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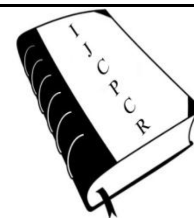

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DPPH SCAVENGING AND REDUCING POWER ANALYSIS OF AQUEOUS EXTRACT OF *DOLICHANDRONE FALCATE* STEM BARK

A.Zechariah Jebakumar[*1], Eidan Musa Al Zahrani[2], Hassan S. Nondo[1]

[*1]Dept. of Research and Scientific studies, Prince Sultan Military college of Health Sciences, Dhahran-31932, Kingdom of Saudi Arabia.
[2]Director, Prince Sultan Military College of Health Sciences, Dhahran-31932, Kingdom of Saudi Arabia.

ABSTRACT

Dolichandrone falcata Seem.(Family: Bignoniaceae), has reported for different biological activities such as anti-allergic, anti-inflammatory, anti-cancer, antiestrogenic and anxiolytic activities. Therefore, the present study was estimated the *invitro* antioxidant effect of aqueous extract of stem bark of *Dolichandrone falcate* by using DPPH scavenging test and reducing power method. The AEDF exhibited a significant dose dependent inhibition of DPPH activity. AEDF and reference standard ascorbic acid have showed similar anti-oxidant activity with reducing power method also.

Key words: *Dolichandrone falcate,* Antioxidant, Reducing Power Method, DPPH radical scavenging.

INTRODUCTION

Dolichandrone falcata Seem., Bignoniaceae, is a is a small deciduous tree with bluish grey bark, peeling in irregular woody scales and also commonly known as Medshingi. It growing on hedges of cultivated fields and frequently in hill forest, occasionally seen in dry scrub forests. *Dolichandrone falcata* bark is traditionally used in the treatment of fractured bones and used as a fish poison. In this plant Chrysin (flavone) was identified and reported for different biological activities such as anti-allergic, anti-inflammatory, anti-cancer, antiestrogenic and anxiolytic activities by previous authors [1-7]. In Ayurveda, the stem bark of *Dolichandrone falcata* is used for cure the ulcer, pain and epilepsy. But still no depth scientific study has been performed on *Dolichandrone falcata* stem-bark pharmacological properties. The aim of present study was estimated the *invitro* antioxidant effect of aqueous extract of stem bark of *Dolichandrone falcate*.

MATERIALS AND METHODS

Plant collection and Preparation of plant extract

The stem-bark of *Dolichandrone falcata* was collected from the forest of Agasthyamalai hills, Tirunelveli district, Tamilnadu, India. It was identified and authenticated by Dr.V.Chelladurai, Research Officer Botany. C.C.R.A.S., Govt. of India. The collected stem-bark of *Dolichandrone falcata* was shadow/air dried in room temperature without sunlight. The dried material was extracted in 1 litre of boiling water for 2-3 h and concentrated to half of the volume by boiling in a water bath. The yielded brownish extract was cooled and filtered using Whatman filter paper. The filterate extract was concentrated up to 100 ml on rotavapour under reduced pressure. The yield value was found to be 12.5%.

Phytochemical analysis

The aqueous extract of *Dolichandrone falcata*was subjected to qualitative analysis for the various phyto-constituents. Standard methods were used for preliminary qualitative phytochemical analysis of extract [8].

ANTIOXIDANT ACTIVITY

DPPH Radical scavenging test

Corresponding Author :- **A.Zechariah Jebakumar** Email:- zacbiostat@gmail.com

The free radical scavenging activity of the aqueous extract of *Dolichandrone falcate* (AEDF) was determined by using 2, 2 Diphenyl-1-picryl hydrazyl radical (DPPH) using UV-Spectrometry [9] at 517nm. The DPPH solution was prepared in 95% aqueous. The AEDF was mixed with 95% aqueous to prepare the stock solution (10mg/100ml or 100μg/ml). From the stock solution 2ml, 4ml, 6ml, 8ml and 10ml of this solution were taken in five test tubes and by serial dilution with same solvent were made the final volume of each test tube up to 10ml whose concentration was then 20μg/ml, 40μg/ml, 60μg/ml, 80μg/ml and 100μg/ml respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of their test tubes. Containing AEDF (20μg/ml, 40μg/ml, 60μg/ml, 80μg/ml and 100μg/ml) and after 10 min, the absorbance was taken at 517nm, using a spectrophotometer (SHIMADZU UV-1700, UV-visible spectrophotometer). Ascorbic acid was used as a reference standard. It is dissolved in distilled water to make stock solution with the same concentration of AEDF control sample was prepared without extract and reference ascorbic acid. 95% aqueous was used as blank % scavenging of the DPPH free radical was measured using following equation.

