Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(1): 1504-1507
Received: 25-11-2018
Accepted: 28-12-2018

Meenakshi SC
Department of Environmental Science, Gulbarga University, Kalaburagi, India

Basavaraj S Beldal
Department of Environmental Science, Gulbarga University, Kalaburagi, India

Ramesh L Londonkar
Department of Environmental Science, Gulbarga University, Kalaburagi, India

Correspondence
Ramesh L Londonkar
Department of Environmental Science, Gulbarga University, Kalaburagi, India

Evaluation of methanolic extract of *Bombax ceiba* bark for *in-vitro* antioxidant and Anti-inflammatory activities

Meenakshi SC, Basavaraj S Beldal and Ramesh L Londonkar

Abstract
The objective of the study was to investigate the anti-oxidant and anti-inflammatory effect of Methanolic extract of *Bombax ceiba* bark. The antioxidant activities of the extract Was evaluated using *in vitro* Ferric reducing power, other activities like Free radical scavenging activity by DPPH method, hydroxyl radical scavenging activity, Nitric oxide radical scavenging activity, Super oxide anion Radical Scavenging Activity and total antioxidant capacity were also carried out. The Anti-inflammatory potential of the extract was evaluated using Hyaluronidase inhibition assay. The effect was compared with a known antioxidant agent (BHA/Ascorbic acid). It was found that the extract at different concentration was exhibited a significant dose dependent antioxidant and anti-inflammatory effect.

Keywords: *Bombax ceiba*, free radical, DPPH, hyaluronidase inhibition

Introduction
Bombax ceiba, commonly known as the silk cotton tree and as Semul, belongs to the family Bombacaceae. It is an important medicinal plant widely cultivated in Pakistan, India, China and Australia [1]. The plant 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 [2]. 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 [3]. An imbalance between the concentration of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the antioxidant defence mechanisms of the body plays a pivotal role in many pathologies. Antioxidants are the compounds that can scavenge free radicals play a significant role in preventing damage of cell proteins, lipids, carbohydrates, nucleic acids as well as bio membranes caused by reactive oxygen species [4]. Many antioxidant compounds present naturally in plants have been identified as free radical or active oxygen and nitrogen scavengers. The flavonoids are authentically considered as the most important group among the natural antioxidants.
Inflammation is local response of living tissues to the injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reactions that can contribute to the multiple symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation can represent such component of inflammation [5].

Materials and Methods
DPPH free radical scavenging activity [6].
Different concentrations (10µg, 50µg and 100µg) of samples in Dimethyl sulfoxide (DMSO) adjusted to 500µl by adding methanol. Five milliliters of a 0.1 mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH; from Sigma –Aldrich, Bangalore) was added. A control without the test compound, but with an equivalent amount of methanol was maintained. The absorbance of the samples measured at 517 nm after 20 min of incubation at room temperaure. Butylated Hydroxy Anisole (BHA) was used as reference standard, and free radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = \frac{(control\ \text{OD} - sample\ \text{OD})}{Control\ \text{OD}} X\ 100$$

Nitric oxide radical Scavenging Activity [6].
Various concentrations (10µg, 50µg and 100µg) of samples and Butylated hydroxy anisole

(BHA) were taken in different test tubes and made up to 3ml with 0.1M phosphate buffer (pH 7.2). Sodium Nitroprusside (5mM) prepared in buffered saline (pH7.2) was added (1ml) to each tube. The reaction mixture was incubated for 30 min at RT. A control without the test compound, but with an equivalent amount of methanol was maintained. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1-Naphthylethylenediamine dihydrochloride). The absorbance of the samples was measured at 546 nm.

Hydroxyl Radical Scavenging Activity [7].

Various concentrations (10µg, 50µg, and 100µg) of samples in DMSO were taken in different test tubes and made up to 250µl with 0.1M phosphate buffer. One milliliter of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of Dimethyl sulphoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. These reaction mixtures were incubated at room temperature for 15 min. The reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5%w/v). Three milliliters of Nash reagent (150 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone was mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at412 nm against reagent blank. Ascorbic acid (AA) was used as reference standard.

The percentage of hydroxyl radical scavenging activity was calculated by the following formula

$$\%\ \text{hydroxyl radical scavenging activity} = 1 - \frac{\text{Difference in absorbance of sample}}{\text{Difference in absorbance of blank}} \text{x} 100$$

Ferric Reducing Antioxidant Power [8].

Various concentrations of sample (10µg, 50µg and 100µg) was mixed with 2.5mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid (w/v) was added, 5 mL of above solution was mixed with 5 mL of distilled water and 1 mL of 0.1% of ferric chloride, the absorbance was measured spectrophotometrically at 700 nm. Ascorbic acid(AA) was used as standard antioxidant.

