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Research Article

EVALUATION OF ANTIOXIDANT ACTIVITY OF *CORCHORUS AESTUANS* LINN LEAVES EXTRACTS

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ABSTRACT
The leaves of *Corchorus aestuans* Linn are said to cure gonorrhea and used in making an injection for urethral discharge. In the present study, the antioxidant potency of *Corchorus aestuans* Linn crude methanol and its fractionated extracts (hexane, ethyl acetate and water) have been investigated, employing three different established *in vitro* testing systems, such as scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, reducing power assay and β-carotene method. The data obtained in these testing systems clearly establish the antioxidant potency of *Corchorus aestuans* Linn. As such, this is the first report on the antioxidant activities of *Corchorus aestuans* Linn.
KEY WORDS: Antioxidant activity, Tiliaceae, *Corchorus aestuans*, leaves extracts

INTRODUCTION

Free radicals are considered as important factors in the etiology of cancer, and components with antioxidant activity have received particular attention as potential inhibitors of several cancers. [1] BHA and BHT have been found to be anti-carcinogenic as well as carcinogenic in experimental animals. There are reports in the literature saying that BHA appeared to have tumor-initiating and tumor-promoting action. [2] Therefore, there has been considerable interest to develop natural antioxidants from botanical sources, especially edible medicinal plants, to replace synthetic antioxidants due to the long-term safety and negative consumer perception of synthetic antioxidants. [3] The natural antioxidants generally function as free radical scavengers and chain breakers, complexes of pro-oxidant metal ions and quenchers of singlet-oxygen formation.[4]

Corchorus aestuans Linn (Syn. *Corchorus acutangulus* Lam.), family-Tiliaceae, is an annual herb occurring throughout the hotter parts of the Subcontinent, Indo-china, Australia, Tropical Africa, West Indies, and Central America.[5, 6] It is popularly known as Jute. The roots and leaves are said to cure gonorrhea and used in making an injection for urethral discharge. The seeds are stomachic and used in pneumonia.[7, 8] The plant is said to possess anticancer, antipyretic, anticonvulsant, stomachic and digitalis glycosides like action.[9]

In the present study, the antioxidant potency of *Corchorus aestuans* Linn crude methanol and its fractionated extracts (hexane, ethyl acetate and water) have been investigated, employing three different established *in vitro* testing systems, such as scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, reducing power assay and β-carotene method. To our knowledge, there is no antioxidant study reported for *Corchorus aestuans* Linn. Thus, antioxidant activity of *Corchorus aestuans* Linn was evaluated as it had not been determined previously.

MATERIALS AND METHODS

Collection and identification of the plant material

The plant *Corchorus aestuans* Linn was collected from the local area of Kheda district, Gujarat, India, in the month of August 2009 and its authentication was confirmed by Dr. M. S. Jangid, Botany Department, Sir P. T. Science College, Modasa, Gujarat, India. Herbarium of the plant has been deposited at Department of Pharmacognosy, B. Pharmacy College, Rampura, Kakanpura, Dist. Panchmahal, Gujarat, India for future reference.

Preparation of sample

The freshly cut leaves were washed with distilled water and air-dried to constant weight at room temperature for 15 days, after which the leaves were powdered into powdered form (using mixer grinder), labeled and kept in the air tight glass jars in a refrigerator.

Preparation of extracts

100 g of the air-dried powdered leaves of *Corchorus aestuans* L. were extracted with 500 ml of each of hexane, ethyl acetate, methanol and distilled water using Soxhlet apparatus. The extracts were concentrated in a rotary evaporator apparatus at approximately 60°C. The concentrated extracts were kept in a desiccator until analyses.[10]

Chemicals

Gallic acid, BHA, ascorbic acid, DPPH, potassium ferricyanide, linoleic acid, Folin-Ciocalteu phenol reagent, Trichloro acetic acid, Tween-80, methanol, hexane, ethyl acetate and β-carotene.

