ORIGINAL ARTICLE

Comparative evaluation of antioxidant and hemolytic potential of ornamental plants *Ficus benjamina*, *Antigonon leptopus*, and *Amaranthus tricolor*

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Abstract

Aim: *Ficus benjamina*, *Amaranthus tricolor,* and *Antigonon leptopus* are belonging to the families Moraceae, Amaranthaceae, and Polygonaceae, respectively. **Materials and Methods:** The dried powder of these leaves was extracted with methanol, chloroform, petroleum ether, diethyl ether, and ethyl acetate. These extracts were tested to antioxidant free radical scavenging assay and hemolytic activity. Evaluation of antioxidant activity of these extracts was performed using two assays such as 2,2,-diphenyl-picrylhydrazyl (DPPH) and 2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) free radical scavenging assay using quercetin as standard. **Results:** In DPPH radical scavenging assay, methanol, chloroform, petroleum ether, diethyl ether, and ethyl acetate showed remarkable antioxidant activity having respective IC_{50} values such as 31.44, 38.21, 42.2, 26.02, and 17.40 µg/ml, respectively. In ABTS radical scavenging assay, petroleum ether, diethyl ether, methanol, and ethyl acetate showed remarkable antioxidant activity having respective IC_{50} values such as 13.7, 77.67, 13.90, and 18.33 µg/ml, respectively. Hemolytic activity showed positive results for all the plant extracts tested for their hemolysis abilities. **Conclusion:** The study concludes that, among the tested plant extracts, ethyl acetate extract of *A. leptopus* showed highest antioxidant potential. This study gives a summary of the antioxidant and antihaemolytic potential of different extracts of *F. benjamina*, *A. leptopus* and *A. tricolor*.

Key words: 2,2,-diphenyl-picrylhydrazyl, 2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid, *Amaranthus tricolor*, *Antigonon leptopus*, *Ficus benjamina*, Hemolysis, Quercetin

INTRODUCTION

Plants can synthesize a large variety of chemical substances that are of physiological importance. Many plants contain antioxidant compounds and these compounds protect cells against the free radicals.[1] During cell metabolism free radicals are produced in the cells, majorly through mitochondrial oxidative phosphorylation. Free radicals like reactive nitrogen species (RNS) and reactive oxygen species (ROS) (i.e., oxygen species such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite) are being produced.[2] RNS are generated from oxygen in the presence of nitric oxide synthetase. ROS and RNS can be harmful as they damage cellular lipids, sugars, proteins, and nucleic acids, thus inhibiting the normal function. Oxygen free radicals have been shown to be responsible for many pathological conditions in human beings such as atherosclerosis, ischemic heart disease, aging process, inflammation, and diabetes. Thus, there is a continuous requirement of antioxidants for free radical inactivation.[3] Antioxidants are a group of substances which, when present at low concentrations in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves. Therefore, the present study focuses to evaluate the DPPH and ABTS radical scavenging assay of various extracts from *A. tricolor*, *A. leptopus* and *F. benjamina* in detail.[4]

Plants contain many chemical substances that might have a hemolytic effect on human erythrocytes. Interaction between

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blood and biomaterials may induce erythrocyte lysis, particularly during prolonged contact or during the contact of blood with large surfaces. Plant extracts can positively affect the red cell membrane and may also induce adverse effects, which include induction of hemolytic anemia.[5] Therefore, many of the commonly available plants need to be evaluated for their potential hemolysis activity. The present study was carried out the determination of hemolysis activity of *F. benjamina, A. tricolor,* and *A. leptopus.*

MATERIALS AND METHODS

Collection of Plant Materials

F. benjamina and *A. tricolor* plants were collected in January at Chikkaballapura. *A. leptopus* plant material was collected at Hebbala (Bengaluru). The latitude, longitude, and elevation of Chikkaballapura and Hebbala are 13.4716°N, 77.7412°E, and 915 m (3002 ft) and 13.0354°N, 77.5988°E, and 900 m (2953 ft), respectively. The voucher specimen was deposited in the form of herbarium at the Department of Botany, BUB. The plants were authenticated by Dr. K P Srinath, Professor, Department of Botany, BUB. The plant material was made into herbarium.[6]

Chemicals Used

DPPH (EEC NO.217-591-8, Sigma, USA) stored at <0°C. Methanol high-performance liquid chromatography (HPLC) grade (Ranbaxy chemicals), quercetin (5 mg dissolved in 100 ml methanol), DPPH (1.3 mg/ml in HPLC grade methanol), phosphate-buffered saline (PBS), ABTS, ammonium persulfate (APS), sodium dodecyl sulfate (SDS), and erythrocytes suspension.[7]

