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In vitro antioxidant properties of the traditional medicinal plant species, *Ehretia microphylla* Lam. and *Erythroxylon monogynum* Roxb.

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ABSTRACT

In the present investigation, *in vitro* antioxidant properties of methanolic aerial parts extracts of the two traditional medicinal plant species, *Ehretia microphylla* and *Erythroxylon monogynum* were assessed by using reducing power assay, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging activities. From the results, methanolic aerial parts extracts of the species, *Ehretia microphylla* and *Erythroxylon monogynum* showed remarkable antioxidant activity in terms of all the three assays studied. However, the species, *Ehretia microphylla* was determined to be more prominent than the other species studied. Therefore, both the species were found to be the most effective potent source of natural antioxidants.

Keywords: *Ehretia microphylla*, *Erythroxylon monogynum*, antioxidant activity, reducing power, $DPPH^{\bullet}$, $ABTS^{\bullet+}$.

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INTRODUCTION

Oxidation reactions can produce 'free radicals' which induce oxidative damage to biomacromolecules *viz.,* DNA, proteins, lipid membranes and carbohydrates [1]. These free radicals are natural by-products of our own metabolism and cause lipid peroxidation in foods [2]. Antioxidants are vital substances as they can protect the body from the damage caused by the free radicals [3]. Generally fruits and vegetables are concentrated source of antioxidants. The free radical scavenging activity of antioxidant substances in plants are investigated by many workers [4-6].

The species, *Ehretia microphylla* belongs to the family, Boraginaceae is a shrub, distributed in tropical regions of Malaysia, China, India and the Solomon Islands. The fresh root is sweet and slightly pungent and used as an antidote to vegetable poisoning. A decoction of the leaves is prescribed for cough and stomach troubles in traditional medicinal practice in Southern India [7]. The plant is also used in the treatment of skeletal fractures in the indigenous system of medicine in Sri Lanka [8]. It is prepared like tea and this form of herbal medicine is effective in treating intestinal motility and also used as a mouth wash as the leaf of this shrub has high fluoride content [7]. The other species, *Erythroxylon monogynum*, (Erythroxylaceae) is a small tree and it has been used in traditional medicinal practice for stomachic, diaphoretic, stimulant, diuretic, dyspepsia and continued fever, and also in dropsy as an adjuvant to some other and more active medicines [9]. Despite this wide spectrum of therapeutic uses, very little works only carried out in these two species. Hence, the present study was made to evaluate the antioxidant properties of methanolic aerial extracts of these two species in terms of reducing power assay and $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and radical scavenging activities.

MATERIALS AND METHODS

Collection of plant materials

The aerial parts of *Ehretia microphylla* and *Erythroxylon monogynum* were collected from Maruthamalai, Coimbatore, Tamil Nadu, India.

Extraction of plant materials

Freshly harvested aerial parts were shade dried at room temperature and ground to fine powder. About 50g of powder was extracted with methanol (250mL) in a soxhlet extractor for 8 to 10 h. The extracts were then concentrated and finally dried to a constant weight and kept at 20°C. For stock solutions, 1mg/1mL of methanolic extracts were dissolved in DMSO (Dimethyl Sulfoxide).

IN VITRO ANTIOXIDANT ACTIVITIES

Reducing power activity

The Fe^{3+} reducing power of the extracts was determined by the method of Yildrim *et al* [10]. Various concentrations of plant extracts (300-700µg/mL) were mixed with 1.0mL of

0.2M sodium phosphate buffer (pH 6.6) and 1.0mL of freshly prepared 1% potassium ferric cyanide. The mixture was incubated in water bath at 50°C for 20min. Then 1.0mL of 10% trichloro acetic acid (TCA) was added and centrifuged at 3000rpm for 10min. The supernatant (2.0mL) was mixed with 2.0mL of distilled water and 500µL of 1% ferric chloride (freshly prepared). The absorbance was read at 700nm. Higher absorbance of the reaction mixture indicates greater reducing power. Ascorbic acid was used as standard antioxidant.