Scavenging activity on 1, 1- diphenyl-2-picrylhydrazyl radicals

The scavenging activity of *C aestuans* L. extracts on DPPH radicals was measured according to the method described by Cheung *et al*. with some modifications[11, 12]. A commercially available and stable free radical DPPH, soluble in methanol was used. DPPH in its radical form has an absorbance peak at 517nm, which disappears on reduction by an antioxidant compound. 1ml of different concentrations of the isolated compound/standard (10-100 µg/ml) were added to 2ml of freshly prepared methanolic solution of 90 µM DPPH and the volume was made up to 4ml with methanol. The reaction mixtures were kept at room temperature in the dark and after 1 hour the absorbance was measured at 517nm using

spectrophotometer. A blank was performed excluding the isolated compound. The optical density of the sample, and the blank was measured by comparing the methanol.

The scavenging activity (%) on DPPH radical was calculated according to the following equation:

$$\text{scavenging activity}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Where,

$A_{control}$ = Absorbance of the control and

A_{sample} = Absorbance of the extract/standard.

All assays were conducted in triplicate. Ascorbic acid and BHA were used as positive reference standards in this study. The scavenging ability of the extracts was expressed as EC_{50} value, which is the effective concentration at which 50% of DPPH radicals were scavenged. The EC_{50} value was obtained from the graph of scavenging activity (%) versus concentration of extracts.

Reducing Power Assay

The reducing power of the prepared extracts was determined according to method described by Oyaizu.[13] Briefly, each extract in varying amounts of 5, 10, 15 and 20 mg was dissolved in 1.0 ml of methanol to which was added 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50ºC for 20 min. Following this, 2.5 ml of 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged at 1000 rpm for 10 min. A 2.5-ml aliquot of the upper layer was combined with 2.5 ml of distilled water and 0.5 ml of a 0.1% (w/v) solution of ferric chloride. Absorbance of the reaction mixture was read spectrophotometrically (Perkins Elmer, USA) at 700 nm. Increased absorbance of the reaction mixture indicates greater reducing power. Mean values from three independent samples were calculated for each extract. Ascorbic acid and BHA were used as positive reference standards.

β - Carotene bleaching method

The antioxidant activity of the prepared *C. aestuans* L. extracts was determined according to the β-carotene bleaching method described by Cheung *et al.*[14] A reagent mixture containing 1 ml of β-carotene solution (0.2 mg/ml in chloroform), 0.02 ml of linoleic acid and 0.2 ml of Tween 80 was pipetted into a round-bottomed flask. After removing the chloroform by using a rotary evaporator, 50 ml of oxygenated distilled water was added to the flask. The mixture was stirred vigorously to form a liposome solution. Aliquots (5 ml) of the liposome solution were transferred to a series of test tubes containing 0.2 ml of extract with different concentrations (4-20 mg/ml). Methanol or water (instead of extract) was used as control while the blank contained all the earlier chemicals (0.02 ml of linoleic acid and 0.2 ml of Tween 80 in 50 ml of oxygenated distilled water) except β-carotene solution. The absorbance of each extract was measured immediately (t = 0 min) at 470 nm using a spectrophotometer (Hitachi U2000). Subsequently, the reaction mixtures were incubated at 50ºC. The absorbance was measured again at time intervals of 20 min for 2 h (t = 120 min). All samples were assayed in triplicate. BHA was used as standard.

The rate of β-carotene bleaching (R) was calculated according to the equation:

$$R = \ln(A_0/A)/t$$

Where,

ln = natural logarithm

A_0 = Absorbance at time 0

A_t = Absorbance at time t

t = 20, 40, 60, 80, 100 or 120 min.

The antioxidant activity (%) was calculated in terms of percentage inhibition relative to the control, using the equation:

$$\text{antioxidant activity}(\%) = \left(\frac{R_{control} - R_{sample}}{R_{control}}\right) \times 100\%$$

Statistical analysis

All the data were represented as Mean ± S.E.M., n=3, and analyzed by One-way ANOVA followed by Dunnett's t-test for the possible significant interrelation between the various extracts. Values representing the concentrations of investigated extracts that cause 50% of neutralization/inhibition (EC_{50}) were determined by the linear regression analysis.

