IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

ANTIOXIDENT ACTIVITY OF VARIOUS EXTRACTS OF *DOLICHANDRONE FALCATA* SEEM BARK

Roopa Kulkarni[1] and Paramjyothi Swamy[2*]

[1]Research Scholar, Department of Post Graduate Studies and Research in Biochemistry,

Gulbarga University, Kalaburagi, Karnataka, India.

[2]Professor, Department of Post Graduate Studies and Research in Biochemistry,

Gulbarga University, Kalaburagi Karnataka, India.

***Corresponding Author:**
Dr. Paramjyothi Swamy
Professor, Department of Post Graduate Studies and Research in Biochemistry,
Gulbarga University, Kalaburagi-585 106.
Karnataka, India.
Email: *paramjyothiswamy@gmail.com*

IJFANS International Journal of Food And Nutritional Sciences
Official Publication of International Association of Food and Nutrition scientists

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

ABSTRACT

Antioxidants are an essential component that protects health. Research evidences suggest that antioxidants may lower the risk of chronic illnesses of human being. Present investigation was carried out to appraise the photochemical constituents and antioxidant activity of *Dolichandrone falcata* seem bark. The bark sample was subjected to Soxhlet extraction with solvents like methanol, chloroform, and pet ether. The antioxidant potential was tested using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Ascorbic acid was used as a standard control. All the three extracts exhibited antioxidant activity with DPPH inhibition in a dose dependent manner. IC_{50} values methanol, chloroform, and pet ether extract of *Dolichandrone falcata* seem bark being 429 µg/ml, 545µg/ml, and 905 µg/ml respectively. Methanol extract showed significant DPPH radical scavenging activity followed by chloroform and pet ether.

Keywords; *Dolichandrone falcata*, Antioxidant activity, Bark extract, DPPH assay.

INTRODUCTION

Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits, and vegetables.[1] Plant sourced antioxidants like Vitamin C, Vitamin E, carotenes, phenolic acids etc., have been recognized as having the potential to reduce disease risk.[2] Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties.[3] A rapid, simple, and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity.[4]

The DPPH assay method is based on the reduction of DPPH, a stable free radical.[5] The free radical DPPH with an odd electron gives a maximum absorption at 517nm (purple colour). When antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical- scavenging antioxidant) and is reduced to DPPH and as consequence the absorbance's decreased from the DPPH.[6] Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured.[7] More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug.[8] When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Diphenyl picrylhydrazine; non radical) with the loss of this violet colour although there would be expected to be a residual pale-yellow colour from the picryl group still present.[9]

Dolichandrone falcata seem (Figure 1) belong to family Bignoniaceae, Malawi. Tanzania, South Africa, and India are all home to the vine Rajputana. Bundelkhand, Bihar, Deccan, Mysore, and Maharashtra are among the Indian states where it can be found. The plant is mainly used for diabetes, anti-inflammatory, anxiolytic, analgesic, antiestrogenic, antimicrobial, antinociceptive, antibacterial, and immunomodulatory activities. The leaves are also used in the treatment of leukaemia and menorrhagia. *Dolichandrone falcata seem* contains alkaloids, carbohydrates, saponin, phenolic, terpenoids, cardiac glycosides, steroids, and amino acids.[10]

Figure 1. Showing plant of *Dolichandrone falcata* seem

With this background, present study was undertaken to evaluate antioxidant potential of bark of this plant extracted with three different solvents.

MATERIALS AND METHODS

Collection and authentication of plant material

Dolichandrone falcata seem plant was collected from the Gulbarga university campus, Kalaburagi, Karnataka India. The taxonomic identity was confirmed in Department of Botany, Gulbarga University. Kalaburagi (Voucher specimen no GCAK-117).

Solvent extraction

The bark of *Dolichandrone falcata* seem was cleaned and shade dried at room temperature and finely powdered in the domestic mixer. 30gm of coarsely powdered sample was subjected to Soxhlet extraction with different solvents like methanol, chloroform, and petroleum ether. The solvents were evaporated to obtain semi-solid masses, which were further used in the study.