$DPPH^{\bullet}$ radical scavenging activity

The method prescribed by Blois [11] was used with some modifications. 0.2mM solution of $DPPH^{\bullet}$ in methanol was prepared, and 500µL of this solution was added to different concentrations of the extracts (250-450µg/mL). The mixture was shaken vigorously and allowed to stand for 30min at room temperature. Control was prepared as above but without the sample extracts, and methanol was used for the baseline correction. Then changes in the absorbance of the plant samples were measured at 517nm using spectrophotometer. A lower absorbance value indicates the higher radical scavenging activity. Results were compared with the standard antioxidant BHT. The ability of $DPPH^{\bullet}$ radical scavenging activity was calculated by using the following formula:

$$DPPH^{\bullet} \text{ scavenging effect (\% of inhibition)} = (A_0\text{-}A_1) \text{ X } 100/A_0$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extracts. The IC_{50} (the microgram of extract to scavenge 50% of the radicals) value was calculated using linear regression analysis. Lower IC_{50} value indicates greater antioxidant activity.

$ABTS^{\bullet+}$ radical scavenging activity

The $ABTS^{\bullet+}$ assay was assessed according to the method of Siddhuraju and Manian [12]. ABTS radical cation was generated by oxidation of $ABTS^{\bullet+}$ (7mmol/L) with potassium persulfate (2.45mmol/L) which was dissolved in 5.0mL of distilled water. After incubation for 12-16 h at room temperature in dark condition blue/green $ABTS^{\bullet+}$ chromophore was produced. The $ABTS^{\bullet+}$ solution was diluted with ethanol (1:89 v/v) and adjusted to equilibrate the absorbance of 0.700±0.001 at 734nm. The generated $ABTS^{\bullet+}$ solution (2.0mL) was mixed with 20µL of sample extracts or trolox standards (0-15µM). The absorbance values were read at 734nm exactly after 30min. The total antioxidant activity unit was defined as the concentration of trolox having the equivalent antioxidant activity expressed as µmol/g sample extracts on dry weight basis.

Statistical Analysis

Statistical analysis was carried out by one way analysis of variance (ANOVA) test using a statistical package program (SPSS 10.0) and the significance of the difference between means was determined by Duncan's Multiple Range Test (DMRT) at $p<0.05$ significant level. Analysis was carried out in triplicate and mean ± SD of three parallel measurements.

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RESULTS AND DISCUSSION

IN VITRO ANTIOXIDANT ACTIVITY

Reducing power assay

The reducing power abilities of plant extracts generally depends on the presence of reductone, which have been shown to impart antioxidant action by breaking the free radical chain by donating a hydrogen atom. The presence of reductones in the plant extract reduces Fe^{3+}/ferricyanide complex to Fe^{2+} form [13]. The data on reducing power assay for methanolic aerial parts extracts of the two studied species, *Ehretia microphylla* and *Erythroxylon monogynum* were presented in Table 1. The absorbance values were increased steadily with the increase in the concentration of extract from 300 to 700µg/mL. The reducing ability was varying from 0.633 (300µg/mL) to 1.605 (700µg/mL) and 0.495 (300µg/mL) to 1.262 (700µg/mL) absorbance for *Ehretia microphylla* and *Erythroxylon monogynum* respectively. The results indicate that the species, *Ehretia microphylla* possessed greater reducing power than the other species, *Erythroxylon monogynum.* Hence it is presumed that the extracts may have high amount of reductones. Similar trend of results obtained for reducing ability of extracts of some other plant species [14,15].

