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IN VITRO EVALUATION OF ANTIOXIDANT POTENTIAL OF VARIOUS EXTRACTS OF WHOLE PLANT OF *Bridelia Scandens* (Roxb) Willd.

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ABSTRACT

The antioxidant activities of various extracts of whole plant of Bridelia scandens (Roxb) Willd, was investigated in different in-vitro methods. The antioxidant activity was evaluated by Total antioxidant activity (Phosphomolybdic acid method), FRAP assay with reference standard Ascorbate and total flavonoids content respectively. The methanolic extract of Bridelia scandens was the most effective total antioxidant activity among the extracts. The IC_{50} values of the methanolic extract of Bridelia scandens and ascorbate was found to be 100µg/ml and 410µg/ml respectively. The methanolic extract of Bridelia scandens was found more effective in FRAP assay than that of petroleum ether and ethyl acetate extracts. But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the Bridelia scandens showed the significant result. The methanolic extract of Bridelia scandens contains high amount of flavonoids than that of other two extracts. Moreover, the results were observed in a concentration dependent manner. 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 significant 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, Total antioxidant activity, FRAP assay, Total flavonoids.*

Introduction:

Reactive oxygen species (ROS), sometimes called active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O^{2-}) and hydroxyl radicals (OH'), as well as nonfree- radical species such as hydrogen peroxide (H_2O_2) [1,2]. In living organisms various ROS can form in different ways, including normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionising radiation, certain pollutants, organic solvents, and pesticides [3, 4, 5].

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Free radicals can cause lipid peroxidation in foods, which leads to their deterioration[6, 7]. In addition, reactive oxygen species have been implicated in more than 100 diseases, including malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes, and cancer [8,9,10]. When produced in excess, ROS can cause tissue injury. However, tissue injury can itself cause ROS generation[11]. Nevertheless, all aerobic organisms, including human beings, have antioxidant defences that protect against oxidative damages, and numerous damage removal and repair enzymes to remove or repair damaged molecules [12, 13.14]. Therefore, the importance of search for natural antioxidants has increased in the recent years so many researchers focused the same[15].

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[16]. 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[17]. Taraxenone was isolated from roots hexane extract[18] .Based on the literature survey also revealed that 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.

Material and Methods

Collection and Identification of Plant materials

The whole plant of *Bridelia scandens* (Roxb) Willd, were collected from Kilikulam, 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[19] for 24 hrs. And the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then marc 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:

Total antioxidant activity (Phosphomolybdic acid method) [20]

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999) [20]. An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95^{0}C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

FRAP assay[21]

A modified method of Benzie and Strain (1996)[21] was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mM HCl and 20 mM $Fecl_3.6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3.6H_2O$. The temperature of the solution was raised to 37^0 C before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Total flavonoids[22]

0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and

these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

Results and Discussion

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[23]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity[24]. Therefore, research for the determination of the natural antioxidants source is important.

Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of petroleum ether extract of *Bridelia scandens* presented in Table 1. The petroleum ether extract of *Bridelia scandens* exhibited a maximum total antioxidant activity of 76.43 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} values of the petroleum ether extract of *Bridelia scandens* and ascorbate were found to be 290µg/ml and 410µg/ml respectively.

Table 1: Total antioxidant activity of Petroleum ether extract of *Bridelia scandens* by Phosphomolybdic acid method.

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	27.94 ± 0.015	26.87 ± 0.076
2	250	46.58 ± 0.074	30.30 ± 0.054
3	500	60.54 ± 0.021	60.64 ± 0.022
4	1000	76.43 ± 0.068	55.23 ± 0.014
		IC_{50} = 290 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of total antioxidant activity of ethyl acetate extract of *Bridelia scandens* presented in Table 2. The ethyl acetate extract of *Bridelia scandens* exhibited a maximum total antioxidant activity of 86.30 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} values of the ethyl acetate extract of *Bridelia scandens* and ascorbate were found to be 260µg/ml and 410µg/ml respectively.

