Antioxidant Activities of *Dendrophthoe falcata* (L.f.) Etting

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Abstract: *Dendrophthoe falcata* is a parasitic plant used in traditional medicine as an aphrodisiac, astringent, and diuretic and for pulmonary tuberculosis, asthma, menstrual disorder, swelling, and wound. The present study primarily describes the antioxidant activities of methanolic and aqueous extracts of the stem of *D. falcate* in different *in vitro* models. Both methanolic and aqueous extracts exhibited antioxidant activity, with IC_{50} values at 18 and 26 µg/mL for DPPH, 22 and 29 µg/mL for ABTS, 62 and 75 µg/mL for NO scavenging activities, 260 and 180 µg/mL for total antioxidant capacity, respectively. Total phenolic content for methanolic and aqueous extracts were found to be 1.5 and 1.1 mg/g while total flavonoid content were found to be 0.140 and 0.029 mg/g. The overall antioxidant activity might be attributed to its polyphenolic, flavonoid and phytosterol constituents.

Keywords: *Dendrophthoe falcate*, antioxidant activity, flavonoids, phenolics, DPPH, ABTS, NO scavenging activity.

INTRODUCTION

Dendrophthoe falcata (L.f.) Ettingsh. (mistletoe), also known as *Loranthus longiflorus* Desr., is a perennial climbing woody parasitic plant. It is indigenous to tropical regions especially in India, Srilanka, Thailand, China, Australia, Bangladesh, Malayasia and Myanmar. In India it is widely distributed throughout upto 900 m [1]. Preliminary phytochemical screening conducted in our department revealed the presence of carbohydrates, phytosterols, flavonoids, glycosides and phenolic compounds [2].

Warrier (1993) reported that the entire plant is used extensively in traditional system of medicine as an aphrodisiac, astringent, narcotic, diuretic, and for the treatment of pulmonary tuberculosis, asthma, menstrual disorders, swellings, wounds, and ulcers [3]. In view of its wide use and its chemical composition, the methanolic and aqueous extracts of *D. falcata* stem were determined for their *in vitro* anti oxidative activities.

MATERIAL AND METHODS

Chemical and Reagents

1,1-Diphenyl-2-picryl hydrazine (DPPH), 2, 2-azino bis (3-ethyl benzo thiazoline-6-sulphonic acid (ABTS), and quercetin were purchased from Sigma Chemicals, USA. Gallic acid was obtained from Nice Chemicals, Mumbai. Ascorbic acid (Ranbaxy Fine Chemicals Ltd.) was used as standard for the study.

Plant Material and Preparation of the Extracts

The stem of *D. falcata* was collected in August 2009 from Manipal. The plant was identified by Dr. Gopalakrishna Bhat, Taxonomist, Poorna Prajna College, Udupi, Karnataka. A voucher specimen (PP 564) is deposited in the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences (Manipal, India).

100 g powder of shade dried stems were extracted with methanol by hot extraction process (soxhlet) for 72 h. After completion of the extraction the solvent was recovered by distillation and concentrated *in vacuo.* The aqueous extract was prepared by maceration process with 100 g of the stem powder using chloroform:water (1:99) for seven days, after completion of the extraction the solvent was recovered by distillation and concentrated *in vacuo*.

Antioxidant Activity Assays

1. 1-Diphenyl-2-Picryl Hydrazine (DPPH) Radical Scavenging Activity

The antiradical activity for the plant extracts was assessed on the basis of the radical-scavenging effect of the stable DPPH free radical. The concentration of DPPH was kept at 300 µM in MeOH. 1 mg of the extracts dissolved separately in 1 mL of MeOH, from which different concentrations (5-200 µg/mL) were prepared. 10 µL of each extract solution was allowed to react with 200 µL DPPH at 37°C for 30 min in a 96-well microliter plate. After incubation, decrease in absorption for each solution was measured at 490 nm using a microplate reader. Ascorbic acid was used as reference [4].

2. 2-Azino Bis (3-Ethyl Benzo Thiazoline-6-Sulphonic Acid (ABTS) Radical Scavenging Activity

For ABTS assay, the stock solutions of 7.4 mM ABTS and 2.6 mM potassium persulfate were prepared and the working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 16 h at room temperature in a dark place. The solution was then diluted by mixing $ABTS^{\cdot+}$ solution with methanol to

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obtain an absorbance of 1.00 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{\bullet+}$ solution was prepared for each assay. Different concentrations of standard solution of ascorbic acid were prepared. The 1 mg of the extracts dissolved separately in 1mL of MeOH, from which different concentrations (5-200 µg/mL) were prepared. 200 µL of each concentration was allowed to react with 4 µL of the $ABTS^{\bullet+}$ for 2 h in a dark place. Then, the absorbance was read at 734 nm [5].

Nitric Oxide (NO) Scavenging Activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which interacts with oxygen to produce nitrite ions. The nitrite ions can be determined by Griess Illosvoy reaction [6]. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radical scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = [(A_c - A_t) / A_c \times 100]$$

Where A_c was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract/Standard [7].