Preliminary photochemical screening

Photochemical analysis of methanol, chloroform, and pet ether extracts of bark of *Dolichandrone falcata* seem plant were carried out using the procedures as described by Trease, and Evans, Harborne, Soni and Sosa.[11-13]

DPPH Radical scavenging activity

The DPPH radical scavenging assay was performed using 1,1 diphenyl-2-picrylhydrazvl (DPPH) according to the method described by Brand-Williams et al., with some modifications.[14] Briefly five different concentrations of the studied plant extracts (62.5,125, 250, 500, 1000µg/ml) were prepared in methanol chloroform and pet ether. L-ascorbic acid (10µg/ml) was used as a standard. 1 ml of each test sample was transferred into a clean test tube into which 1ml of 0.1 mM DPPH in methanol was added. The mixture was shaken and left to stand in the dark at room temperature for 15 minutes. 1ml of methanol was used as blank. The negative control comprised 1ml of DPPH solution only and positive control comprised of DPPH and Ascorbic acid (10µg/ml). After incubation in the dark for 30 min the absorbance values were measured at 517 nm using spectrophotometer. The experiments were performed in triplicate. The DPPH radical scavenging activity was estimated using the following equation. The half maximum inhibitory concentration (IC_{50}) of the extracts was computed from the plot of percentage DPPH free radical inhibition versus the extract concentration using the following formula:

DPPH Radical Scavenging Activity= (Abs of Control-Abs of Sample)/Abs of Control] × 100

Statistical analysis

Samples were analysed in triplicate and the results are given in mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Table 1 represents phytochemical screening of methanol, chloroform, and pet ether extract of *Dolichandrone falcata* seem bark. Methanol extract showed presence of total phenols, flavonoids, glycosides, and steroids to a greater extent than tannins, alkaloids and terpenoids. On the other hand, chloroform extract showed presence of more total phenols and steroids, when compared to tannins, flavonoids, alkaloids and terpenoids, no glycerides were shown to be present in both chloroform and pet ether extracts. Rather pet ether extract showed least presence of almost all the phytoconstituents mentioned above. Patil et al., have reported presence of alkaloids.[15] Flavonoids, saponins, phenolics, terpenoids, glycosides and steroids in the leaves of *Dolichandrone falcata* seem extracted in different solvents. Ekode and Manik have reported important secondary metabolites in *Dolichandrone falcat*a seem leaves using GC-MS.[16]

Table 1: Phytochemical constituents of methanol, chloroform and pet ether extracts of *Dolichandrone falcata* seem bark

Phytoconstituents	Methanol Extract	Chloroform Extract	Pet ether Extract
Total phenol	+++	+++	+
Tannins	++	++	++
Flavonoids	+++	++	+
Alkaloids	++	++	+
Saponins	+	+	+
Glycosides	+++	-	-
Terpenoids	++	++	+
Steroids	+++	+++	+

+++ Strongly present; ++Present; +Weakly present; -Absent

The results of absorbance at 517nm and of DPPH inhibition with various concentration of standard and text extracts in methanol is represented in Table 2. The values of absorbance at 517nm for standard i.e., ascorbic acid was found to be 0.40 at 10 µg/ml. A gradual decline in absorbance of test sample was observed from 0.61 to 0.004 with increase in concentration of sample from 62.5 to 1000 µg/ml. However, an increase in percentage DPPH inhibition was observed. The test extracts at concentration of 500 and 1000 µg/ml exhibited 65.03 and 94.07 percentage DPPH inhibition respectively, which was quite high compared to that of standard i.e., 44.85. The concentration of test samples required to scavenge 50% of the DPPH radical (IC_{50}) was determined using linear regression equation and was found to be 429 µg/ml for methanol extract (Figure 2).

