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In-vitro antioxidant potential of various extracts of whole plant of *Bridelia scandens* (Roxb) wild

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Abstract

The present investigation was to examine the in-vitro antioxidant potential of various extracts of whole plant of Bridelia scandens by different in-vitro methods. The antioxidant activity was determined by Hydroxyl radical scavenging activity, Nitric oxide radical scavenging activity with reference standard Ascorbate and total phenol content respectively. An IC_{50} value was found that methanolic extract of Bridelia scandens is more effective in hydroxyl radical scavenging activity than that of petroleum ether and ethyl acetate extracts. The methanolic extract of Bridelia scandens was found to more effective in the nitric oxide radical scavenging activity. The IC_{50} values of the methanolic extract of Bridelia scandens and ascorbate were found to be 130µg/ml and 410µg/ml respectively. But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the Bridelia scandens showed the better result. In addition, the methanolic extract of Bridelia scandens was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. So, the in-vitro studies clearly showed that the methanolic extract of Bridelia scandens has a significant antioxidant activity. 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.

Key words: Whole plant of *Bridelia scandens*, *In-vitro* antioxidant, Hydroxyl radical scavenging activity, Nitric oxide radical activity, Total Phenol.

INTRODUCTION

There is a considerable epidemiological evidence indicating association between diets rich in fresh fruits and vegetables and a decreased risk of cardiovascular disease and certain forms of cancer. Free-radicals are generated continuously in the body due to metabolism and disease [1]. In order to protect themselves against free radicals, organisms are endowed with endogenous (catalase, superoxide dismutase, glutathione peroxidase/ reductase) and exogenous (C and E vitamins, carotene, uric acid) defenses; yet these defense systems are not sufficient in critical situations (oxidative stress, contamination, UV exposure, etc.) where the production of free

radicals significantly increases [2]. Free radicals can cause lipid peroxidation in foods, which leads to their deterioration [3]. Antioxidants are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation [4].

Antioxidants are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years [5]. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There is a plethora of plants that have been found to possess strong antioxidant activity [6]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases [7]. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties.

Bridelia scandens belongs to the family Euphorbiaceae. It is distributed in the warm regions of India and Southeast Asia. This plant used as antimicrobial activity [8]. 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 [9]. Taraxenone was isolated from roots hexane extract [10] .Based on the literature survey also revealed that lack of scientific report regarding antioxidant activity of the whole plant of *Bridelia scandens* (Roxb) Willd. However, no data are available in the literature on the antioxidant activity of whole plant of *Bridelia scandens*. Therefore we undertook the present investigation to examine the antioxidant activities of various extract of whole plant of *Bridelia scandens* through different *in vitro* models.

RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases[15].They are also involved in autoimmune disorders like rheumatoid arthritis etc[16].

Hydroxyl radical scavenging activity

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins [17]. The percentage of Hydroxyl radical scavenging activity of petroleum ether extract of *Bridelia scandens* presented in Table 1. The petroleum ether extract of *Bridelia scandens* was exhibited a maximum Hydroxyl radical scavenging activity of 48.27 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 75.23 % at 1000 µg/ml. The IC_{50} values of the petroleum ether extract of *Bridelia scandens* and ascorbate were found to be 1100µg/ml and 410µg/ml respectively.

The percentage of hydroxyl radical scavenging activity of ethyl acetate extract of *Bridelia scandens* presented in Table 2. The ethyl acetate extract of *Bridelia scandens* was exhibited a maximum hydroxyl radical scavenging activity of 56.21 % at 1000 µg/ml whereas for ascorbate

(standard) was found to be 75.23 % at 1000 µg/ml. The IC_{50} values of the ethyl acetate extract of *Bridelia scandens* and ascorbate were found to be 810µg/ml and 410µg/ml respectively.

Table 1: Hydroxyl radical scavenging activity of Petroleum ether extract of *Bridelia scandens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	22.11 ± 0.042	26.87 ± 0.076
2	250	37.21 ± 0.034	30.30 ± 0.054
3	500	43.36 ± 0.078	60.64 ± 0.022
4	1000	48.27 ± 0.024	75.23 ± 0.014
		IC_{50} = 1100 µg/ml	**IC_{50} = 410 µg/ml**

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

Table 2: Hydroxyl radical scavenging activity of Ethyl acetate extract of *Bridelia scandens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	23.18 ± 0.081	26.87 ± 0.076
2	250	38.59 ± 0.067	30.30 ± 0.054
3	500	48.38 ± 0.042	60.64 ± 0.022
4	1000	56.21 ± 0.039	75.23 ± 0.014
		IC_{50} = 810 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of hydroxyl radical scavenging activity of methanolic extract of *Bridelia scandens* presented in Table 3. The methanolic extract of *Bridelia scandens* was exhibited a maximum hydroxyl radical scavenging activity of 89.64 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 75.23 % at 1000 µg/ml. The IC_{50} of the methanolic extract of *Bridelia scandens* and ascorbate were found to be 50µg/ml and 410µg/ml respectively.