Table 1. Reducing power activity of methanolic aerial parts extracts of *Ehretia microphylla* and *Erythroxylon monogynum.*

S. No.	Sample concentration (µg/mL)	Absorbance at 700 nm		Standard, Ascorbic acid	
		Ehretia microphylla	*Erythroxylon monogynum*	concentration (µg/mL)	Absorbance at 700 nm
1.	300	0.633±0.03[a]	0.495±0.03[a]	20	0.417±0.03[a]
2.	400	0.696±0.01[a]	0.539±0.01[a]	40	0.648±0.02[b]
3.	500	1.085±0.50[b]	0.610±0.50[b]	60	0.856±0.01[c]
4.	600	1.354±0.21[c]	1.230±0.21[c]	80	1.098±0.05[d]
5.	700	1.605±0.12[d]	1.262±0.12[d]	100	1.393±0.02[e]

Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different ($p<0.05$).

DPPH$^{\bullet}$ radical scavenging activity

DPPH$^{\bullet}$ assay is the most widely reported method for screening antioxidant activity of many plant drugs [16]. DPPH is a stable, nitrogen-centered free radical which produces violet colour in methanol solution. It was reduced to yellow colour, with the addition of plant extract in a concentration dependent manner [17]. In the present investigation, the methanolic aerial parts extracts of the study species, *Ehretia microphylla* and *Erythroxylon monogynum* have showed increased DPPH$^{\bullet}$ radical scavenging activity with increasing concentration from 250-450µg/mL. The IC_{50} values for the methanolic extract of *Ehretia microphylla* was162µg/mL which have higher radical scavenging activity than that of *Erythroxylon monogynum* (172µg/mL) (Table 2).

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Table 2. DPPH$^{\bullet}$ radical scavenging activity of methanolic aerial parts extracts of *Ehretia microphylla* and *Erythroxylon monogynum.*

Sample concentration (µg/mL)	*Ehretia microphylla*		*Erythroxylon monogynum.*		IC_{50} for the standard BHT
	Percentage activity	IC_{50} (µg/mL)	Percentage activity	IC_{50} (µg/mL)	
250	66.66± 0.40^{a}	162	65.53± 0.40^{a}	172	34.74 ± 00.26^{a}
300	69.49± 0.82^{b}		74.57± 0.81^{b}		
350	74.57± 0.49^{c}		80.79± 1.63^{c}		
400	75.58± 1.63^{c}		81.92± 1.22^{cd}		
450	85.31± 0.65^{d}		82.87± 0.81^{d}		

Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different (p<0.05).

ABTS$^{\bullet+}$ radical scavenging activity

The ABTS$^{\bullet+}$ assay is based on inhibition of free radicals by antioxidants [18]. In the present study, the methanolic aerial parts extracts of *Ehretia microphylla* were able to quench radicals more effectively (5109.7±187-3 µmol TE/g extract) than the other species, *Erythroxylon monogynum* (4245.7±90.7 µmol TE/g extract) (Table 3). The higher ABTS$^{\bullet+}$ value would imply greater antioxidant activity of the sample [19]. The speculating ABTS$^{\bullet+}$ quenching activity may be contributed due to the presence of hydrogen-donating compounds. Having this ABTS radical scavenging property, the plant extracts can be accepted as equal to trolox in terms of antiradical property.

Table 3. ABTS$^{\bullet+}$ radical scavenging activity of methanolic aerial parts extracts of *Ehretia microphylla* and *Erythroxylon monogynum.*

Species	Total antioxidant Activity (µmol TE/g extract)
Ehretia microphylla	5109.7± 187.3^{b}
Erythroxylon monogynum	4245.7± 090.7^{a}

Values were performed in triplicates and represented as mean ± SD.
Mean values followed by different superscript in a column are significantly different (p<0.05).

CONCLUSION

From the above results it is known that the methanolic aerial parts extracts of the study species, *Ehretia microphylla* and *Erythroxylon monogynum* are having better antioxidant properties. However, the species, *Ehretia microphylla* exhibits slightly more pronounced radical scavenging effect than the other species, *Erythroxylon monogynum*. Hence, both species could be promising sources of natural antioxidants. Nonetheless, further studies on isolation and purification of compounds responsible for antioxidant activity are needed.

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