$$\%\ \text{DPPH radical-scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{(\text{Absorbance of control})} \times 100$$

Reducing Power Method

The assay of reducing power method [10] is one to determine the antioxidant activity. In this 1 ml of plant extract of AEDF solution mixed with 2.5 ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml Potassium Ferricyanide [K_3Fe (CN6)] (10g/l), the mixture was incubated at 50°C for 20 minutes. 2.5 ml of Tri chloroacetic acid (100g/l) was added to mixture. This was centrifuged at 3000 rpm for 10 min. Finally 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (lg/L) and absorbance measured at 700nm in UV-visible spectrophotometer (SHIMADZU UV-1700, UV-visible spectrophotometer). Ascorbic acid was used as standard and phosphate buffer used as blank.

DPPH radical scavenging activity of aqueous extracts of *Dolichandrone falcate* (AEDF) added to aqueous solution of DPPH and radical scavenging activity was measured as 517 nm as compared to standard Ascorbic acid. Values are the average of triplicate experiments.

Table 1. Antioxidant activity by DPPH method

S. No.	Concentration (μg/ml)	Absorbance of ascorbic acid	Absorbance of AEDF	% scavenging DPPH of Ascorbic acid	%scavenging DPPH of AEDF
1	20μg/ml	0.174	0.142	38.12	43.16
2	40μg/ml	0.126	0.094	56.22	62.42
3	60μg/ml	0.110	0.082	64.24	72.12
4	80μg/ml	0.080	0.054	76.42	84.17
5	100μg/ml	0.064	0.026	92.17	88.19

Table 2. Antioxidant activity by reducing power method

S.No.	Concentration (mg/ml)	Absorbance of Ascorbic acid	Absorbance of AEDF
1	0.1	0.22	0.14
2	0.2	0.27	0.25
3	0.3	0.36	0.35
4	0.4	0.45	0.42
5	0.5	0.57	0.49

Reducing power of aqueous extract of *Dolichandrone falcate* (AEDF) of as compared to Ascorbic acid. Values are the average of triplicate experiments.

RESULTS AND DISCUSSION

The results of preliminary phytochemical investigation of the aqueous extract of *Dolichandrone falcate* stem-bark(AEDF) shows the presence of phenols, flavanoids, glycosides, terpenes, alkaloids, tannins, and saponins. In this present study the aqueous extract of stem-bark *Dolichandrone falcate* were investigated by using DPPH scavenging test and reducing power method. The stem-bark of AEDF showed by their two methods effectively when compared with reference standard ascorbic acid. In the DPPH scavenging method is based on the capability of DPPH radical to decolorize in the presence of antioxidants. The DPPH radical is considered to be model of a stable lipophilic radical a chain reaction. In lipophilic radicals was initiated by the lipid auto-oxidation antioxidants react with DPPH reducing a number of DPPH molecules equal to number of their hydroxyl groups. Therefore, the absorption at 517 nm was proportional to the amount of residual DPPH [11-13]. The AEDF exhibited a significant dose dependent inhibition of DPPH activity.

The reducing power method based on the capability of a reducing the compound due to presence of reductants which are breaking the free radical chain by

donating hydrogen atom. The stem-bark of AEDF exhibited the antioxidant activity due to presence of reductants (i.e., antioxidants). The reduction of Fe^{3+}/Ferricyanide complex to ferrous form, in this main principle is increasing the absorbance of the reaction mixture indicates the antioxidant activity that leads to reducing power of the samples. AEDF was very potent and the power of extract was increased with quantity of sample. By comparing the reference standard Ascorbic acid, the AEDF showed potent antioxidant activity [14,15].

CONCLUSION

It is concluded from the data, that the aqueous extract of *Dolichandrone falcata* possess significant Antioxidant activity and may prove to be effective for the treatment of various diseases caused by free radicals. The antioxidant activity may be rich in flavanoids in this plant. However further studies required to elucidate the exact mechanism of action for develop its as potent antioxidant.

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