RESULTS AND DISCUSSION

The antioxidant activity of *Corchorus aestuans* L. leaves extracts (Hexane, Ethyl acetate, Methanol and Water) was carried out by DPPH, Reducing power assay and β-Carotene bleaching methods.

a) Scavenging activity on 1, 1 - diphenyl-2-picrylhydrazyl radicals

The proton radical-scavenging action is known to be one of the various mechanisms for anti-oxidation. DPPH radical scavenging activity is determined by a colorimetric assay. This assay is sensitive, requiring only small amount of samples and allows testing of both lipophilic and hydrophobic substances.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The use of the stable DPPH radical has the advantage of being unaffected by side reactions, such as enzyme inhibition and metal chelation. Scavenging of DPPH free radical determines the free radical scavenging capacity or antioxidant potential of the test sample, which shows its effectiveness, prevention, interception and repair mechanism against injury in a biological system.

The scavenging activity (EC_{50} values) of extracts on DPPH radicals are shown in [Table 1] and [Table 2]. Lower EC_{50} value indicates stronger ability of the extract to act as DPPH scavenger while the higher EC_{50} value indicates the lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction. The methanol extract of leaves of *C. aestuans* L. showed the best DPPH scavenging activity with the lowest EC_{50} 34 µg/ml, followed by the ethyl acetate (EC_{50} 39 µg/ml), hexane (EC_{50} 48 µg/ml) and water extract (EC_{50} 54 µg/ml). This indicated that the methanol extract may well react with free radicals, terminating the chain reaction of free radicals.

The results [Table 1], [Table 2] and [Figure 1], [Figure 2] show that there is a correlation between higher DPPH scavenging activity and larger amount of total phenolics in the methanol extract. This finding is supported by previous reports which showed that phenolic compounds generally

correlate with antioxidant capacities measured by DPPH assay. Thus, this indicated that phenolic compounds in the methanol extract may contribute to the DPPH scavenging activity although other antioxidants may probably be present in the extract.

b) Reducing power assay

Antioxidant effect often correlates with reductive activity. In the reducing power assay, the presence of antioxidants in the samples results in the reduction of the ferric cyanide complex to the ferrous form which can be monitored by measuring the formation of Pearl's Prussian blue at 700 nm. The increased absorbance at 700 nm indicates an increase in reducing power of samples. The extracts that showed comparable absorbance readings with ascorbic acid and BHA are considered to have high reducing power.

The reducing power of *C. aestuans* L. extracts and positive reference standards is shown in [Table 3] and [Figure 3]. The reducing power of *C. aestuans* L. extracts increased steadily with increasing concentrations [Figure 3] and varied significantly with different concentrations ($P<0.05$) [Table 3]. The methanol and ethyl acetate extracts appeared to possess ($P<0.05$) the highest significant reducing activity among the extracts [Figure 3]. The reducing power of methanol extract was 1.452±0.05, 1.925±0.04, 2.142±0.02 and 2.523±0.03 when tested at concentrations of 5, 10, 15 and 20 mg/ml, respectively, while the reducing power of ethyl acetate extract was 0.958±0.02, 1.557±0.04, 2.014±0.05 and 2.460±0.02 when tested at concentrations of 5, 10, 15 and 20 mg/ml, respectively. The reducing power of water extract was significantly ($P<0.05$) lowest at 0.165±0.01, 0.212±0.02, 0.345±0.01 and 0.425±0.01 when tested at concentrations of 5, 10, 15 and 20 mg/ml, respectively. However, the reducing power of the positive reference standards (ascorbic acid and BHA) were relatively more pronounced than the tested extracts. The stronger reducing power in the methanol and ethyl acetate extracts was probably due to the concentration of antioxidant compounds in the extract.

c) β-Carotene bleaching activity

In the β-carotene bleaching assay, the linoleic acid free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As a result, β-carotene molecules lose their double bonds by oxidation in this model system. In the absence of an antioxidant, the β-carotene molecule loses its chromophore and undergoes rapid discoloration, which can be monitored spectrophotometrically.

