Evaluation of Antioxidant Activity of *Cedrela toona Roxb.* Leaf Extracts

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RESEARCH ARTICLE

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ABSTRACT

Antioxidant potency of fruits of *Cedrela toona Roxb.* crude methanol and its fractionated extracts (hexane, acetone 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 methanol extract of fruits of *Cedrela toona Roxb.* showed the best DPPH scavenging activity with the lowest EC 50 34 µg/ml, followed by the acetone (EC 50 39 µg/ml), hexane (EC 50 48 µg/ml) and water extract (EC 50 54 µg/ml). The reducing power of *Cedrela toona Roxb.* fruit extracts increased steadily with increasing concentrations and varied significantly with different concentrations. The methanol and acetone extracts appeared to possess the highest significant reducing activity among the extracts. The stronger reducing power in the methanol and acetone extracts was probably due to the concentration of antioxidant compounds like flavonoids and phenolics in the extract. In the β-carotene bleaching assay, the antioxidant activities of all the fruits extracts gradually increased with increasing concentration of the extracts and varied significantly with different concentrations. The water extract showed the lowest significant antioxidant activity, while the methanol extract showed the highest significant antioxidant activity. The high antioxidant activity of methanol extract tested using β-carotene model may be correlated with the high phenolic content of the methanol extract. In conclusion, antioxidant study suggested that fruits of *Cedrela toona Roxb*. are potential source of natural antioxidants. However, further investigations on in vivo antioxidant activities are highly recommended.

In the present study, the antioxidant potency of *Cedrela toona Roxb*. crude methanol and its fractionated extracts (hexane, acetone 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. There is no antioxidant study reported for *Cedrela toona* Roxb.Thus, antioxidant activity of *Cedrela toona* Roxb. was evaluated as it had not been determined previously.

Keywords: *Cedrela toona*, Antioxidant, DPPH, Reducing power, β-carotene

1. Introduction

Literature survey reveals that *Cedrela toona* Roxb. is medium sized to large deciduous tree with brown to grey scaly bark. Leaves 15 – 45 cm long usually paripinnate but sometimes with a terminal leaflet in juvenile growth, leaflets mostly 8-20, ± ovate, often falcate, 4-15 cm long, 15-50 mm wide, apex acuminate, base strongly asymmetric, margins entire, mostly glabrous, domatia present as small hair – tuffs; petiole 4-11 cm long, petiolules 5-12 mm long. Penicles 20-40 cm long. Petals 5-6 mm long, white. Capsule ellipsoid, 10-20 mm long, 6-8 mm diameter; seeds winged at both ends.(1-4) Traditionally the bark is astringent, antidysentric, antiperiodic.(5) Flowers are emmenagogue, leaf is spasmolytic, hypoglycaemic and antiprotozoal.(6) Bark and heartwood yielded tetraterpenoids, including toonacillin. Heartwood also gave a coumarin gerarnyl gernalol as its fatty esters. Toonacillin and its 6 – hydroxyl derivatives are antifeedent.(5)

Materials and Methods (7-12)

Preparation of sample

The fresh fruits were washed with distilled water and air-dried to constant weight at room temperature for 15 days, after which the fruits 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 fruits of *Cedrela toona* Roxb.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.

Chemicals

Gallic acid, BHA, ascorbic acid, DPPH, potassium ferricyanide, linoleic acid, Trichloro acetic acid, Tween-80, methanol, hexane, acetone and β-carotene.

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

The scavenging activity of *Cedrela toona* Roxb.extracts on DPPH radicals was measured according to the method described by Cheung *et al*. with some modifications. 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. 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) Trichloro-acetic 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 at 700 nm [Table 3.30 & Figure 3.34]. 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 *Cedrela toona* Roxb.extracts was determined according to the β-carotene bleaching method described by Cheung *et al.* 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. 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:

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

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:

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

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.

The antioxidant activity of *Cedrela toona* Roxb. leaf extracts (Hexane, Acetone, Methanol and Water) was carried out by DPPH, Reducing power assay and β-Carotene bleaching methods.

Result and Discussion

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 *Cedrela toona* Roxb.showed the best DPPH scavenging activity with the lowest EC_{50} 34 µg/ml, followed by the ethylacetate (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.

