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IN VITRO ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF WHOLE PLANT OF *BRIDELIA SCANDENS* (Roxb) Willd

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ABSTRACT

The objective of this investigation was to evaluate the *in vitro* antioxidant activities of various extracts of whole plant of *Bridelia scandens.* All the three extracts were examined for DPPH (α,α-diphenyl-β-picrylhydrazyl) radical scavenging activity, superoxide anion scavenging activity and iron chelating activity with reference standard rutin, quercetin and EDTA, respectively through *in vitro* models. The methanolic extract of *Bridelia scandens* was found to be most active than that of other two extracts of *Bridelia scandens* in scavenging DPPH radicals with rutin used as reference standard and an IC_{50} value was found to be 90 μg/mL and 480 μg/mL, respectively. Scavenging superoxide radical and iron chelating activity of the three extracts were carried out with quercetin and EDTA as reference standards. Both the methods significantly showed the methanolic extract of *Bridelia scandens* was most effective than that of petroleum ether and ethyl acetate extracts. It is concluded that the methanolic extract of *Bridelia scandens* showed strong scavenging activity against free radical as compared to all other extracts.

Key words: *Bridelia scandens*, *In vitro* antioxidant, DPPH assay, Superoxide anion, Iron chelating activity.

INTRODUCTION

Oxygen-derived free radicals such as superoxide anion and hydroxyl radical are cytotoxic and promote tissue injuries. Antioxidants act as a major defense against radical-mediated toxicity by protecting against the damages caused by free radicals[1]. The cellular antioxidant status determines the susceptibility to oxidative damage and is usually altered in response to oxidative stress[2].

Antioxidants may be defined as radical scavengers, which protect the human body

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against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties[3-10].

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[11]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity[12]. It is commonly recognized that antioxidants can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutations and therefore, helps to prevent cancer or heart disease.

Recently, there is a growing interest in the discovery of natural antioxidants, mainly for two reasons : (i) there are epidemical and clinical evidences suggesting that consumption of vegetables and fruits reduces the risk of developing chronic disease, e.g. Cancer and (ii) phytochemicals are generally safer than synthetic chemicals[13]. Therefore, the importance of search for natural antioxidants has increased in the recent years and many researchers focused the same[14].

Bridelia scandens belongs to the family Euphorbiaceae. It is distributed in the warm regions of India and Southeast Asia. This plant is used for antimicrobial activity[15]. The bark decoction has been used in the traditional medicine for the treatment of asthma, intestinal worms and cough and leaves are used against colics. Tannins were isolated from the bark. The fatty alcohol, $C_{22}H_{46}O$, named bridelyl alcohol besides fatty acids and a phlobatannin were isolated from the leaves of *Bridelia* scandens[16]. Taraxenone was isolated from roots by hexane extract[17]. Literature survey also revealed a lack of scientific report regarding antioxidant activity of the whole plant of *Bridelia scandens* (Roxb) Willd. Hence, the aim of the present study was to evaluate the antioxidant activity of various extracts of *Bridelia scandens* through various *in vitro* models.

EXPERIMENTAL

Material and methods

Collection and identification of plant materials

The whole plant of *Bridelia scandens* (Roxb) Willd, were collected from Sengottai, 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 *Bridelia scandens* (Roxb) Willd, 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[18] for 24 hrs. and the mark was subjected to ethyl acetate (76-78^{0}C) for 24 hrs. Then mark was further 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

DPPH photometric assay[19]

The effect of extract on DPPH radical was assayed using the method of Mensor et al.[19] A methanolic solution of 0.5 mL of DPPH (0.4 mM) was added to 1 mL of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$\text{Scavenging activity (\%)} = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100 \qquad ...(1)$$

Where A_{518} control is the absorbance of DPPH radical + methanol; A_{518} is the sample absorbance of DPPH radical + sample extract/ standard.

