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Research Article

ANTIOXIDANT POTENTIAL OF VARIOUS EXTRACTS FROM WHOLE PLANTS OF *DOLICHOS BIFLORUS* (LINN) EVALUATED BY THREE *IN VITRO* METHODS

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ABSTRACT
In the present investigation was to evaluate the antioxidant capacity of various extracts from whole plants of *Dolichos biflorus* with the help of three *in-vitro* antioxidant models were carried out for total antioxidant activity (phosphomolybdic acid method), ferric-reducing antioxidant potential (FRAP) assay and iron chelating method. Methanolic extract of *Dolichos biflorus* was showed more effective in free radical scavenging activity in total antioxidant activity, FRAP and iron chelating methods than that of other two extracts. The results obtained in the present study indicate that the methanolic extract of whole plants of *Dolichos biflorus* are a potential source of natural antioxidant.
KEYWORDS: *Dolichos biflorus*, Total antioxidant activity, FRAP assay, iron chelating activity.

INTRODUCTION

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by- products of cell metabolism[1, 2]. Free radicals form when oxygen is metabolized or formed in the body and are chemical species those posses an unpaired electron in the outer (valance) shell of the molecule. This is the reason, why the free radicals are highly reactive and can react with proteins, lipids, carbohydrates and DNA. These free radicals attack the nearest stable molecules, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction, finally resulting in the description of a living cell[3].

Overproduction of free radicals (oxidative stress), which occurs when the cell's natural antioxidant systems are overwhelmed, results in severe damage to biological molecules, especially to DNA, proteins and lipids. Oxidative stress has been associated with the progression of chronic conditions such as acquired immune deficiency syndrome (AIDS), cancer, aging, atherosclerosis, inflammation, cardiovascular disease, diabetes, and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases[4]. Natural compounds, which are present in herbal products, vegetables, fruits and grains, possess the ability to reduce oxidative damage by acting as antioxidants[5]. The oxidation process proceeds as a chemical reaction that transfers electrons from a reducing substance to an oxidizing agent, forming chain reactions that can be difficult to contain.

Dolichos biflorus Linn. Syn. *Dolichos uniflorus* (Family - Fabaceae) is a branched, sub-erect and downing herb, native to most parts of India and is found up to altitudes of 1000 m. It is a fast growing annual vine with trifoliate leaves and brown, flat, curved pods filled with seeds[6]. The seeds can be cooked and eaten. In Ayurveda, the seed is used in the treatment of piles, pain, constipation, wounds, urinary calculi, cough, edema, asthma etc. The soup prepared from seeds is also beneficial in enlarged liver and spleen. The seeds of *D. biflorus* have been reported to show antilithiatic[7], antihepatotoxic[8] and hypolipidemic[9] activity and involved in lowering the level of blood sugar and total cholesterol[10]. Two Ayurvedic preparations[11, 12], having *D. biflorus* as an ingredient, have shown their antinephrotoxic and free radical scavenging activity. The present study is aimed to evaluate the antioxidant capacity of various extract from whole plants of *D. biflorus* with the help of three in-vitro antioxidant models.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The whole plant of *Dolichos biflorus* (Linn), were collected form Sankaran koil, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Dolichos biflorus* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[13]for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then marc was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by in vitro Techniques:

Total antioxidant activity (Phosphomolybdic acid method) [14]

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999)[14]. An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95^{0}C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

FRAP assay

A modified method of Benzie and Strain (1996)[15] was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mMHCl and 20 mM$Fecl_3$. $6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3.6H_2O$. The temperature of the solution was raised to 37^0 C before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Iron chelating activity

The method of Benzie and strain (1996)[15] was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

RESULTS AND DISCUSSION

Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of petroleum ether extract of *Dolichos biflorus* depicted in Table 1. The petroleum ether extract of *Dolichos biflorus* exhibited a maximum total antioxidant activity of 51.58% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50}values of the petroleum ether extract of *Dolichos biflorus* and ascorbate were found to be 970µg/ml and 410µg/ml respectively.

The percentage of total antioxidant activity of ethyl acetate extract of *Dolichos biflorus* depicted in Table 2. The ethyl acetate extract of *Dolichos biflorus* exhibited a maximum total antioxidant activity of 58.67% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50}values of the ethyl acetate extract of *Dolichos biflorus* and ascorbate were found to be 620µg/ml and 410µg/ml respectively.

The percentage of total antioxidant activity of methanolic extract of *Dolichos biflorus* presented in Table 3. The methanolic extract of *Dolichos biflorus* exhibited a maximum total antioxidant activity of 79.38% at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} of the methanolic extract of *Dolichos biflorus* and ascorbate were found to be 260µg/ml and 410µg/ml respectively.

Based on the result clearly indicated methanolic extract of *Dolichos biflorus* was found to be more effective than that other two extracts. But when compare all the extracts with standard the methanolic extract of *Dolichos biflorus* was found strong antioxidant activity. The IC_{50} of the methanolic extract of *Dolichos biflorus* and ascorbate were found to be 260µg/ml and 410µg/ml respectively.

FRAP assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

The FRAP assay showed greater variability between various extracts. Table 4 was illustrated the FRAP values of petroleum ether extract of *Dolichos biflorus* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml). The maximum reducing ability at 1000µg/ml for petroleum ether extract and ascorbate were found to be 48.33% and 98.07% respectively. The IC_{50} values of petroleum ether extract and ascorbate were recorded as 1050µg/ml and 50µg/ml respectively.

The reducing ability of the ethyl acetate extract of *Dolichos biflorus* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were illustrated in Table 5. The maximum reducing ability at 1000µg/ml for ethyl acetate extract and ascorbate were found to be 57.42% and 98.07% respectively. The IC_{50} values of ethyl acetate extract and ascorbate were recorded as 780µg/ml and 50µg/ml respectively.