Super oxide Anion Radical Scavenging Activity [9].

Nitroblue tetrazolium (NBT;150 µM in Tris-HCl buffer 16mM pH 8.0; 1ml) and Nicotinamide adenine dinucleotide (NADH; 234 µM in Tris-HCl buffer 16mM pH 8.0; 1ml) were mixed in a series of test tube. Various concentrations (10µg, 50µg and 1000µg) of samples and Ascorbic acid (AA) were added to these test tubes and made up to 3ml with Tris-HCl buffer(16mM; pH 8.0).Ascorbic Acid (AA) was used as reference standard for comparison. Phenazine methosulphate solution was added(40 µM; 1ml) to each test tube, The reaction mixture was incubated for 5 min at RT. A control without the test compound was maintained. The absorbance of the samples was measured at 560 nm. Super oxide radical scavenging activity was calculated using the following formula:

$$\%\ \text{SO radical scavenging activity} = \frac{(control\ \text{OD} - sample\ \text{OD})}{Control\ \text{OD}} X\ 100$$

Total antioxidant capacity [10].

Various concentrations of samples (10µg, 50µg and 100 µg) were taken in a series of test tubes. To this, 1.9 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. and allowed to cool. The absorbance of the aqueous solution of each was measured at 695 nm against a blank. Antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. Butylated hydroxy anisole (BHA) was used as reference standard. The values are expressed as ascorbic acid equivalents in µg per mg of extract.

Hyaluronidase Inhibition Activity [11].

The assay medium consisting of 3 - 5U hyaluronidase (from Sigma –Aldrich, Bangalore) in 100µl of 20mM sodium phosphate buffer (pH 7.0) with 77mM sodium chloride, 0.01% BSA was preincubated with different concentrations (10,50, 100 µg/ml) of the test compound for 15 min at 37 °C. The assay was commenced by adding 100µl hyaluronic acid (from Sigma –Aldrich, Bangalore; 0.03% in 300mM sodium phosphate, pH 5.35) to the incubation mixture and incubated for a further 45 min at 37 °C. The undigested hyaluronic acid was precipitated with 1ml acid albumin solution made up of 0.1% bovine serum albumin in 24mM sodium acetate and 79mM acetic acid (pH 3.75). After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in the absence of enzyme was used as the reference value for maximum inhibition. The inhibitory activity of test compound was calculated as the percentage ratio of the absorbance in the presence of test compound *vs.* absorbance in the absence of enzyme. The enzyme activity was checked by control experiment run simultaneously, in which the enzyme was pre incubated with 5µl vehicle instead, and followed by the assay procedures described above. Compound was tested in a range of 10µg -100µg in the reaction mixture. Indomethacin (Indo) was used as reference standard.

Results and Discussion

The antioxidant activity of *Bombax ceiba* extract, at all the concentrations has exhibited a significant effect. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidant. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The extract and fractions are able to reduce the stable free radical to the yellow coloured diphenyl picryl hydrazine. The potential decrease in the concentration of DPPH radical due to the scavenging ability of *Bombax ceiba* (L) at different concentration 10, 50,100µg was found to be 28.63%, 42.56%, 61.82% respectively. table: 1. Similar observations were reported by Vieria *et al*., (2009). 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.

Nitric oxide is a very unstable species under aerobic condition. it is react with O_2 to produce stable product nitrate and nitrite to intermediates NO_2, N_2O_4, and N_3O_4. It is estimated by using Griess reagent. In presence of test compound which is a scavenger has decreased the amount of nitrous acid. The potency of the extract was evaluated for its Nitric Oxide radical scavenging activity. The extract at various concentrations showed dose dependent effect as depicted in (table: 2). In the present study, the nitrite produced by the incubation of solution of sodium nitrprusside in

phosphate buffer was reduced by the extract, This may be due to the antioxidant principles in the extracts which complete with oxygen to react with nitric oxide and thus inhibit the generation of nitrite

The extract of *Bombax ceiba* was examined for its ability to act as OH-radicals scavenging agent. The extract at the different concentrations i.e. 10, 50 &100µg produced dose dependent hydroxyl radical scavenging activity i.e. 67.61 %, 74.43%, 79.83 % respectively table: 3.

The Ferric Reducing Antioxidant Power and Super oxide Anion Radical Scavenging Activity are shown in table: 4 and 5 respectively.