[Table 4] and [Figure 4] show the antioxidant activities of the *C. aestuans* L. leaves extracts and BHA, as measured by the bleaching of β-carotene. The antioxidant activities of all the extracts gradually increased with increasing concentration of the extracts [Figure 4] and varied significantly with different concentrations ($P<0.05$) [Table 4]. As shown in [Figure 4], the water extract showed the lowest significant antioxidant activity 17.54±2.78, 38.34±2.54 , 52.72±1.97 and 62.43±1.89 ($P<0.05$) when tested at concentrations of 4, 8, 16 and 20 mg/ml, respectively, while the methanol extract showed the highest significant antioxidant activity 54.46±1.05, 62.35±1.85, 72.46±2.02 and 82.59±2.56 when tested at concentrations of 4, 8, 16 and 20 mg/ml, respectively.

The methanol extract of leaves of *C. aestuans* L exhibited 82.59±2.56% antioxidant activity at 20 mg/ml which was comparable to that of BHA standard at 20 mg/ml (94.42±0.59%). The high antioxidant activity of methanol extract tested using β-carotene model may be correlated with the high phenolic content of the methanol extract.

CONCLUSION

The results obtained from the present study indicated that the antioxidant activity of methanol, ethyl acetate, hexane and water extracts was probably due to the concentration of antioxidant compounds like flavonoids, tannins and phenolics in the respective extracts. In conclusion, antioxidant study of *C. aestuans* L. leaves extracts suggested that *C. aestuans* L. is a potential source of natural antioxidants. However, further investigations on *in vivo* antioxidant activities are highly recommended.

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Table 1: DPPH radical scavenging activity of *C. aestuans* L. extracts (Absorbance of test samples and standards)

Conc. (µg/ml)	Absorbance					
	Hexane Extract	Ethyl acetate Extract	Methanol Extract	Water Extract	BHA (Std)	Ascorbic acid (Std)
10	0.352	0.342	0.312	0.359	0.298	0.205
20	0.310	0.298	0.296	0.328	0.215	0.178
50	0.178	0.142	0.137	0.275	0.134	0.098
60	0.109	0.094	0.088	0.149	0.067	0.045
80	0.087	0.055	0.052	0.099	0.048	0.036
100	0.056	0.050	0.042	0.086	0.036	0.031
Blank	0.413	0.414	0.415	0.415	0.413	0.423

Table 2: DPPH radical scavenging activity of *C. aestuans* L. extracts (% Inhibition of test samples and standards)

Conc. (µg/ml)	DPPH Radical Scavenging activity (% inhibition)					
	Hexane Extract	Ethyl acetate Extract	Methanol Extract	Water Extract	BHA (Std)	Ascorbic acid (Std)
10	14.77±0.04	17.39±0.22	24.82±0.18	13.49±0.19	27.84±0.09	52.78±0.11
20	24.94±0.07	28.02±0.25	28.67±0.13	20.96±0.11	47.94±0.15	57.92±0.08
50	56.90±0.07	65.70±0.12	66.99±0.10	33.73±0.07	67.55±0.19	76.83±0.11
60	73.61±0.15	77.29±0.28	78.79±0.09	64.09±0.32	83.78±0.11	89.36±0.51
80	78.93±0.06	86.71±0.19	87.47±0.10	76.14±0.34	88.38±0.23	91.49±0.21
100	86.44±0.21	87.92±0.07	89.88±0.08	79.28±0.25	91.28±0.28	92.67±0.14
EC_{50} values	48	39	34	54	28	8

***Values are Mean ± SEM, n=3, A value of P<0.0001 was considered statistically significant (By one way ANOVA, Dunnett's test).**