Preparation of Extracts by Decoction Method

The freshly collected leaves were washed under running tap water to remove dirt and shade dried for 8–10 days. The dried leaves were ground into fine powder using mixer grinder. Fifteen grams of powdered plant material were soaked into 90 ml of organic solvents, namely, methanol, chloroform, petroleum ether, diethyl ether, and ethyl acetate for 4–5 h in water bath at 50°C. The extracts were filtered through the Whatman No.1 filter paper. The extract was then allowed to evaporate. The condensed extracts were stored in Eppendorf vials at 4°C till further investigation.[8]

Antioxidant Free Radical Scavenging Assay

DPPH radical scavenging assay

The free radical scavenging activity of plants extract for DPPH was determined as described by Rajakumara *et al.* (1994). 1.3 mg/ml of 75 µl DPPH methanol solution was added to a 1.25 µg/ml solution of the plant sample of various concentrations (200 and 400 µg/ml) or standard, quercetin (0.10–20 µg/ml). The reaction mixture was mixed, incubated at 25°C for 15 min. The absorbance was measured at 517 nm using ultraviolet (UV) spectrophotometer (PerkinElmer Lambda). A control reaction was carried out without the test sample.[9-12] The percentage inhibition of DPPH radical scavenging assay is calculated using the formula as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test sample)}}{\text{Absorbance (control)}} \times 100$$

ABTS radical scavenging assay

The free radical scavenging activity of plants extract for ABTS assay was performed as per Auddy *et al.* (2003). Ten milliliters of ABTS (7 mM) and 10 ml of APS (2.45 mM) solutions are mixed and allowed to incubate at room temperature in dark for 16 h. The solution thus obtained is further diluted with PBS to give an absorbance of 1.000. Different concentrations of the plant samples (1.25–100 µg/ml) and the reference standard were quercetin (0.30–20 µg/ml) added to 950 µl of ABTS working solution to give a final volume of 1 ml made up by adding (PBS – 125 mM NaCl in 10 mM sodium phosphate buffer; pH 7.4). The absorbance recorded immediately at 734 nm using UV spectrophotometer (PerkinElmer Lambda).[13-16] The percentage inhibition of ABTS radical scavenging assay is calculated using the formula as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test sample)}}{\text{Absorbance (control)}} \times 100$$

Determination of Hemolysis Activity

Take a 50 µl of 10 dilution of erythrocytes suspension into 2 ml of new Eppendorf tube and add 100 µl of test samples (plant extract). This reaction mixture is incubating at 37°C water bath for 60 min. Adjust the volume of reaction mixture to 1 ml by adding 850 µl of 1× PBS (as negative control). Finally, reaction mixture was centrifuged at 300 rpm for 3 min and the resulting hemoglobin in supernatant was measured at 540 nm using UV spectrophotometer to determine the concentration of hemoglobin. One hundred microliters of 1% SDS were used as positive control.[5,17-20] The percentage of hemolysis can be calculated using the formula as follows:

$$\% \text{ Haemolysis} = \frac{(\text{Control OD} - \text{Sample OD})}{(\text{Control OD})} \times 100$$

RESULTS

DPPH Assay

Both methanol and chloroform extracts of *F. benjamina* showed significant antioxidant activity in the DPPH assay.

Among the two extracts, methanol extract demonstrated higher antioxidant potential with a significant IC_{50} value of 99.74 µg/ml. Among the methanol and ethyl acetate extract of *A. leptopus*, the ethyl acetate extract showed the highest significant antioxidant potential with an IC_{50} value of 17.4 µg/ml. Among the four extracts of *A. tricolor*, the highest antioxidant potential was demonstrated by diethyl ether extract with the highest significant IC_{50} value of 26.02 µg/ml. Among all the three different plant samples and eight different extracts, the most significant antioxidant potential was demonstrated by ethyl acetate extract of *A. leptopus*, with the lowest IC_{50} value of 17.4 µg/ml. The results of the antioxidant activity assay are tabulated in Table 1.