Table 2: Total antioxidant activity of Ethyl acetate extract of *Bridelia scandens* by Phosphomolybdic acid method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	38.35 ± 0.066	26.87 ± 0.076
2	250	49.58 ± 0.047	30.30 ± 0.054
3	500	74.79 ± 0.072	60.64 ± 0.022
4	1000	86.30 ± 0.039	55.23 ± 0.014
		IC_{50} = 260 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of total antioxidant activity of methanolic extract of *Bridelia scandens* presented in Table 3. The methanolic extract of *Bridelia scandens* exhibited a maximum total antioxidant activity of 87.12 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} of the methanolic extract of *Bridelia scandens* and ascorbate were found to be 100µg/ml and 410µg/ml respectively.

Table 3: Total antioxidant activity of Methanolic extract of *Bridelia scandens* by Phosphomolybdic acid method

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	51.50 ± 0.012	26.87 ± 0.076
2	250	76.98 ± 0.049	30.30 ± 0.054
3	500	86.02 ± 0.036	60.64 ± 0.022
4	1000	87.12 ± 0.024	55.23 ± 0.014
		IC_{50} = 100 µg/ml	**IC_{50} = 410 µg/ml**

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

Based on the result showed the methanolic extract of *Bridelia scandens* was found to more effective than petroleum ether and ethyl acetate extract. But when compare all the extracts with standard the methanolic extract of *Bridelia scandens* was found strong antioxidant activity. The IC_{50} of the methanolic extract of *Bridelia scandens* and Ascorbate were found to be 100µg/ml and 410µg/ml respectively.

FRAP assay

The antioxidant potential of *Bridelia scandens* was ascertained from FRAP assay based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the petroleum ether extract of *Bridelia scandens* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 4. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 37.52% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 1300µg/ml and 50µg/ml respectively.

Table 4: Reducing ability of Pet. ether extract of *Bridelia scandens* on FRAP assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	18.39 ± 0.077	72.04 ± 0.014
2	250	23.42 ± 0.027	82.05 ± 0.034
3	500	33.97 ± 0.022	86.04 ± 0.026
4	1000	37.52 ± 0.041	98.07 ± 0.041
		IC_{50} = 1300 µg/ml	**IC_{50} = 50 µg/ml**

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

The reducing ability of the ethyl acetate extract of *Bridelia scandens* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 5. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 52.79% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 950µg/ml and 50µg/ml respectively.

Table 5: Reducing ability of Ethyl acetate extract of *Bridelia scandens* on FRAP assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	13.50 ± 0.016	72.04 ± 0.014
2	250	31.85 ± 0.011	82.05 ± 0.034
3	500	47.81 ± 0.029	86.04 ± 0.026
4	1000	52.79 ± 0.021	98.07 ± 0.041
		IC_{50} = 950 µg/ml	**IC_{50} = 50 µg/ml**

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

The reducing ability of the methanolic extract of *Bridelia scandens* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 6. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 78.82% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 190µg/ml and 50µg/ml respectively.

Table 6: Reducing ability of Methanolic extract of *Bridelia scandens* on FRAP assay

S. No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	33.33 ± 0.044	72.04 ± 0.014
2	250	53.43 ± 0.029	82.05 ± 0.034
3	500	64.59 ± 0.036	86.04 ± 0.026
4	1000	78.82 ± 0.013	98.07 ± 0.041
		IC_{50} = 190 µg/ml	**IC_{50} = 50 µg/ml**

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

Based on the above results indicated, the methanolic extract of *Bridelia scandens* was found to most effective than that of petroleum ether & ethyl acetate extract. But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the *Bridelia scandens* showed the moderate result.

Total flavonoids

Flavonoids present in food of plant origin are also potential antioxidants[25], [26]. Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities [27]. The total amount of flavonoids content of various extract of whole plant of *Bridelia scandens* was present in Table 7.

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

S.No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Petroleum ether extract of *Bridelia scandens*	0.027 ± 0.002
2	Ethyl acetate extract of *Bridelia scandens*	1.015 ± 0.017
3	Methanolic extract of *Bridelia scandens*	2.062 ± 0.025

*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 flavonoids than that of petroleum ether and ethyl acetate.

Conclusion

The results of the present investigation indicated that the methanolic extract of *Bridelia scandens* can be used as easily accessible source of natural antioxidants and as a possible food supplement in pharmaceutical industry. However, the methanolic extract of *Bridelia scandens* was found high content of flavonoids. So it can be concluded that these components might be involved in the antioxidant activity of *Bridelia scandens.* Therefore, it is suggested that further work should be performed on the isolation and identification of the antioxidant components in *Bridelia scandens.*

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