Table 3: Reducing powers at various concentrations

Extracts	Concentrations of extracts (mg/ml)			
	5	10	15	20
Hexane	0.654±0.04	0.857±0.03	0.952±0.02	1.345±0.01
Ethyl acetate	0.958±0.02	1.557±0.04	2.014±0.05	2.460±0.02
Methanol	1.452±0.05	1.925±0.04	2.142±0.02	2.523±0.03
Water	0.165±0.01	0.212±0.02	0.345±0.01	0.425±0.01
Ascorbic acid*	2.345±0.04	2.462±0.02	2.499±0.03	2.582±0.04
BHA*	2.431±0.02	2.456±0.03	2.547±0.02	2.621±0.03

***Positive reference standard, Absorbance values are expresses as Mean ± S.E.M of triplicate measurements. A value of P<0.05 was considered statistically significant by One way ANOVA followed by Dunnett's t-test.**

Table 4: Antioxidant activity (%) measured by β -carotene bleaching method

Extracts	Concentrations of extracts (mg/ml)			
	4	8	16	20
Hexane	18.54±1.37	42.52±0.03	59.43±2.63	69.82±1.97
Ethyl acetate	40.98±1.56	58.96±1.24	67.45±1.76	74.12±2.43
Methanol	54.46±1.05	62.35±1.85	72.46±2.02	82.59±2.56
Water	17.54±2.78	38.34±2.54	52.72±1.97	62.43±1.89
BHA*	71.24±1.07	81.00±0.54	87.58±0.62	94.42±0.59

***Positive reference standard, Absorbance values are expresses as Mean ± S.E.M. of triplicate measurements. A value of P<0.05 was considered statistically significant One way ANOVA followed by Dunnett's t-test.**