Table 1: Results of 2,2,-diphenyl-picrylhydrazyl antioxidant assay

Sample	Concentration (µg/ml)	*Ficus benjamina*	*Antigonon leptopus*	*Amaranthus tricolor*
Control	0	0	0	0
Quercetin	0.3125	7.44	2.92	4.42
	0.625	13.85	6.62	11.04
	0.125	27.12	25.18	21.28
	2.5	34.2	33.2	37.1
	5	48.05	55.08	46.08
	10	66.4	74.54	60.9
	IC_{50} (µg/ml)	5.86	4.435	6.542
Methanol	0	0	0	0
	3.125	4.7	8.57	13,947
	6.25	10.25	16.95	23.989
	12.5	17.14	26.97	39.749
	25	26.93	39.43	46.862
	50	36.38	55	69.874
	100	52.84	71.07	83.961
	IC_{50} (µg/ml)	99.74	37.51	31.44
Chloroform	3.125	8.25	-	10.879
	6.25	16.05	-	22.455
	12.5	22	-	27.755
	25	30.43	-	36.425
	50	41.34	-	52.536
	100	59.6	-	64.452
	IC_{50} (µg/ml)	142.3	-	38.21
Diethyl ether	3.125	-	-	7.321
	6.25	-	-	23.989
	12.5	-	-	30.404
	25	-	-	49.126
	50	-	-	66.064
	100	-	-	80.866
	IC_{50} (µg/ml)			26.02
Ethyl acetate	0	-	0	-
	3.125	-	19.75	-
	6.25	-	27.06	-
	12.5	-	39.21	-
	25	-	67.13	-
	50	-	85.92	-
	100	-	86.67	-
	IC_{50} (µg/ml)		17.4	

ABTS Assay

Both methanol and chloroform extracts of *F. benjamina* showed significant antioxidant activity in the DPPH assay. Among the two extracts, methanol extract demonstrated higher antioxidant potential with a significant IC_{50} value of 109.5 µg/ml. Among the methanol and ethyl acetate extract of *A. leptopus*, the ethyl acetate extract showed the highest significant antioxidant potential with an IC_{50} value of 13.9 µg/ml. Among the four extracts of *A. tricolor*, the highest antioxidant potential was demonstrated by diethyl ether extract with the highest significant IC_{50} value of 77.67 µg/ml.

Table 2: Results of ABTS antioxidant assay

Sample	Concentration (µg/ml)	*Ficus benjamina*	*Antigonon leptopus*	*Amaranthus tricolor*
Control	0	0	0	0
Quercetin	3.13	28.3	12.06	6.7
	6.25	35.8	26.77	13.2
	12.5	58.4	40.46	19.1
	25	69.4	66.67	36.1
	50	80.72	86.09	42.8
	100	85.6	95.85	61.7
	IC_{50} (µg/ml)	9.37	1.786	8.564
Methanol	0	0	0	0
	6.25	3.47	18.94	-
	12.5	5.51	28.96	-
	25	13.98	48.83	12.98
	50	25.9	52.52	29.09
	100	37.76	80.33	42.9
	200	53.34	83.83	64.75
	IC_{50} (µg/ml)	109.5	18.33	126.1
Chloroform	3.125	2.41	-	-
	6.25	6.16	-	-
	12.5	12.68	-	-
	25	22.86	-	14.57
	50	34.73	-	24.7
	100	51.52	-	36.51
	IC_{50} (µg/ml)	143.9		187.2
Diethyl ether	3.125	-	-	-
	6.25	-	-	-
	12.5	-	-	-
	25	-	-	15.87
	50	-	-	27.91
	100	-	-	46.3
	IC_{50} (µg/ml)	-		77.67
Ethyl acetate	0	-	0	-
	3.125	-	19.77	-
	6.25	-	26.29	-
	12.5	-	49.99	-
	25	-	60.39	-
	50	-	74.31	-
	100	-	84.77	-
	IC_{50} (µg/ml)		13.9	

ABTS: 2,2'-azinobis-3-ethyl-benzothiazoline-6-sulfonic acid

Among all the three different plant samples and eight different extracts, the most significant antioxidant potential was demonstrated by ethyl acetate extract of *A. leptopus*, with the lowest IC_{50} value of 13.9 µg/ml. The results of the antioxidant activity assay are tabulated in Table 2.

Hemolysis Assay

All the plant extracts of three plants and their seven different extracts were tested for their anti-hemolytic potential. The results of the hemolysis assay are graphically represented in Figure 1.

Similarly, methanol and ethyl acetate leaf extracts of *A. leptopus* showed maximum hemolysis of red blood cell (RBC) at higher concentrations (80, 160, and 320 µg/ml) compared to lower concentrations (10, 20, and 40 µg/ml). The hemolysis percentage is graphically represented in Figure 1a.

In addition, both chloroform and diethyl ether leaf extracts of *A. tricolor* showed maximum hemolysis at higher concentrations (80, 160, and 320 µg/ml) compared to lower concentrations (10, 20, and 40 µg/ml). The hemolysis percentage is graphically represented in Figure 1b.

The leaf extracts of *F. benjamina* showed maximum lysis of RBCs at higher concentration of 320 µg/ml and above while compared to lower concentrations of 160 µg/ml and lower. The results are graphically represented in Figure 1c. Overall, none of the plant samples showed any significant anti-hemolysis.

