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FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS OF WHOLE PLANT OF *Dolichos biflorus* (Linn): AN *IN-VITRO* EVALUATION

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ABSTRACT

The study was designed to examine the *in vitro* antioxidant activities of various extracts of whole plant of *Dolichos biflorus.* The antioxidant activity was evaluated by, Super Oxide Anion Scavenging Activity, Nitric oxide reducing scavenging activity with reference standard Quercetin and Ascorbate respectively and Estimation of total phenolic content also. The methanolic extract of *Dolichos biflorus* was found to more effective in the Superoxide Anion Scavenging Activity. The IC_{50} of the methanolic extract of *Dolichos biflorus* and Rutin were found to be 200µg/ml and 60µg/ml respectively. An IC_{50} value was found that methanolic extract of *Dolichos biflorus* is more effective in scavenging superoxide radical than that of petroleum ether and ethyl acetate extract. But when compare to the all the three extracts with Quercetin (standard), the methanolic extract of the *Dolichos biflorus* showed the similar result. The nitric oxide scavenging activity of the methanolic extract of *Dolichos biflorus* was found to most effective than that of petroleum ether & ethyl acetate extract. The IC_{50} value of methanolic extract and ascorbate were recorded as 100µg/ml and 410µg/ml respectively. The total phenol content of pet ether, ethyl acetate, methanolic extract was found to be 3.15, 6.22 and 10.16mg/g respectively. It is concluded that a whole plant of methanolic extract of *Dolichos biflorus,* which contains large amounts of phenolic compounds, which exhibits high antioxidant and free radical scavenging activities. These *in vitro* assays indicate that this plant extracts is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: *Dolichos biflorus*, *In vitro* antioxidant, Superoxide anion, Nitric oxide reducing scavenging, Total phenol.

INTRODUCTION

Oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species [1]. The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O_2^-) [2]. Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) can be involved in a high number of diseases [3,4].

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Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols [5]. Peroxidation of unsaturated lipids in biologic membranes has been implicated in a wide range of diseases including ageing and cancer [6], diabetes and cardiovascular diseases [7] and rheumatoid arthritis [8]. Current interest has focused on the potential role of anti-oxidants and anti- oxidant enzymes in the treatment and prevention of certain diseases. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin, lignans, catechins and isocatechins [9]. Antioxidant-based drug formulations are used for the prevention and treatment of

complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [10]. In recent years there has been an increasing interest in the use of natural substances, and some questions regarding the safety of synthetic compounds have led to more detailed studies of plant resources.

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 [11]. 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 [12], antihepatotoxic [13] and hypolipidemic activity [14] and involved in lowering the level of blood sugar and total cholesterol [15]. Two Ayurvedic preparations [16,17], having *D. biflorus* as an ingredient, have shown their anti-nephrotoxic 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 sankarankoil, 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 for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark 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:

Superoxide radical scavenging activity

Superoxide radical (O_2^-) was generated from the photo reduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975) [18]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Quercetin was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Nitric oxide method

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the method of Garrat (1964) [19]. The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^{0}C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

Total phenol

The measurement of total phenol is based on Mallick and Singh (1980) [20]. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2^{o}C for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases [21].They are also involved in autoimmune disorders like rheumatoid arthritis etc [22].

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [23]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging

activity [24]. Therefore, the importance of search for natural antioxidants has increased in the recent years so many researchers focused the same [25].

Superoxide anion scavenging activity

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical [26]. Percentage scavenging of superoxide anion examined at different concentrations of petroleum ether extract of *Dolichos biflorus* (Linn) (125, 250, 500, 1000 µg/ml) was depicted in table 1.The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 56.85% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 450µg/ml and 60µg/ml respectively.

Percentage scavenging of superoxide anion examined at different concentrations of ethyl acetate extract of *Dolichos biflorus* (Linn) (125, 250, 500, 1000 µg/ml) was depicted in table 2. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 86.35% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 425µg/ml and 60µg/ml respectively.

Percentage scavenging of superoxide anion examined at different concentrations of methanolic extract of *Dolichos biflorus* (Linn) (125, 250, 500, 1000 µg/ml) was depicted in table 3. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 82.57% and 98.01% respectively. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavonoids. The IC_{50} value of plant extract and Quercetin was recorded as 200µg/ml and 60µg/ml respectively.

Nitric oxide scavenging activity

Nitric oxide is a diffusible free radical which is an important effector molecule in diverse biological systems [28]. Studies in animal models have suggested the role for NO in pathogenesis of inflammation and pain [29,30]. So it is worthful to investigate the NO scavenging potential of the plant extract. The reduction of nitric oxide radical by the petroleum ether extract of *Dolichos biflorus* and ascorbate was illustrated in Table 4. The maximum scavenging activity of petroleum ether extract and ascorbate at 1000 µg/ml were found to be 50.14 % and 55.23% respectively. The IC_{50} value of petroleum ether extract and ascorbate were recorded as 975µg/ml and 410µg/ml respectively.

