ml of ethanol extract of *Bombax malabaricum* and its various fractions (10 mg/ml in methanol) were added in the sequence of glacial acetic acid (2 ml) and $FeCl_3$ solution (one drop). Concentrated H_2SO_4 (1 ml) was added and the formation of brown ring at the interface confirmed the presence of cardiac glycosides.

Test for coumarins

In a vial having 300 mg/ml of the extract and each fraction was plugged with filter paper dipped in 1 N NaOH and boiled in a boiling water bath for few minutes. Yellow fluorescence of filter paper under UV light confirmed the presence of coumarins.

Test for phlobatannins

An amount of 80 mg of the extract and various fractions was boiled in 1% HCl. Development of red precipitate indicated the existence of phlobatannins[27].

Test for flavonoids

Mixture of methanol extract and various fractions of *Bombax malabaricum* were prepared by adding 50 mg of each sample to 100 ml of distilled water and filtered. An aliquot of 5 ml of dilute ammonia solution was mixed with 10 ml of the filtrate. Appearance of yellow coloration by addition of few drops of concentrated sulfuric acid indicated the presence of flavonoid.

Test for tannins

A mixture was prepared by mixing 50 mg of methanol extract and each fraction in 20 ml of distilled water and boiled. Appearance of brownish green or blue-black coloration after mixing few drops of 0.1% $FeCl_3$ confirmed the existence of tannins[28].

Determination of in-vitro scavenging activities[34]:

DPPH radical scavenging activity:

The free radical scavenging activity of the extract and fractions was evaluated using the stable DPPH free radical. One mL of 0.1 mM DPPH solution in methanol was added to 1.0 mL of standard and extract/fractions solution at different concentrations. The mixture was incubated for 20 min and the absorbance recorded at 517 nm. Ascorbic acid was used as positive control. DPPH radical scavenging activity was calculated using the formula:

Percent scavenging = $((Ao - At)/Ao) \times 100$; where Ao =Absorbance of control (without extract) and At = Absorbance of sample. All determinations were carried out in triplicate[29].

ABTS radical scavenging activity:

ABTS free radical was generated by reacting 7 mM ABTS solution with 2.45 mM potassium per sulphate. The mixture was allowed to stand for 15 h in dark at room temperature. ABTS solution was diluted with methanol to obtain the absorbance of 0.7 ± 0.2 units at 750 nm. The standard/extract solutions were prepared at different concentrations in methanol and 20 µL of test solutions were added to 180 µL of ABTS free radical solution. The absorbance was measured after 20 minutes incubation at 750 nm. Ascorbic acid was used as positive control. The ABTS free radical scavenging activity was calculated using the formula:

$$\text{Percent scavenging} = ((Ao - At)/Ao) \times 100;$$

where Ao = Absorbance of control (without extract) and At = Absorbance of sample[30]. All the tests were performed in triplicate.

Total antioxidant capacity:

The total antioxidant capacities of the extract and fractions of *Bombax malabaricum* were determined using phosphomolybdenum method. Briefly, 0.1 mL of standard/ extract solution was mixed with 0.3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. The mixture was cooled down to room temperature and absorbance recorded at 695 nm. The blank solution contained all the reagents except the test sample. Ascorbic acid was used to plot the standard curve. The results were expressed as ascorbic acid equivalents[31]. All the tests were performed in triplicate.

o-Phenanthroline assay/Iron chelating activity:

The 1, l0-Phenanthroline-iron (III) reagent was prepared by mixing 0.198 g of l, l0-phenanthroline monohydrate, 2 mL of 1 M hydrochloric acid and 0.16 g of ferric ammonium sulphate in 100 mL water. Briefly, 0.2 mL standard/extracts were mixed with 0.2 mL 1, l0- phenanthroline-iron (III) reagent, 0.6 mL methanol and 4 mL water. The solutions were incubated at 50°C for 30 min and absorbance read at 510 nm. Ascorbic acid was used as positive control. A higher absorbance indicated higher iron chelating activity

Percentage scavenging was calculated by using the following formula:

Percent scavenging = $((At - Ao)/At) \times 100$; where Ao = Absorbance of control (without extract) and At = Absorbance of sample[32,33]. All the tests were performed in triplicate.