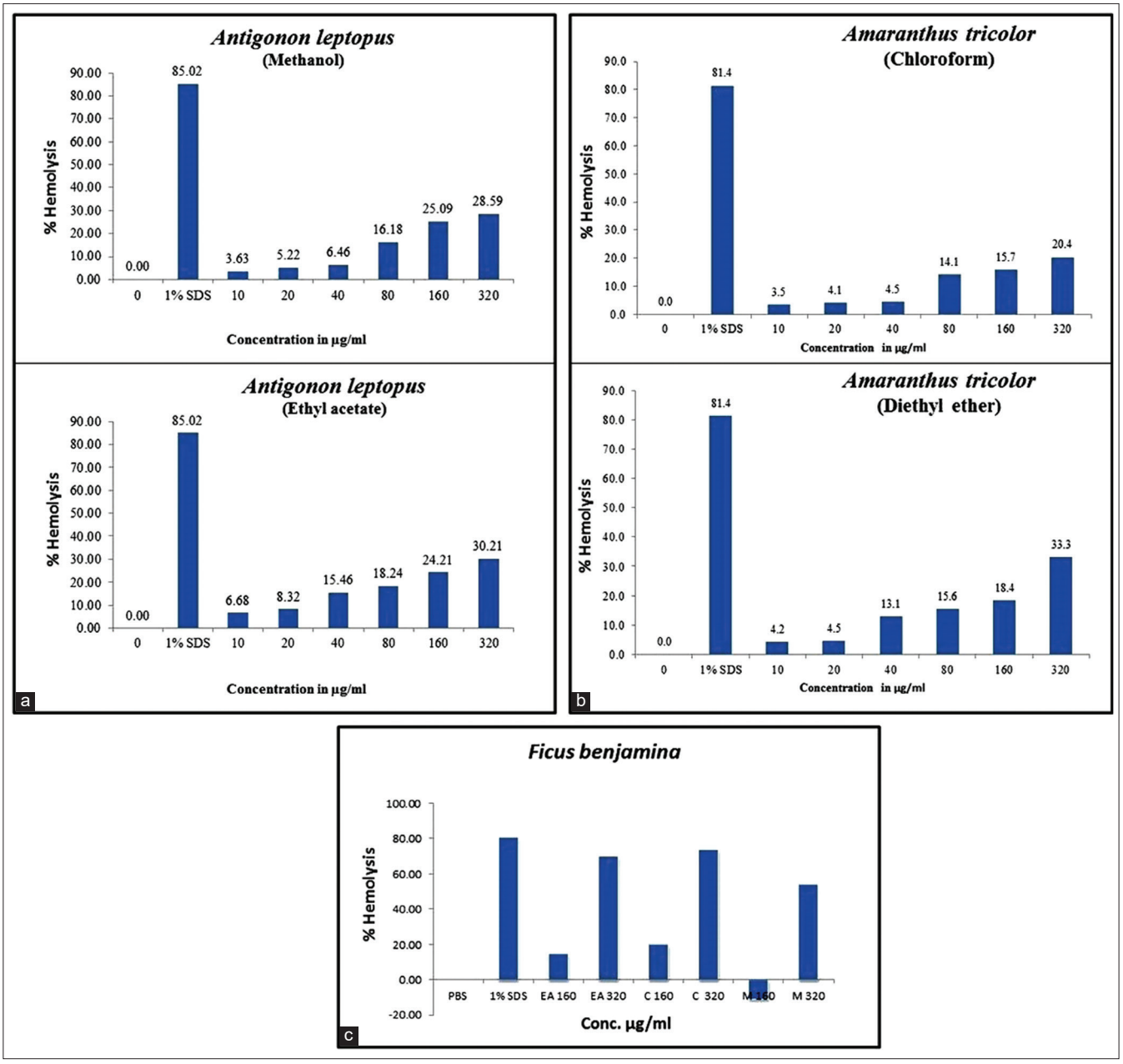

Figure 1: Hemolysis activity of crude extracts of (a) *Antigonon leptopus,* (b) *Amaranthus tricolor,* (c) *Ficus benjamina*

CONCLUSIONS

Three different plant samples were subjected to DPPH and ABTS radical scavenging assay, among the three different plants, the ethyl acetate leaf extract of *A. leptopus* showed highest significant antioxidant potential with IC_{50} value of 17.4 µg/ml and 13.9 µg/ml in both DPPH and ABTS assay compared to other leaf extracts. However, other leaf extracts were also showed significant antioxidant potential with their respective IC_{50} values.

In hemolytic assay, all the leaf extracts of three different plants showed maximum hemolysis of erythrocytes (RBCs) at higher concentrations compared to lower concentrations. Therefore, the present work reveals that lower concentrations of all the leaf extracts of three different plant samples showed minimum hemolysis of erythrocytes (RBCs).

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