The reduction of nitric oxide radical by the ethyl acetate extract of *Dolichos biflorus* and ascorbate was illustrated in Table 5. The maximum scavenging activity of ethyl acetate extract and ascorbate at 1000 µg/ml were found to be 52.47% and 55.23% respectively. The IC_{50} value of ethyl acetate extract and ascorbate were recorded as 925µg/ml and 410µg/ml respectively.

The reduction of nitric oxide radical by the methanolic extract of *Dolichos biflorus* and ascorbate was noted to be concentration dependent and was illustrated in Table 6. The maximum scavenging activity of methanolic extract and ascorbate at 1000 µg/ml were found to be 62.76% and 55.23% respectively. The IC_{50} value of methanolic extract and ascorbate were recorded as 100µg/ml and 410µg/ml respectively.

Table 1. Effect of Petroleum ether extract of *Dolichos biflorus* (Linn) on Superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Quercetin)
1	125	23.45 ±0 .167	73.81 ± 0.006
2	250	42.18 ± 0.034	91.31 ± 0.011
3	500	52.25 ± 0.054	92.99 ± 0.024
4	1000	56.85 ± 0.035	98.01 ± 0.012
		IC_{50} = 450 µg/ml	**IC_{50} = 60 µg/ml**

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

Table 2. Effect of Ethyl acetate extract of *Dolichos biflorus* (Linn) on Superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	20.73 ± 0.010	73.81 ± 0.006
2	250	38.55 ± 0.060	91.31 ± 0.011
3	500	56.35 ± 0.012	92.99 ± 0.024
4	1000	86.35 ± 0.012	98.01 ± 0.012
		IC_{50} = 425 µg/ml	**IC_{50} = 60 µg/ml**

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

Table 3. Effect of Methanolic extract of *Dolichos biflorus* (Linn) on Superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	43.16 ± 0.012	73.81 ± 0.006
2	250	62.25 ± 0.120	91.31 ± 0.011
3	500	77.47 ± 0.008	92.99 ± 0.024
4	1000	82.57 ± 0.026	98.01 ± 0.012
		IC_{50} = 200 µg/ml	**IC_{50} = 60 µg/ml**

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

Table 4. Nitric oxide scavenging 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	20.06 ± 0.021	26.87 ± 0.076
2	250	33.60 ± 0.655	30.30 ± 0.054
3	500	44.74 ± 0.020	60.64 ± 0.022
4	1000	50.14 ± 0.003	55.23 ± 0.014
		IC_{50} = 975 µg/ml	**IC_{50} = 410 µg/ml**

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

Table 5. Nitric oxide scavenging 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	22.15 ± 0.020	26.87 ± 0.076
2	250	30.25 ± 0.012	30.30 ± 0.054
3	500	42.47 ± 0.012	60.64 ± 0.022
4	1000	52.47 ± 0.012	55.23 ± 0.014
		IC_{50} = 925 µg/ml	**IC_{50} = 410 µg/ml**

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

Table 6. Nitric oxide scavenging activity of Methanolic extract of *Dolichos biflorus* (Linn)

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	50.58 ± 0.014	26.87 ± 0.076
2	250	54.61 ± 0.017	30.30 ± 0.054
3	500	59.76 ± 0.028	60.64 ± 0.022
4	1000	62.76 ± 0.028	55.23 ± 0.014
		IC_{50} = 100 µg/ml	**IC_{50} = 410 µg/ml**

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

Table 7. The total Phenolic content of various extracts of whole plant of *Dolichos biflorus*

S. No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1	Petroleum ether extract of *Dolichos biflorus*	3.15 ± 0.010
2	Ethyl acetate extract of *Dolichos biflorus*	6.22 ± 0.021
3	Methanolic extract of *Dolichos biflorus*	10.16 ± 0.026

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

Total phenol

Phenolic compounds are known as powerful chain breaking antioxidants [31]. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidative action [32]. The total amount of phenolic content of various extract of whole plant of *Dolichos biflorus* was present in Table 7.

Based on the result the methanolic extract of *Dolichos biflorus* was found higher content of phenolic components than that of petroleum ether and ethyl acetate extract of *Dolichos biflorus.*

CONCLUSION

The present study was clearly indicated the methanolic extract of *Dolichos biflorus* showed strong antioxidant activity by inhibiting Super oxide anion scavenging activity, Nitric oxide radical scavenging activities when compared with standard Quercetin and Ascorbate. But the ethyl acetate extract showed moderate

activity when compared with standard Ascorbate. In addition, the methanolic and ethyl acetate extract of *Dolichos biflorus* was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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