Table 2: DPPH inhibition percentage of methanol extract of *Dolichandrone falcata seem* bark

Conc. of *Dolichondrone falcata* bark (µg/ml)	**Abs. at 517 nm**	**DPPH inhibition (%)**
DPPH alone	0.73	0.00 ± 0.00
Ascorbic acid - 10µg/ml	0.40	44.85 ± 0.02
62.5	0.61	17.04 ± 0.09
125	0.57	22.36 ± 0.06
250	0.48	34.01 ± 0.01
500	0.26	65.03 ± 0.09
1000	0.004	94.07 ± 0.06

Values are expressed as Mean ± SEM; n=3

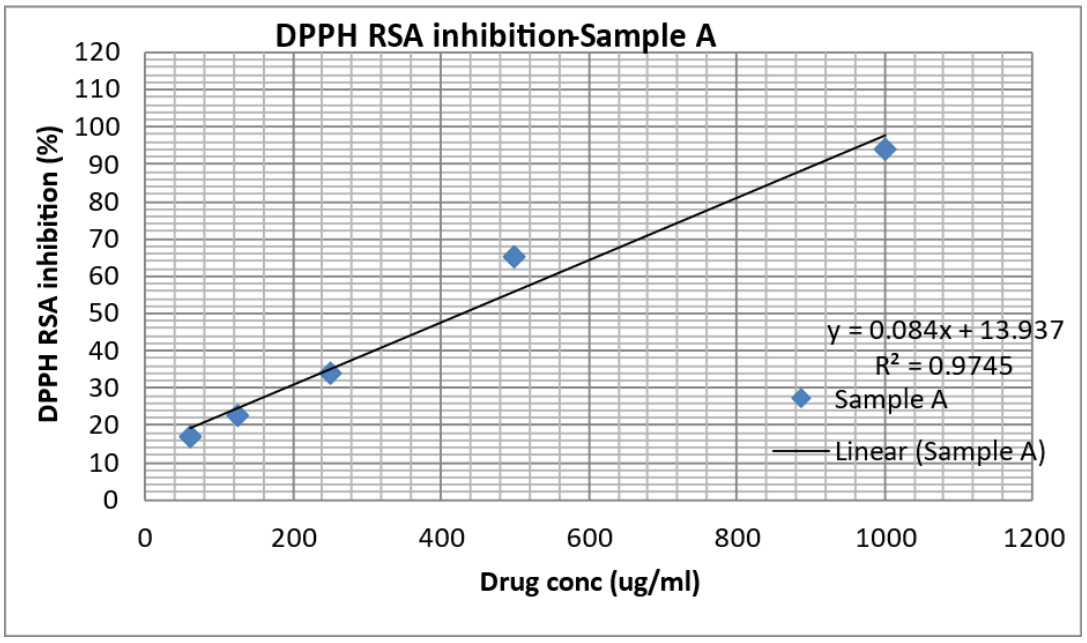

Figure 2. DPPH inhibition percentage of methanol extract of *Dolichandrone falcata* seem bark (Sample A)

Table 3 represents absorbance and percentage of DPPH inhibition of standard and test samples extracted in chloroform. The values for absorbance at 517nm were found to be 0.37 for standard. Here also a decline in absorbance (from 0.68 to 0.30) and increase in percentage DPPH inhibition (from 7.67 to 58.68) was observed with increase in concentration of test samples, IC_{50} value was found to be 545 µg/ml (Figure 3).

Table 3: DPPH inhibition percentage of chloroform extract of *Dolichandrone falcata seem* bark

Conc. of Dolichondrone falcata bark (µg/ml)	Abs. at 517 nm	DPPH inhibition (%)
DPPH alone	0.74	0.00 ± 0.00
Ascorbic acid - 10µg/ml	0.37	47.56 ± 0.02
62.5	0.68	7.67 ± 0.01
125	0.67	9.43 ± 0.00
250	0.64	13.43 ± 0.01
500	0.58	20.83 ± 0.02
1000	0.30	58.68 ± 0.02