Table 1. DPPH radical scavenging activity of *Cedrela toona* Roxb. Extracts (Absorbance of test samples and standards)

Con. (µg/ml)	***Absorbance***					
	Hexane	***Ethyl acetate***	***Methanol***	***Water***	***BHA***	***Ascorbic***

	Extract	*Extract*	*Extract*	*Extract*	*(Std)*	*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 *Cedrela toona* Roxb.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).

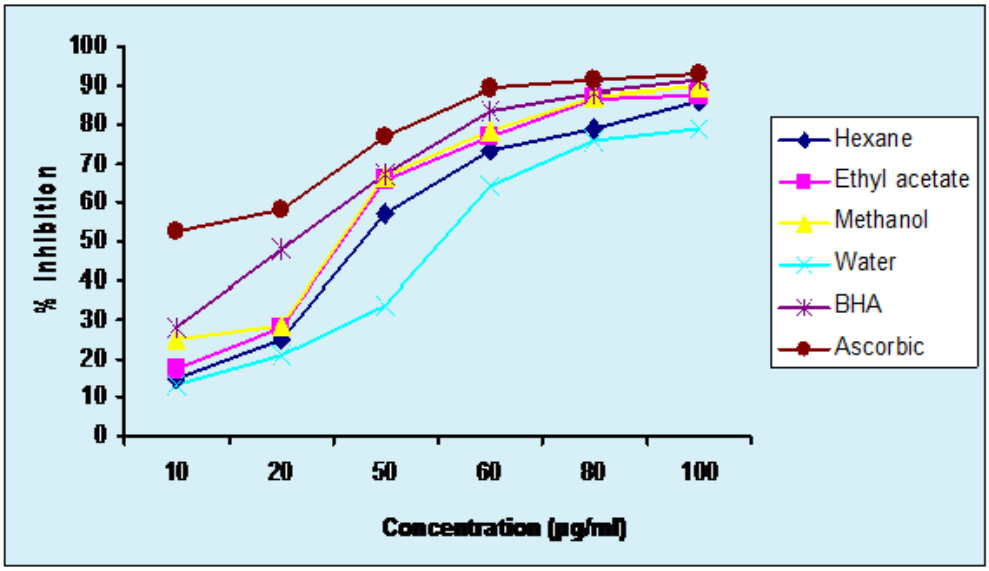

Figure 1: Antioxidant activity (%) measured by DPPH method

The stronger scavenging activity in the methanol, acetone, hexane and water extracts was probably due to the concentration of antioxidant compounds like flavonoids, tannins and phenolics in the respective extracts.

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.