Superoxide radical scavenging activity[20]

Superoxide radical ($O_2^{-\bullet}$) was generated from the photoreduction of riboflavin and was detected 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.[20] The assay mixture contained sample with 0.1 mL 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 wherein 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. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results were averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Iron chelating activity[21]

The method of Benzie and Strain[21] 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 (200 µM) and 2 mL of various concentrations ranging from 10 to 1000 µg 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

Free radical is a molecule with an unpaired electron and it is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases[22]. They are also involved in autoimmune disorders like rheumatoid arthritis etc.[23]

DPPH scavenging activity

The percentage of DPPH radical scavenging activity of petroleum ether extract of *Bridelia scandens* is depicted in Table 1. The IC_{50} values of the petroleum ether extract of *Bridelia scandens* and rutin were found to be 1430 µg/mL and 480 µg/mL, respectively.

Table 1: Effect of petroleum ether extract of *Bridelia scandens* on DPPH assay

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Petroleum ether extract)	Standard (Rutin)
125	17.97 ± 0.070	18.85 ± 0.076
250	18.53 ± 0.010	22.08 ± 0.054
500	23.56 ± 0.015	52.21 ± 0.022
1000	32.62 ± 0.091	69.83 ± 0.014
	IC_{50} = 1430 µg/mL	**IC_{50} = 480 µg/mL**

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

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Bridelia scandens* is depicted in Table 2. The IC_{50} values of the ethyl acetate extract of *Bridelia scandens* and rutin were found to be 1150 µg/mL and 480 µg/mL, respectively.

Table 2: Effect of ethyl acetate extract of *Bridelia scandens* on DPPH assay

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Ethyl acetate extract)	**Standard (Rutin)**
125	14.04 ± 0.081	18.85 ± 0.076
250	24.93 ± 0.067	22.08 ± 0.054
500	39.27 ± 0.042	52.21 ± 0.022
1000	45.98 ± 0.039	69.83 ± 0.014
	IC_{50} = 1150 µg/mL	**IC_{50} = 480 µg/mL**

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

The percentage of DPPH radical scavenging activity of methanolic extract of *Bridelia scandens* is depicted in Table 3. The IC_{50} values of the methanolic extract of *Bridelia scandens* and rutin were found to be 90 µg/mL and 480 µg/mL, respectively.

Table 3: Effect of methanolic extract of *Bridelia scandens* on DPPH assay

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Methanolic extract)	**Standard (Rutin)**
125	51.51 ± 0.012	18.85 ± 0.076
250	54.31 ± 0.049	22.08 ± 0.054
500	62.63 ± 0.036	52.21 ± 0.022
1000	66.60 ± 0.024	69.83 ± 0.014
	IC_{50} = 90 µg/mL	**IC_{50} = 480 µg/mL**

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

IC_{50} values of methanolic extract of *Bridelia scandens* exhibits significant antioxidant activity when compared to that of standard rutin. IC_{50} values of plant extract and rutin were recorded as 90 µg/mL and 480 µg/mL, respectively. But other two extracts showed lower activity, when compared to that of methanolic extract of *Bridelia scandens* and standard rutin.

Superoxide anion scavenging activity

Percentage scavenging of superoxide anion activity of petroleum ether extract of *Bridelia scandens* is depicted in table 4. The IC_{50} value of plant extract and quercetin were recorded as 180 µg/mL and 60 µg/mL, respectively.

Table 4: Effect of petroleum ether extract of *Bridelia scandens* on superoxide anion scavenging activity method

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Petroleum ether extract)	Standard (Quercetin)
125	38.06 ± 0 .015	73.81 ± 0.006
250	76.32 ± 0.049	91.31 ± 0.011
500	87.62 ± 0.030	92.99 ± 0.024
1000	92.80 ± 0.027	98.01 ± 0.012
	IC_{50} = 180 µg/mL	**IC_{50} = 60 µg/mL**

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

Percentage scavenging of superoxide anion activity of ethyl acetate extract of *Bridelia scandens* is depicted in Table 5. The IC_{50} value of plant extract and quercetin were recorded as 380 µg/mL and 60 µg/mL, respectively.