Total Antioxidant Capacity

Total antioxidant capacity was measured according to the method reported by Prieto *et al.* [8]. In brief, 100 µg of extract and 100 µg of ascorbic acid (as standard) were taken in 0.1 mL of alcohol, combined separately in an eppendroff tube with 1.9 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1.9 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the samples. For samples of unknown composition, water-soluble antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicate and values are expressed as ascorbic acid equivalents in µg /mL of extract.

Total Phenolics

Total soluble phenolics in the extracts were determined with Folin-Ciocalteu reagent using gallic acid as a standard phenolic compound. 1.0 mL of extract solution containing 1.0 mg extract was diluted with 46 mL of distilled water in a volumetric flask. 1.0 mL of Folin-Ciocalteu reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0 mL of 2% sodium carbonate was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm. The concentration of total phenols was expressed as gallic acid equivalents in mg/g of dry extract [9].

Total Flavonoids

Aluminum chloride colourimetric method was used for determination of flavonoids. To the 10 mL volumetric flask 2 mL of water and 1 mL of plant extract (1 mg/mL) were added. After 5 min 3 mL of 5 % sodium nitrite and 0.3 mL of 10 % aluminum chloride were added. After 6 min, 2 mL of 1 M sodium hydroxide was added and the volume made up to 10 mL with water. Absorbance was measured at 510 nm. The percentage of total flavonoids were calculated from calibration curve of quercetin (10-250 µg) plotted by using the same procedure and total flavonoids was expressed as quercetin equivalents in milligrams per gram sample [10].

RESULTS AND DISCUSSION

Methanolic and aqueous extracts of *D. falcata* exhibited antioxidant activity in different *in vitro* models (Table **1)**. IC_{50} values of methanolic and aqueous extracts were 260 µg/mL and 180 µg/mL for total antioxidant capacity, 18 µg/mL and 26 µg/mL for DPPH (Fig. **1**) and 22 µg/mL and 29 µg/mL for ABTS (Fig. **2**), 62 µg/mL and 75 µg/mL for nitric oxide radicals with IC_{50} values 62 µg/mL and 75 µg/mL for NO scavenging activity (Fig. **3**), respectively. Total phenolic content for methanolic and aqueous extracts were found to be 1.5 mg/g and 1.1 mg/g respectively (equivalent to gallic acid). The total flavonoid content for methanolic and aqueous extracts were found to be 0.140 mg/g and 0.029 mg/g respectively (equivalent to quercetin).

Phenolics present in fruits and vegetables have received considerable attention because of their potential antioxidant activities [11]. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent [12]. However, it should be noted that some chemical group of amino acids,

Table 1. ***In Vitro*** **Antioxidant Activities of** ***Dendrophthoe falcata*** **Stem Extracts**

Sample	Antioxidant Activity (IC_{50}, µg/mL)			
	DPPH	ABTS	Nitric Oxide Scavenging Activity	Total Antioxidant Capacity
Methanolic Extract	18	22	62	260
Aqueous Extract	26	29	75	180
Ascorbic Acid	8.2	12	26	-

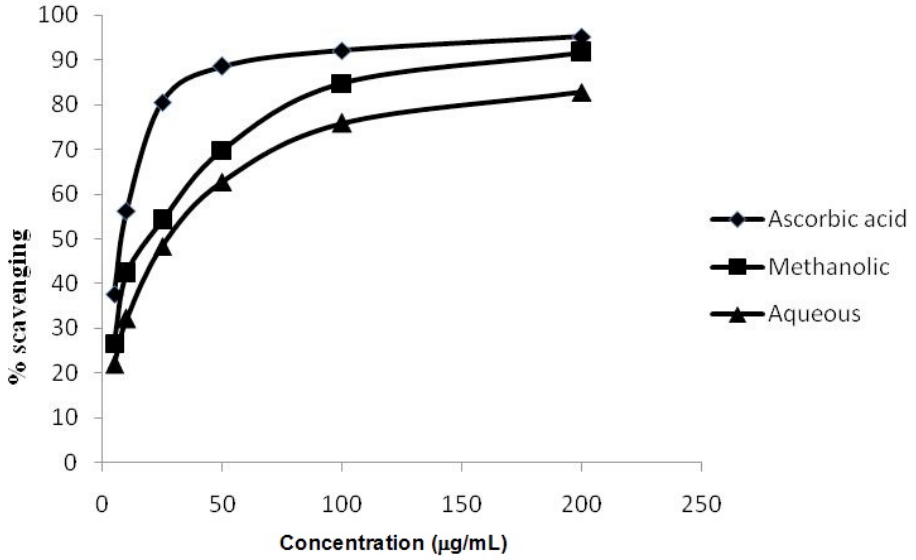

Fig. (1). DPPH scavenging activity of *Dendrophthoe falcata* stem extracts.