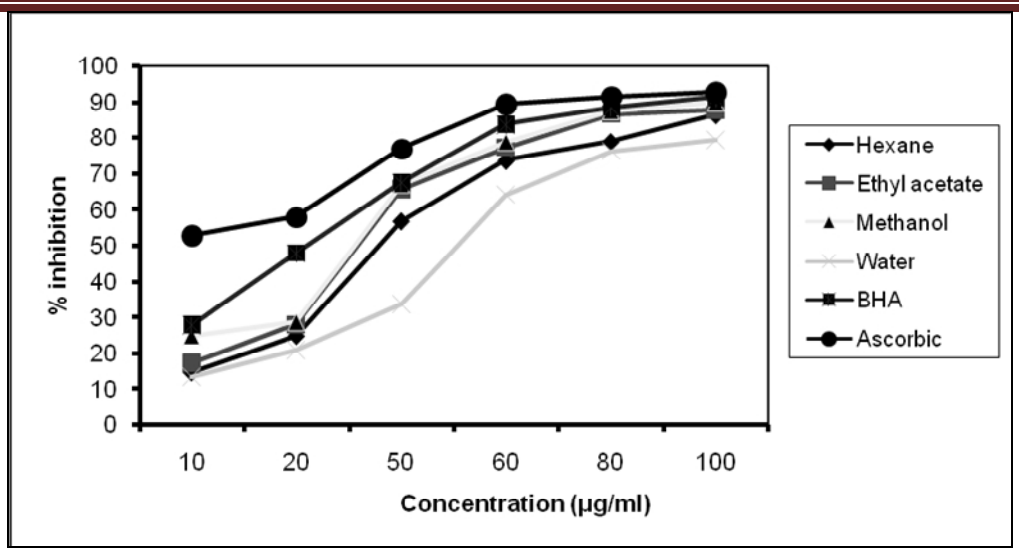
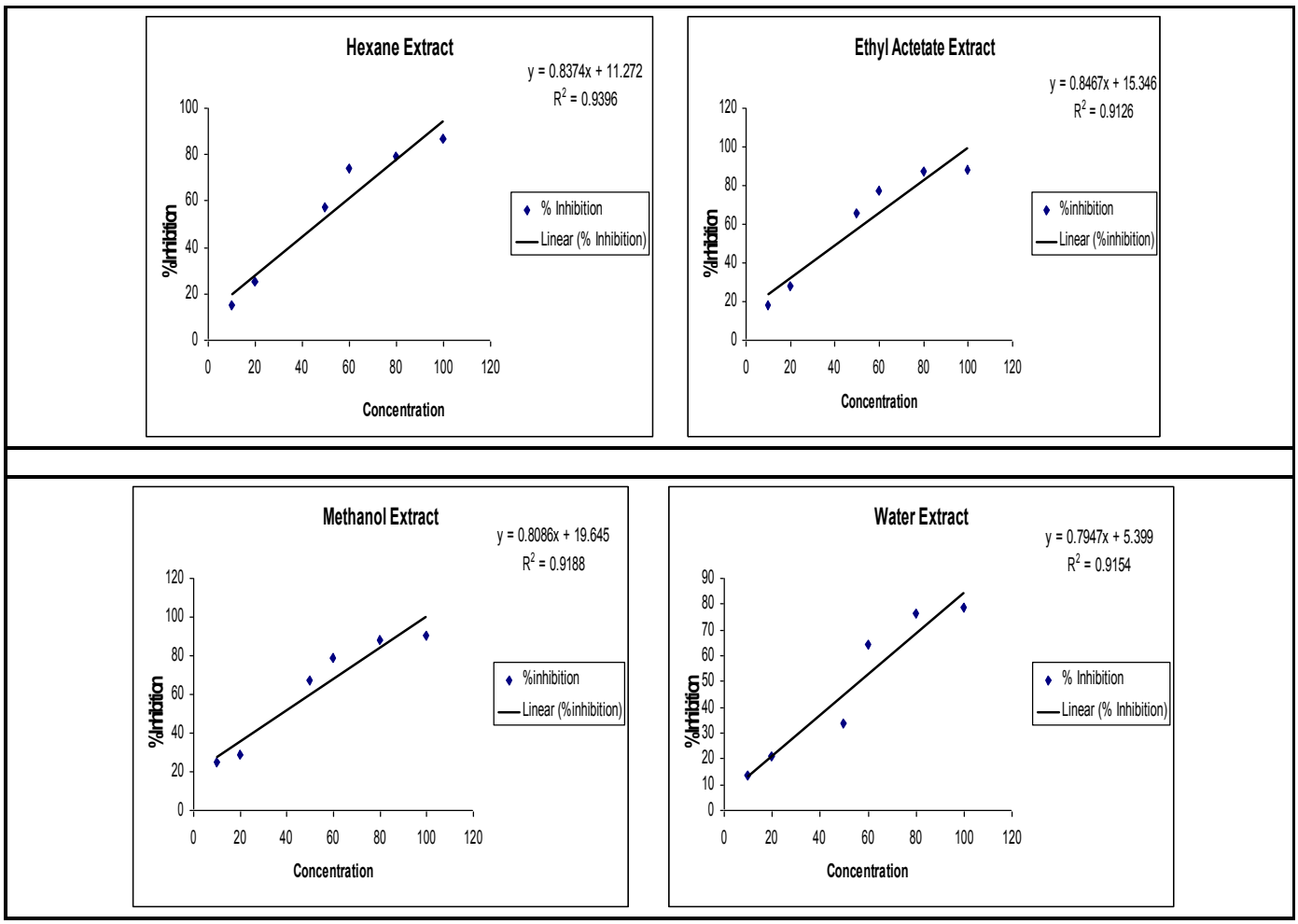


Figure 1: Antioxidant activity (%) measured by DPPH method



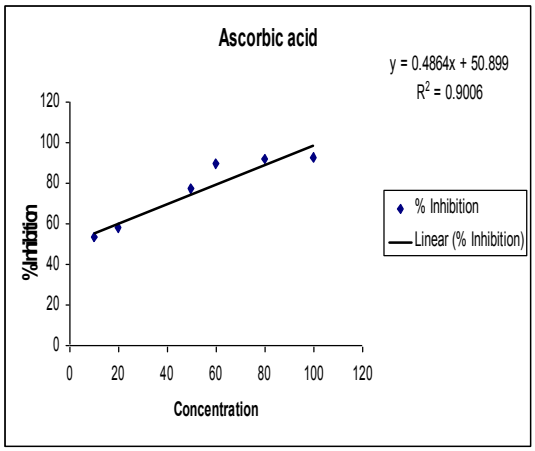


Figure 2: Calibration curves of BHA, Ascorbic acid, Hexane, Ethyl acetate Methanol and Water extracts of *C. aestuans* Linn.