RESULTS AND DISCUSSION

Different concentrations of 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg / ml of alcoholic extracts were compared with their antioxidant activity in different in vitro models. Free radicals are removed from all selected extract fractions in a concentration-based manner until concentration is given in all models. On a comparative basis, ethanol and butanol fraction showed good antioxidant activity compared to remaining fraction these are ether and DCM part (Table 2 and 3).

DPPH is a stable free radical. The in vitro study carried out on this radical is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. This radical reacts with suitable reducing agents, the electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up[35]. From the present results, it may be concluded that the extracts reduce the radical to the corresponding hydrazine when they react with the hydrogen donors in the antioxidant principles. The result show that ethanol and butanol fraction compared to pet. Ether and DCM fraction % SC_{50} found both potent fractions 6.98% and 7.19% respectively by DPPH assay method (Table no. 2 and fig. 1).

In the present study, examined by ABTS radical scavenging assay method the observation shows ethanol and butanol fraction compared to pet. Ether and DCM fraction % SC_{50} found both potent fractions 4.69% and 4.06% respectively (Table no. 2 and fig. 2). This may be due to the antioxidant principles in the ABTS radical cation was produced in the stable form using potassium per sulphate. After getting the stable absorbance, the antioxidant plant extract is added to the reaction medium and the antioxidant power was measured by studying decolorization[36]. ABTS assay is an

excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants[37]. Different artificial free radical species, such as ABTS + radical cation, has been used to assess radical scavenging ability and antioxidant activity.

Ortho-substituted phenolic compounds may exert pro-oxidant effects by interacting with iron. O-phenanthroline quantitatively forms complexes with ferric ion which get disrupted in the presence of chelating agents. The extract fractions interfered with the formation of ferrous-O-phenanthroline complex, thereby suggesting that the extract fractions have metal chelating activity and show in table no. 2 and fig. 3. The result of this assay method shows pet. Ether and DCM fraction compared to ethanol and butanol fractions % SC_{50} found both potent fractions 9.63% and 101.9% respectively.

The total antioxidant activity of the extract fractions was calculated based on them formation of phosphomolybdenum complex which was measured spectrophotometrically[37] the obtained result show in table no. 2. Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like liver and brain. In this study, lipid peroxidation was induced in vitro and the extracts showed concentration dependent prevention towards generation of lipid peroxides.

Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, phenolic compounds, tannins, saponins and flavonoids (show in Table no. 1) in the selected plant[39]. Phenolics, flavonoids, saponins, and tannins have been proved to be responsible for the antioxidant activity of various medicinal plants reported earlier[40]. Hence, these may be responsible for the observed activity in the plant. The present study proved the antioxidant potential of *Bombax malabaricum*.

Table 1: Estimation of phytochemicals of extract and its fractions of *Bombax malabaricum*.

Phytochemical tests	Ethanol	Pet. Ether	Butanol	Dichloromethane	Aqueous
Alkaloids	+	+	-	+	+
Anthraquinones	+	+	-	+	+
Cardiac glycosides	+	-	-	-	+
Coumarins	+	-	+	+	+
Flavonoids	+	+	+	+	+
Saponins	+	-	+	+	+
Phlobatannins	+	-	+	-	+
Tannins	+	+	-	+	+
Terpenoids	+	+	+	+	+

Table No. 2: Effect of different extract/fractions of *Bombax malabaricum*. in various in vitro antioxidant assay

Extract/ Fractions	% SC_{50} values		
	DPPH	ABTS	O-phenanthroline
Ethanol	6.98	4.69	198.4
Pet. Ether	200.06	48.31	101.9
DCM	50.12	31.64	91.63
Butanol	7.19	4.06	298.40
Aqueous	41.04	51.93	189.83