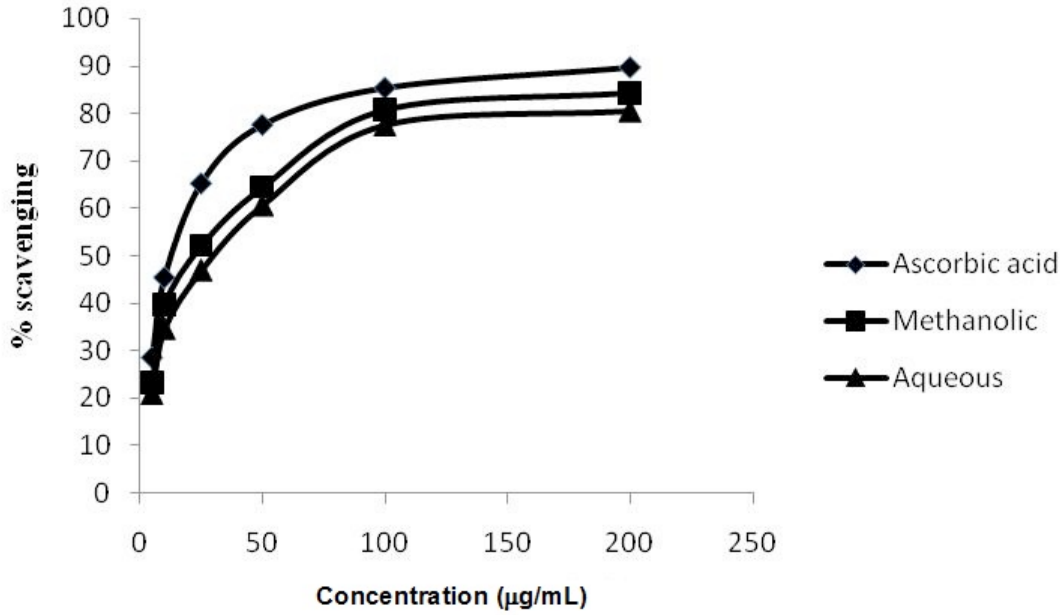

Fig. (2). ABTS scavenging activity of *Dendrophthoe falcata* stem extracts.

proteins, organic acids, sugars and aromatic amines could react with the reagent. In this investigation, *D. falcata* stem was dried before extraction while ascorbic acid was lost during drying process and amino acids, proteins and sugars could be removed from the extraction solvents. Thus, interference from ascorbic acid or other compounds like amino acids, proteins and sugars should be very little [13].

Hydrogen donation is the main mechanism of phenolics as antioxidants. The lower strength of the O–H bond present in phenolics corresponds to a higher scavenging activity. Quercetin identified from *D. falcata* [14] has C2–C3 double bond and a C-3 hydroxyl group while the most active hydroxyl groups for hydrogen donation are those attached to C4 and C3 positions [13]. Thus, the antioxidant activity of flavonoids could be related to the hydroxyl group.

The results obtained in the present study indicate that *D. falcata* stem extracts exhibit potent free radical scavenging and antioxidant activity. The overall antioxidant activity might be attributed to its polyphenolic content and other phytochemical constituents. The findings of the present study suggest that *D. falcata* stem could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases. The presence of phytoconstituents like quercetin can be attributed to the antioxidant property of the plant.

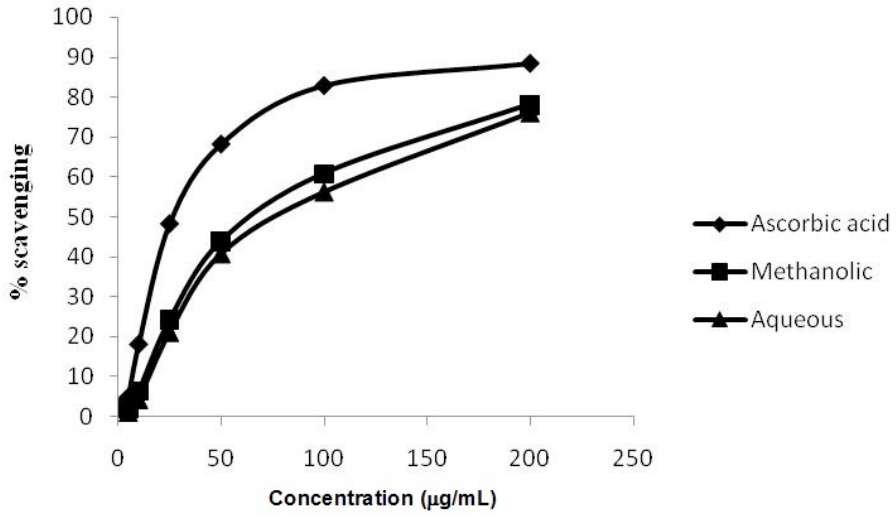

Fig. (3). Nitric oxide scavenging activity of *Dendrophthoe falcata* stem extracts.

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ABBREVIATIONS

ABTS	=	2, 2 – Azino bis (3-ethyl Benzo Thiazoline –6–Sulphonic acid
DPPH	=	1, 1- Diphenyl-2- Picryl Hydrazine
IC_{50}	=	Half maximal Inhibitory concentration
NO	=	Nitric oxide
ROS	=	Reactive oxygen species

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