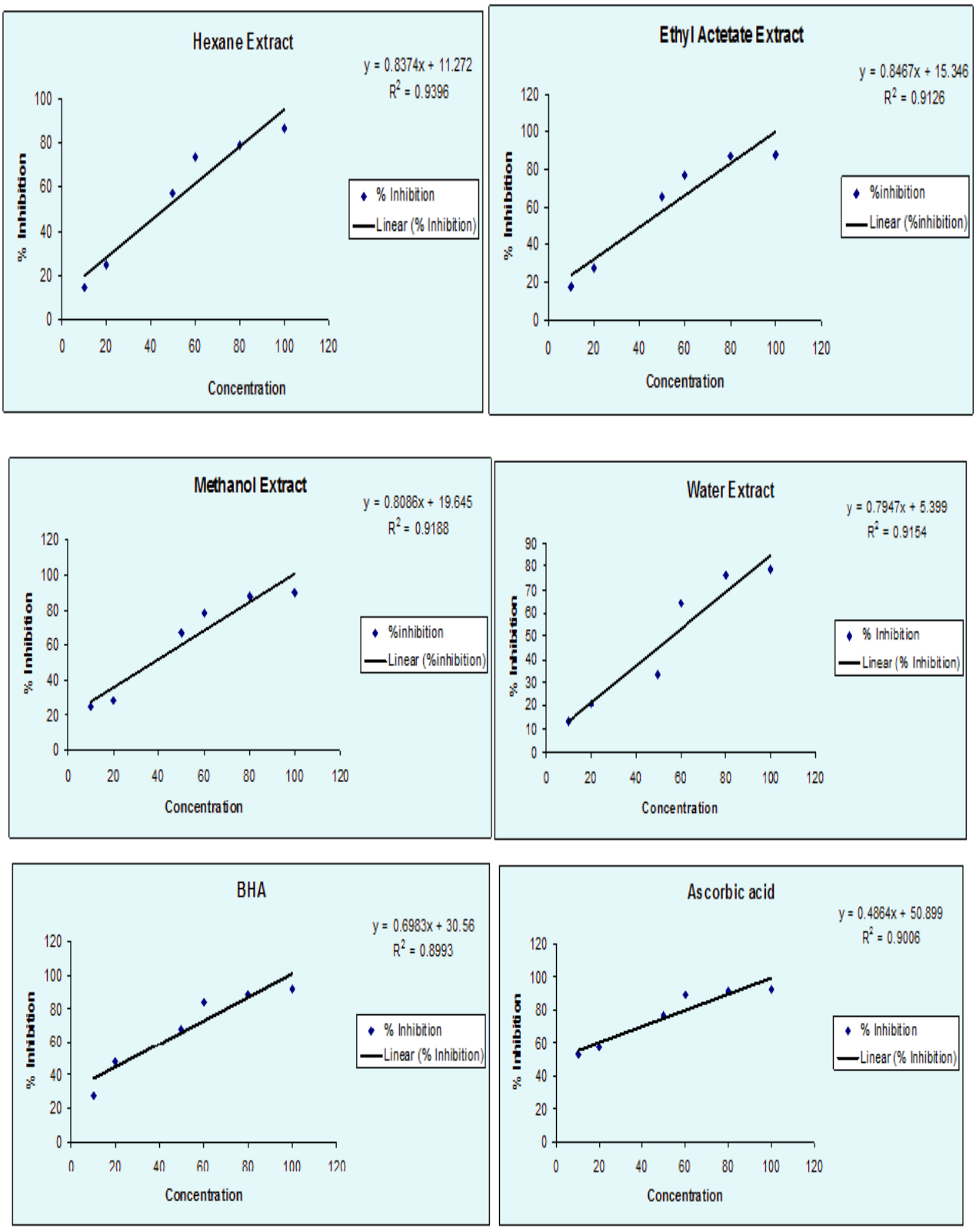

Figure 2: Calibration curves of BHA, Ascorbic acid, Hexane, Acetone, Methanol, and Water extracts of *Cedrela toona* Roxb**.**

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 *Cedrela toona* Roxb.. extracts and positive reference standards is shown in [Table 3] and [Figure 3]. The reducing power of *Cedrela toona* Roxb. extracts increased steadily with increasing concentrations [Figure 3] and varied significantly with different concentrations (P<0.05) [Table 3]. The methanol and acetone 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 acetone extracts was probably due to the concentration of antioxidant compounds in the extract.

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.

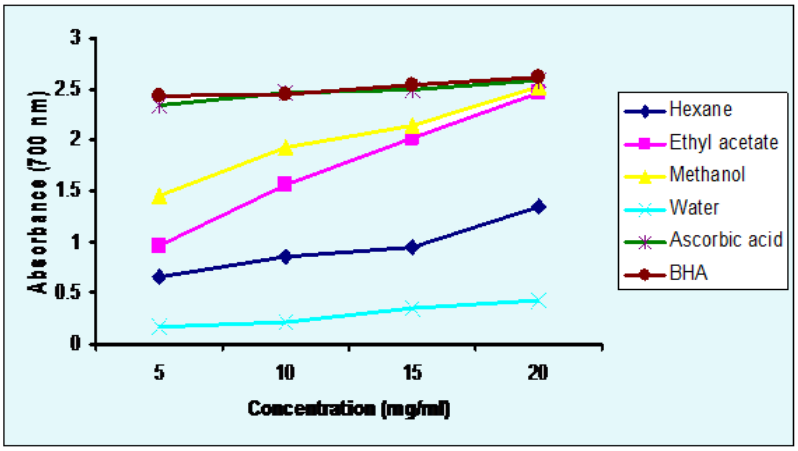

Figure 3: Reducing powers at various concentrations

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 spectrophoto-metrically.

Table 4 and Figure 4 show the antioxidant activities of the *C. toona* Roxb. 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. toona* Roxb.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.