The total antioxidant activity of the extracts was calculated based on the formation of Phosphomolybdenum complex which was measured spectrophotometrically [12]. Table 6

The Hyaluronidase inhibition activity was carried out to evaluate the anti-inflammatory potential of the extract. The potency of the extract was compared with the standard Indomethacin. The extract at different concentrations i.e. 10, 50,100µg showed dose dependent effect i.e. 20.09, 33.71, 48.21% inhibition where as the standard Indomethacin at10µg concentration exhibited 77.32% inhibition but at 50 µg and 100 µg exhibited much higher activity i.e. beyond the measurable range. table: 7.

Table 1: Percentage Free radical scavenging activity

Concentration	% Activity	
	Bc	BHA
10 µg	28.63±0.79	53.05±0.97
50 µg	42.56±0.74	68.53±1.41
100 µg	61.82±0.3	89.77±81

Table 2: % Nitric oxide scavenging activity

Concentration	% Activity	
	Bc	BHA
10 µg	10.11±0.27	52.81±1.79
50 µg	14.61±0.59	80.34±0.5
100 µg	23.60±1.2	93.26±1.04

Table 3: Percentage hydroxyl radical scavenging activity

Concentration	µg equivalent to AA	
	Bc	AA
10 µg	67.61±0.59	76.41±0.57
50 µg	74.43±0.67	82.10±0.74
100 µg	79.83±0.39	96.88±0.37

Table 4: Ferric Reducing Antioxidant Power

Concentration	Absorbance (700 nm)	
	Bc	AA
10 µg	0.049±0.003	0.062±0.004
50 µg	0.183±0.001	0.213±0.015
100 µg	0.671±0.01	1.000±0.1

Table 5: Percentage Superoxide radical scavenging activity

Concentration	% Activity	
	Bc	AA
10 µg	6.40±0.14	37.36±0.84
50 µg	12.80±0.32	46.88±0.36
100 µg	26.32±0.46	59.12±0.26

Table 6: Total antioxidant capacity

Concentration	µg equivalent to AA	
	Bc	AA
10 µg	08.65±0.79	12.65±0.55
50 µg	13.00±0.54	21.00±0.8
100 µg	22.55±0.29	39.50±0.7

Table 7: Percentage inhibition of hyaluronidase enzyme by the samples

Concentration	% Activity	
	B1	Indomethacin
10 µg	20.09±0.36	42.86±0.34
50 µg	33.71±0.49	87.95±0.15
100 µg	48.21±0.27	98.21±0.37

Bc- *Bombax ceiba*, AA – Ascorbic Acid

Conclusion

The methanolic extract of *B. ceiba* showed significant antioxidant potential against ROS and RNS and the anti-inflammatory potential of the extract provided the effectiveness of the extract in a dose dependent manner for inhibition of different enzymes responsible for oxidation and inflammation. This property may be related to its pharmacological activities. The Antioxidative effect and the anti-inflammatory effect of the plant extract is possibly due to the presence of flavonoids and phenolic components. Further studies will be aimed at the isolation and characterization of the substances responsible for the antioxidant and anti-inflammatory potential of the plant extract.

Acknowledgment

The authors are thankful to DST for financial support in the form of Inspire fellowship.

References

1. Chanda YR. The Wealth of India, Raw Material. New Delhi: CSIR, 1962, 175-183.
2. Nadkarni KM. The Indian Materia Medica, Popular Parkashan, Bombax, 1976, 207-209.
3. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proc National Acad Sci. 1993; 90:7915-7922.
4. Halliwell B, Gulteridge JM. Role of free radicals and catalytic metal ions in human diseases. Methods Enzymol. 1990; 105:105-114.
5. Paschapur MS, Patil MB, Kuma R, Patil SR. Evaluation of anti-inflammatory activity of ethanolic extract of *Borassus flabellifer* L. Male flowers(inflorescences) in experimental animals. J Medicinal Plants Res. 2009; 3(2):049-054.
6. Kumar S, Kumar D, Manjusha Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract, Acta. Pharm. 2008; 58:215-220.

7. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. Biochemistry. 1991; 20:6006-6012.
8. Barreira JCM, Ferreira ICFR, Oliveira MBPP, Pereira JA. Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars, Food Chem. Toxicol. 2008; 46(6):2230-2235.
9. Gulcin I, Alici HA, Cesur M. Determination of *in vitro a*ntioxidant and radical scavenging activities of Propofol. Chem. Pharm. Bull. 2005; 53(3):281-285.
10. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E1. Analytical Biochemistry. 1999; 269:337-341.
11. Ling SK, Tanaka T, Kouno I. Effects of iridoids on lipoxygenase and hyaluronidase activities and their activation by β-glucosidase in the presence of amino acids. Biol Pharm Bull. 2003; 26(3):352-356.
12. Kharya MD, Bhardwaj S, Sharma A. Screening methods of antioxidant activity, Pharmacog Reviews. 2007; 1:232-237.