Table 3: Hydroxyl radical scavenging activity of Methanolic extract of *Bridelia scandens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	76.72 ± 0.012	26.87 ± 0.076
2	250	84..87 ± 0.049	30.30 ± 0.054
3	500	88.42 ± 0.036	60.64 ± 0.022
4	1000	89.64 ± 0.024	75.23 ± 0.014
		IC_{50} = 50 µg/ml	**IC_{50} = 410 µg/ml**

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

Based on the above result the methanolic extract of *Bridelia scandens* (IC_{50} = 50 µg/ml) was found more effective than that of standard (IC_{50} = 410 µg/ml). The methanolic extract of *Bridelia scandens* was found to more effective than that of petroleum ether and ethyl acetate extracts.

Nitric oxide scavenging activity

Nitric oxide is a diffusible free radical which is an important effector molecule in diverse biological systems [18]. The animal studies suggested the role for NO in pathogenesis of inflammation and pain [19], [20]. 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 *Bridelia scandens* and ascorbate were illustrated in Table 4. The maximum nitric oxide scavenging activity of petroleum ether extract and ascorbate at 1000 µg/ml were found to be 46.37 % and 75.23% respectively. The IC_{50} value of petroleum ether extract and ascorbate were recorded as 1080µg/ml and 410µg/ml respectively.

Table 4: Nitric oxide scavenging activity of Petroleum ether extract of *Bridelia scandens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	11.58 ±0 .015	26.87 ± 0.076
2	250	20.06 ± 0.049	30.30 ± 0.054
3	500	31.48 ± 0.030	60.64 ± 0.022
4	1000	46.37 ± 0.027	75.23 ± 0.014
		IC_{50} = 1080 µg/ml	**IC_{50} = 410 µg/ml**

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

The reduction of nitric oxide radical by the ethyl acetate extract of *Bridelia scandens* and ascorbate were illustrated in Table 5. The maximum scavenging activity of ethyl acetate extract and ascorbate at 1000 µg/ml were found to be 64.37% and 75.23% respectively. The IC_{50} value of ethyl acetate extract and ascorbate were recorded as 380µg/ml and 410µg/ml respectively.

Table 5: Nitric oxide scavenging activity of Ethyl acetate extract of *Bridelia scan dens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	32.25 ± 0.051	26.87 ± 0.076
2	250	48.61 ± 0.029	30.30 ± 0.054
3	500	52.03 ± 0.031	60.64 ± 0.022
4	1000	64.37 ± 0.019	75.23 ± 0.014
		IC_{50} = 380 µg/ml	**IC_{50} = 410 µg/ml**

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

The reduction of nitric oxide radical by the methanolic extract of *Bridelia scan dens* and ascorbate were illustrated in Table 6. The maximum scavenging activity of methanolic extract and ascorbate at 1000 µg/ml were found to be 79.31% and 75.23% respectively. The IC_{50} value of methanolic extract and ascorbate were recorded as 130µg/ml and 410µg/ml respectively.

Based on the results given below, the methanolic extract of *Bridelia scandens* was found more effective in scavenging nitric oxide radical than that of ethyl acetate and petroleum ether extract. But when compare to the all the three extracts with Ascorbate (standard), the methanolic extract of the *Bridelia scan dens* showed the better result.

Table 6: Nitric oxide scavenging activity of Methanolic extract of *Bridelia scandens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	49.42 ± 0.015	26.87 ± 0.076
2	250	57.58 ± 0.029	30.30 ± 0.054
3	500	66.60 ± 0.032	60.64 ± 0.022
4	1000	79.31 ± 0.028	55.23 ± 0.014
		IC_{50} = 130 µg/ml	**IC_{50} = 410 µg/ml**

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

Total phenol

Phenolic compounds are known as powerful chain breaking antioxidants [21]. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [22]. The phenolic compounds may contribute directly to antioxidative action [23]. The total phenolic content of various extract of whole plant of *Bridelia scandens* was presented in Table 7.

Table 7: The total Phenolic content of various extracts of whole plant of *Bridelia scandens*

S.No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1	Petroleum ether extract of *Bridelia scandens*	1.50 ± 0.022
2	Ethyl acetate extract of *Bridelia scandens*	2.60 ± 0.072
3	Methanolic extract of *Bridelia scandens*	4.80 ± 0.039

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

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

MATERIALS AND METHODS

Collection and Identification of Plant materials

The whole plant of *Bridelia scandens* (Roxb) Willd, were collected from Senkottai, 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 [11] for 24 hrs then mark 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

Hydroxyl radical scavenging activity [12]

This was assayed as described by Elizabeth and Rao (1990) [12]. The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+} -Ascorbate –EDTA $–H_2O_2$ system (Fenton reaction). The reaction mixture contained 0.1 ml deoxyribose (2.8mM),0.1 ml EDTA (0.1 mM), 0.1 ml H_2O_2 (1mM), 0.1 ml Ascorbate (0.1mM), 0.1 ml KH_2PO_4-KOH buffer, P^H 7.4 (20mM) and various concentrations of plant extract in a final volume of 1 ml. The reaction mixture was incubated for 1 hour at 37^0 C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

Nitric oxide radical scavenging activity [13]

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)[13].The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25^0C 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 [14]

The measurement of total phenol is based on Mallick and Singh (1980)[14]. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2^oC 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.

CONCLUSION

The present study was clearly indicated the methanolic extract of *Bridelia scandens* showed strong antioxidant activity by inhibiting Hydroxyl radical scavenging, Nitric oxide radical scavenging activities when compared with standard Ascorbate. In addition, the methanolic extract of *Bridelia scandens* 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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