Values are expressed as Mean ± SEM; n=3

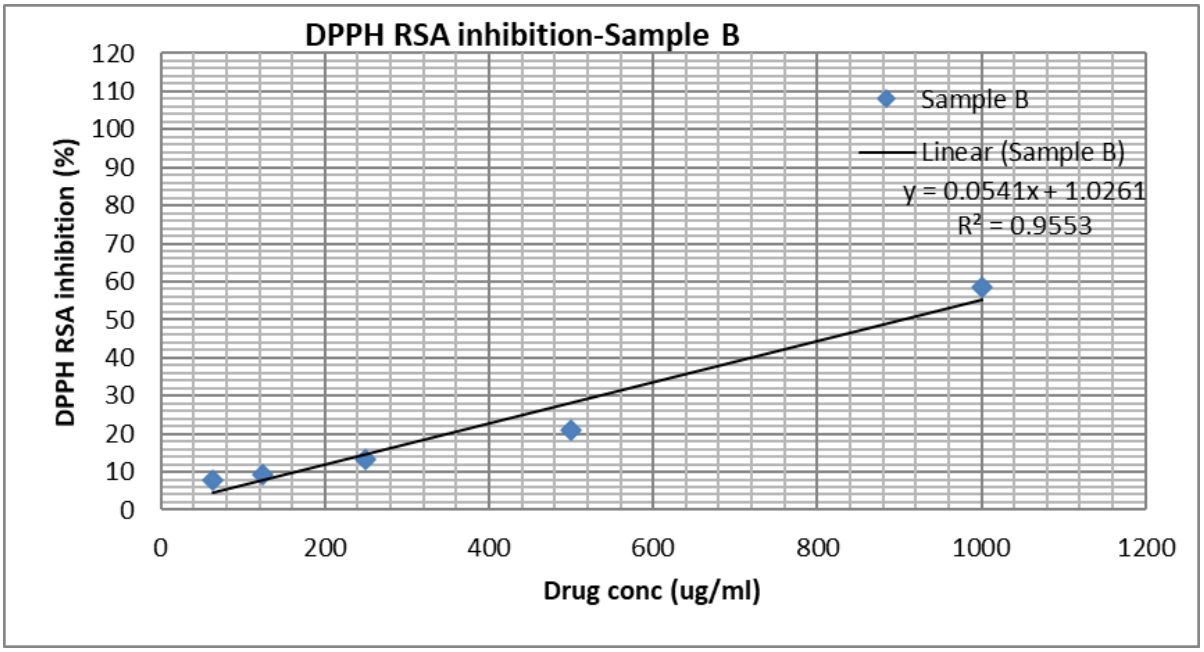

Figure 3. DPPH inhibition percentage of chloroform extract of *Dolichandrone falcata* seem bark (Sample B)

Table 4 represents the results for samples extracted in pet ether, where in absorbance was found to be 0.39 for standard and decline in the absorbance was observed in dose dependent manner. Further percentage DPPH inhibition was found in the range of 7.66% to 55.68% with IC_{50} value of 905 µg/ml (Figure 4).

Table 4: DPPH inhibition percentage of pet ether extract of *Dolichandrone falcata seem* bark

Conc. of Dolichondrone falcata bark (µg/ml)	Abs. at 517 nm	DPPH inhibition (%)
DPPH alone	0.85	0 0.00 ± 0.00
Ascorbic acid - 10µg/ml	0.39	56.65 ± 0.01
62.5	0.72	15.54 ± 0.01
125	0.65	23.32 ± 0.01
250	0.58	32.10 ± 0.01
500	0.43	49.06 ± 0.01
1000	0.18	78.44 ± 0.02

Values are expressed as Mean ± SEM; n=3

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 UGC CARE Listed (Group -I) Journal Volume 11, Iss 13, 2022