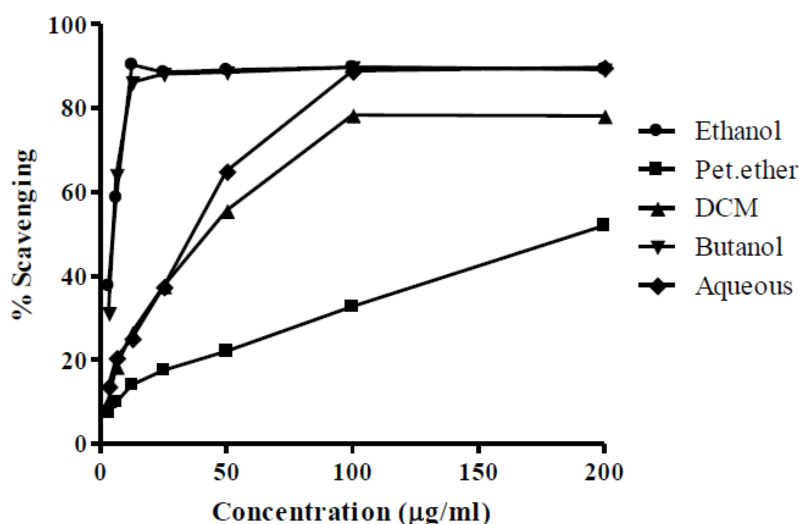

Fig. 1: Effects of different extract/fractions of *Bombax malabaricum*. in in vitro DPPH antioxidant assay.

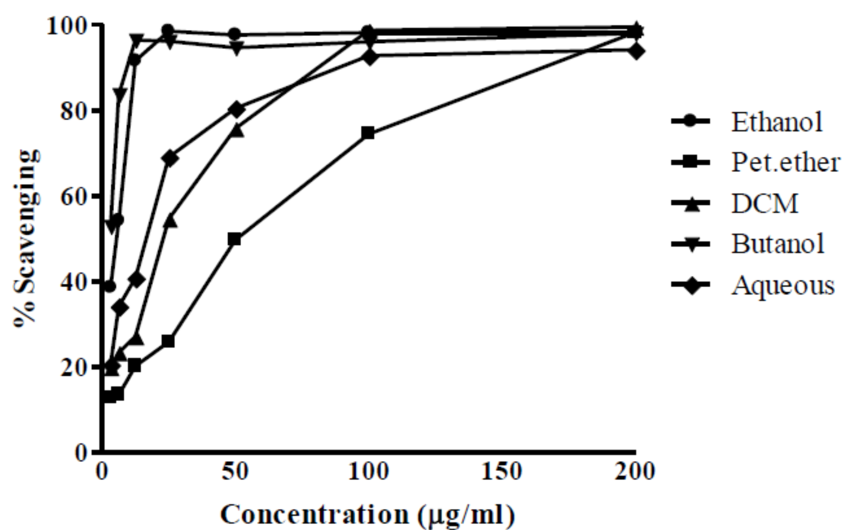

Fig. 2: Effects of different extract/fractions of *Bombax malabaricum*. in in vitro ABTS radical scavenging assay.

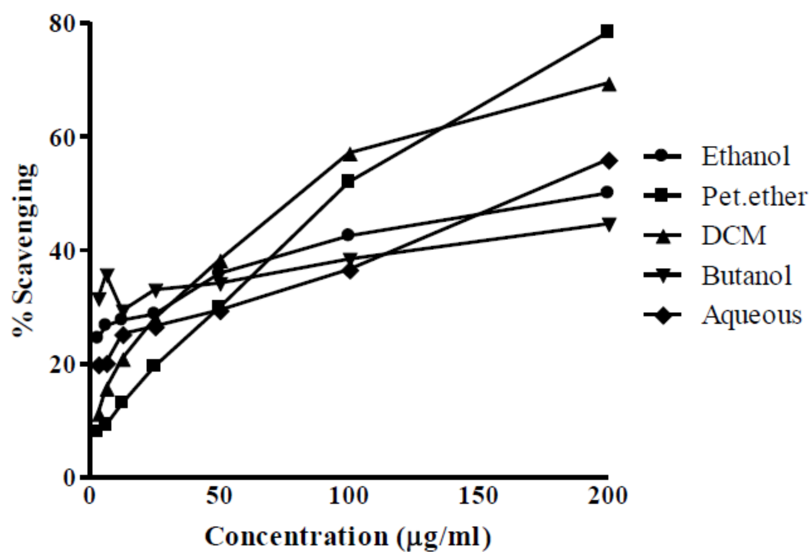

Fig. 3: Effects of different extract/fractions of *Bombax malabaricum*. in in vitro O-phenanthroline assay

Table 3: Estimation of Total Antioxidant Capacity of different extract/fractions of *Bombax malabaricum*.

Extract/Fraction	Concentration equivalent to Ascorbic acid		
Concentration	**(2mg/ml)**	**(5mg/ml)**	**(10 mg/ml)**
Ethanol	535.36	1035.83	1816.07
Pet. ether	204.64	367.74	454.64
DCM	215.36	437.74	836.79
Butanol	635.83	1069.17	908.45
Aqueous	269.88	417.74	498.93

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