Table 5: Effect of ethyl acetate extract of *Bridelia scandens* on superoxide anion scavenging activity method

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Ethyl acetate extract)	Standard (Quercetin)
125	13.48 ± 0.051	73.81 ± 0.006
250	15.54 ± 0.029	91.31 ± 0.011
500	55.43 ± 0.031	92.99 ± 0.024

Cont…

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Ethyl acetate extract)	Standard (Quercetin)
1000	63.98 ± 0.019	98.01 ± 0.012
	IC_{50} = 380 µg/mL	**IC_{50} = 60 µg/mL**

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

Percentage scavenging of superoxide anion activity of methanolic extract of *Bridelia scandens* is depicted in Table 6. The IC_{50} value of plant extract and quercetin were recorded as 50 µg/mL and 60 µg/mL, respectively.

Table 6: Effect of methanolic extract of *Bridelia scandens* on superoxide anion scavenging activity method

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Methanolic extract)	Standard (Quercetin)
125	60.29 ± 0.015	73.81 ± 0.006
250	61.93 ± 0.029	91.31 ± 0.011
500	68.47 ± 0.032	92.99 ± 0.024
1000	85.38 ± 0.028	98.01 ± 0.012
	IC_{50} = 50 µg/mL	**IC_{50} = 60 µg/mL**

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

Based on the above results of the IC_{50} values and percentage scavenging capacity, it was found that methanolic extract of *Bridelia scandens* is more effective in scavenging superoxide radical than that of quercetin (standard).

Iron chelating activity

Iron binding capacity of the petroleum ether extract of *Bridelia scandens* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/mL) were examined and the values are presented in Table 7. The IC_{50} values of plant extract and EDTA were recorded as 370 µg/mL and 65 µg/mL, respectively.

Table 7: Effect of petroleum ether extract of *Bridelia scandens* on iron-chelating method

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Pet. ether extract)	**Standard (EDTA)**
125	38.20 ± 0.020	58.68 ± 0.007
250	48.80 ± 0.037	65.87 ± 0.018
500	63.35 ± 0.029	83.83 ± 0.012
1000	71.35 ± 0.022	97.90 ± 0.019
	IC_{50} = 370 µg/mL	**IC_{50} = 65 µg/mL**

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

Iron binding capacity of the ethyl acetate extract of *Bridelia scandens* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/mL) were examined and the values were presented in Table 8. The IC_{50} value of plant extract and EDTA were recorded as 310 µg/mL and 65 µg/mL, respectively.

Table 8: Effect of ethyl acetate extract of *Bridelia scandens* on iron-chelating method

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Ethyl acetate extract)	**Standard (EDTA)**
125	14.93 ± 0.016	58.68 ± 0.007
250	24.54 ± 0.011	65.87 ± 0.018
500	81.41 ± 0.029	83.83 ± 0.012
1000	87.87 ± 0.021	97.90 ± 0.019
	IC_{50} = 310 µg/mL	**IC_{50} = 65 µg/mL**

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

Iron binding capacity of the methanolic extract of *Bridelia scandens* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/mL) were examined and the values were presented in Table 9. The IC_{50} value of plant extract and EDTA were recorded as 70 µg/mL and 65 µg/mL, respectively.

Table 9: Effect of methanolic extract of *Bridelia scandens* on iron-chelating method

Concentration (µg/mL)	% of activity (± SEM)*	
	Sample (Methanolic extract)	Standard (EDTA)
125	53.65 ± 0.044	58.68 ± 0.007
250	63.35 ± 0.029	65.87 ± 0.018
500	75.39 ± 0.036	83.83 ± 0.012
1000	81.18 ± 0.013	97.90 ± 0.019
	IC_{50} = 70 µg/mL	**IC_{50} = 65 µg/mL**

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

From the results of IC_{50} value, the methanolic extract was found to be more effective than that of EDTA as a standard. The methanolic extract of *Bridelia scandens* exhibited significant antioxidant activity as compared to that of petroleum ether and ethyl acetate extracts of *Bridelia scandens*.

CONCLUSION

In conclusion, the methanolic extract of *Bridelia scandens* was found to show the strongest antioxidant activity as compared to the other two extracts. The prominent antioxidant activity may be due to presence of higher contents of tannins, phenolic, flavonoids and saponoins. Future scope of this work involves isolation and identification of different constituents responsible for this antioxidant activity.

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