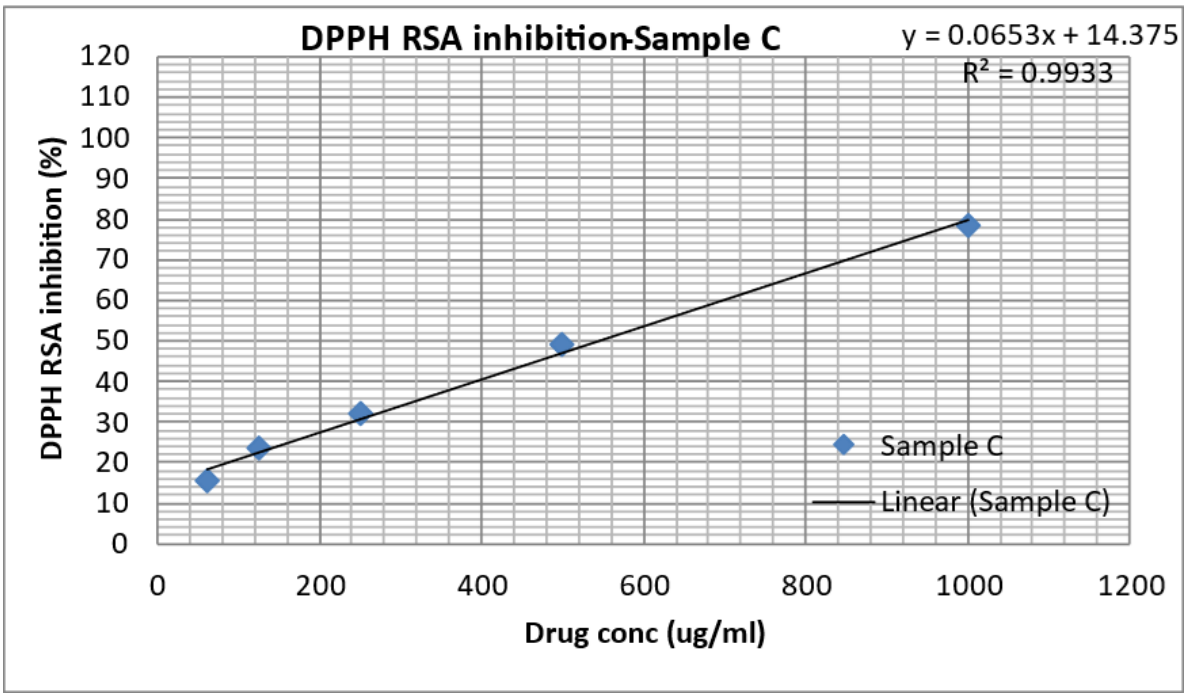

Figure 4. DPPH inhibition percentage of pet ether extract of *Dolichandrone falcata* seem bark (Sample C)

DPPH is a stable free radical that changes colour when reduced by an antioxidant. This approach is useful in determining a compound's ability to scavenge free radicals and prevent oxidative damage. Free radicals have the potential to harm cells and facilitates numerous chronic illnesses including cancer, cardiovascular disease, and neurological disorders. Identification of natural antioxidant sources can therefore aid in the formulation of novel therapies for their disorders.[17]

The reduction capability of DPPH radical is determined by the decrease in absorbance at 517nm and IC_{50} values. The experimental data revealed that bark extracts of *Dolichandrone falcata* seem have the properties of scavenging free radicals, higher in methanolic extract followed by chloroform and pet ether. DPPH scavenging capacity of the test extracts in different solvents exhibited concentration dependent relationship.[18]

The IC_{50} of a compound is inversely related to its antioxidant capacity as it is expressing the amount of antioxidant required to decrease the DPPH concentration by 50%. Further, plants seem to have strong antioxidants if their IC_{50} values are lower than 50mg/ml. The IC_{50} values found in present study were 429µg/ml, 545µg/ml and 905µg/ml for methanol, chloroform and pet ether extracts respectively i. e., very much low than that of expected values of aqueous extract of *Dolichandrone falcata* seem when tested using DPPH scavenging test which exhibited high antioxidant activity.

CONCLUSION

Present study reveals that all the three extracts of *Dolichandrone falcata* seem bark inhibited free radicals and enhanced the antioxidant capacity in a dose dependent manner. Methanol extract exhibited highest antioxidant property. Phenols and flavonoids might be responsible for holding strong antioxidant activity.

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