The reducing ability of the methanolic extract of *Dolichos biflorus* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were illustrated in Table 6. The maximum reducing ability at 1000µg/ml for methanolic extract and ascorbate were found to be 75.74% and 98.07% respectively. The IC_{50} values of methanolic extract and ascorbate were recorded as 180µg/ml and 50µg/ml respectively.

Based on the above results indicated, the methanolic extract of *Dolichos biflorus* was found to most effective than that of petroleum ether & ethyl acetate extract. But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the *Dolichos biflorus* showed the moderate result.

Iron chelating activity

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components[16]. Iron binding capacity of the petroleum ether extract of *Dolichos biflorus* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were summarized in Table 7.Maximum chelating of metal ions at 1000µg/ml for plant extract and EDTA were found to be 47.58% and 97.90% respectively. The IC_{50} value of plant extract and EDTA were recorded as 1050µg/ml and 65µg/ml respectively.

Iron binding capacity of the ethyl acetate extract of *Dolichos biflorus* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 8. Maximum chelating of metal ions at 1000µg/ml for plant extract and EDTA were found to be 61.37% and 97.90% respectively. The IC_{50} value of plant extract and EDTA were recorded as 590µg/ml and 65µg/ml respectively.

Iron binding capacity of the methanolic extract of *Dolichos biflorus* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 9. Maximum chelating of metal ions at 1000µg/ml for plant extract and EDTA was found to be 66.71% and 97.90% respectively. The IC_{50} value of plant extract and EDTA was recorded as 130µg/ml and 65µg/ml respectively.

Based on the findings clearly indicated that, the methanolic extract of *Dolichos biflorus* had showed more effective metal chelating activity than that of other two extracts.

CONCLUSION

In the present work, the high antioxidant capacity observed for methanolic extract of whole plant of *Dolichos biflorus* (Linn) suggest that it may play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases. However, further studies are needed to isolate active principles responsible for the overall antioxidant activity of the extract.

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Table 1: Total antioxidant activity of Petroleum ether extract of *Dolichos biflorus* (Linn)

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	19.65±0.11	26.87 ± 0.08
2	250	26.76±0.91	30.30 ± 0.05
3	500	42.71±0.26	60.64 ± 0.02
4	1000	51.58±0.14	55.23 ± 0.01
		IC_{50} = 970µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 2: Total antioxidant activity of Ethyl acetate extract of *Dolichos biflorus* (Linn)

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	28.30±0.19	26.87 ± 0.08
2	250	33.27±0.16	30.30 ± 0.05
3	500	45.43±0.28	60.64 ± 0.02
4	1000	58.67±0.13	55.23 ± 0.01
		IC_{50} = 620µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 3: Total antioxidant activity of Methanolic extract of *Dolichos biflorus* (Linn)

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	24.63±0.39	26.87 ± 0.08
2	250	49.76±0.31	30.30 ± 0.05
3	500	72.71±0.26	60.64 ± 0.02
4	1000	79.38±0.14	55.23 ± 0.01
		IC_{50} = 260µg/ml	**IC_{50} = 410 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 4: FRAP assay of Petroleum ether extract of *Dolichos biflorus* (Linn)

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	13.42±0.32	72.04 ± 0.01
2	250	35.33±0.19	82.05 ± 0.03
3	500	41.71±0.21	86.04 ± 0.02
4	1000	48.33±0.03	98.07 ± 0.04
		IC_{50} = 1050µg/ml	**IC_{50} = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 5: FRAP assay of Ethyl acetate extract of *Dolichos biflorus* (Linn)

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	16.35±0.21	72.04 ± 0.01
2	250	33.55±0.24	82.05 ± 0.03
3	500	42.66±0.14	86.04 ± 0.02
4	1000	57.42±0.24	98.07 ± 0.04
		IC50 = 780µg/ml	**IC50 = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 6: FRAP assay of Methanolic extract of *Dolichos biflorus* (Linn)

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	40.53±0.21	72.04 ± 0.01
2	250	65.02±0.13	82.05 ± 0.03
3	500	71.55±0.60	86.04 ± 0.02
4	1000	75.74±0.34	98.07 ± 0.04
		IC_{50} = 180 µg/ml	**IC_{50} = 50 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 7: Effect of Petroleum ether extract of *Dolichos biflorus* (Linn) on Iron-chelating method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (EDTA)
1	125	18.83±0.89	58.68 ± 0.01
2	250	29.45±0.12	65.87 ± 0.02
3	500	33.54±0.74	83.83 ± 0.01
4	1000	47.58±0.16	97.90 ± 0.02
		IC_{50} = 1050 µg/ml	**IC_{50} = 65 µg/ml**

*Data presented as the mean ± SEM of three measurements.

Table 8: Effect of Ethyl acetate extract of *Dolichos biflorus* (Linn) on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (EDTA)
1	125	32.54±0.68	58.68 ± 0.01
2	250	39.67±0.87	65.87 ± 0.02
3	500	48.68±0.18	83.83 ± 0.01
4	1000	61.37±0.75	97.90 ± 0.02
		IC50 = 590µg/ml	**IC50 = 65 µg/ml**

*Data presented as the mean ± SEM of three measurements.

Table 9: Effect of Methanolic extract of *Dolichos biflorus* (Linn) on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (EDTA)
1	125	49.11±0.46	58.68 ± 0.01
2	250	56.19±0.45	65.87 ± 0.02
3	500	61.34±0.14	83.83 ± 0.01
4	1000	66.71±0.34	97.90 ± 0.02
		IC_{50} = 130µg/ml	**IC_{50} = 65 µg/ml**

*Data presented as the mean ± SEM of three measurements.

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