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.

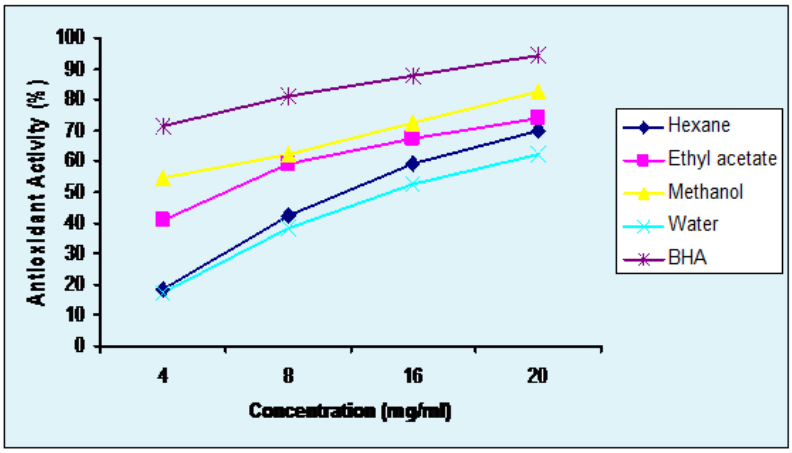

Figure 4: Antioxidant activity (%) measured by β -carotene bleaching method

Discussion

The methanol extract of leaves of *Cedrela toona* Roxb.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 reducing power of *Cedrela toona* Roxb. leaf extracts increased steadily with increasing concentrations and varied significantly with different concentrations. The methanol and ethyl acetate extracts appeared to possess the highest significant reducing activity among the extracts. The stronger reducing power in the methanol and acetone extracts was probably due to the concentration of antioxidant compounds like flavonoids and phenolics in the extract. In the β-carotene bleaching assay, the antioxidant activities of all the fruits extracts gradually increased with increasing concentration of the extracts and varied significantly with different concentrations. The water extract showed the lowest significant antioxidant activity, while the methanol extract showed the highest significant antioxidant activity. 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

In conclusion, antioxidant study suggested that leaves of *Cedrela toona* Roxb. are potential source of natural antioxidants. However, further investigations on *in vivo* antioxidant activities are highly recommended. It is also needed to determine phytoconstituents, which are responsible for the antioxidant activity.

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Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this article.

References

1. Khare, C.P. Indian Medicinal Plant. An Illustrated Dictionary. Published by Springer, 2006; 112 - 113.
2. Loupee D, Oteng- Amoaka A.A, Brink M. Timber 1. Vol1, vol7, PROTA publishers, 2008; 557-559.
3. http://en.wikipedia.org/wiki/Toon.
4. Kashyapa K, Chand R. The useful plants of India. National Institute of Sciences Communication and Information Resources, New Delhi, 2006; 112-113.
5. Nadkarni A K. Indian Materia Medica. Edn 3, Vol I, Popular prakashan, 2009; 1908.
6. Pullaiah, T. Biodiversity in India. Vol 4, Published by Regency Publication, 2006; 160.
7. Pandya N, Santani D, Jain S, Antioxidant activity of ezetimibe in hypercholesterolemic rats, Indian J Pharmacol, 2006; 38(3):205, 206.
8. Sim KS, Sri Nurestri AM, Norhanom AW. Phenolic content and antioxidant activity of *Pereskia grandifolia* Haw. (Cactaceae) extracts. Phcog Mag 2010; 6:248-54.
9. Tanmayee Mishra, Arvind K Goyal, Sushil K Middha, Arnab Sen, "Antioxidative properties of *Canna edulis* Ker-Gawl.", Ind J Nat Prod Resour 2011; 2(3):315-321.
10. Koleva I. I., Van Beek T. A., Linssen J.P.H., De Groot A, Evstatieva L.N. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis. 2002; 13:8-17.
11. Kumaran A. and Karunakaran R. J.; In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India, Food Science and Technology, 2007; 40(2):344-352.
12. Gupta M., Mazumdar U. K., Gomathi P., Kumar R. S.; Antioxidant and Free Radical Scavenging Activities of *Ervatamia coronaria* Stapf. Leaves, Iranian Journal of Pharmaceutical Research, 2004; 2:119-126.