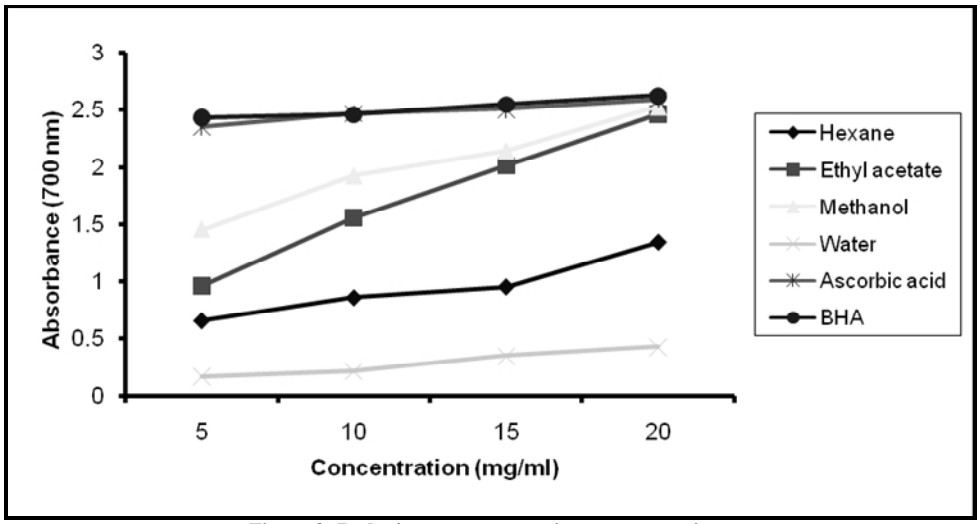


Figure 3: Reducing powers at various concentrations

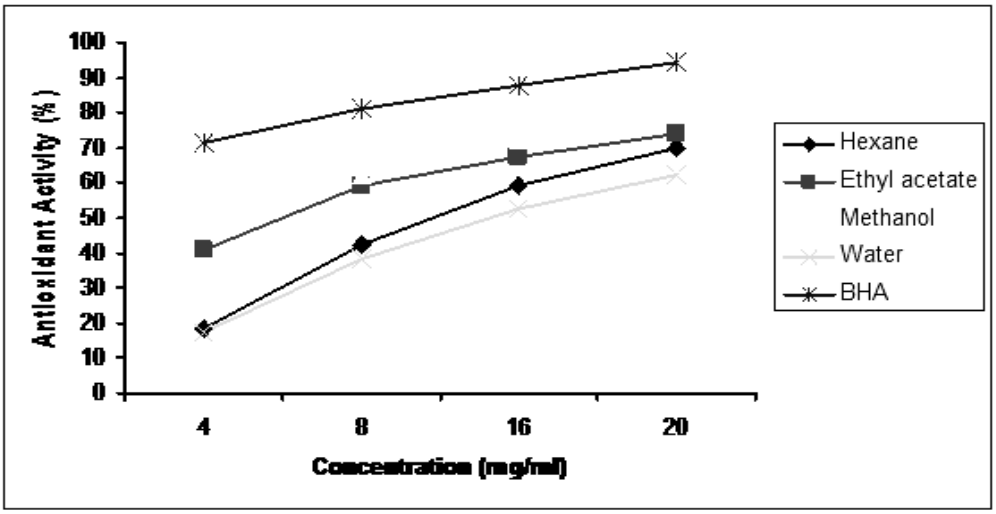


Figure 4: Antioxidant activity (%) measured by β -carotene bleaching method

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