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In- vitro antioxidant studies of various extracts of whole plant of *Borreria hispida* (Linn)

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ABSTRACT

The present investigation to evaluate the antioxidant activities of various extracts of whole plant of *Borreria hispida* (Linn) in different *invitro* methods. The antioxidant activity was evaluated by Total antioxidant activity (Phosphomolybdic acid method), FRAP assay with reference standard Ascorbate and total phenol content respectively. The methanolic extract of *Borreria hispida* was the most effective total antioxidant activity among the three extracts. The IC_{50} values of the methanolic extract of *Borreria hispida* and ascorbate were found to be 160µg/ml and 410µg/ml respectively. The methanolic extract of *Borreria hispida* 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 *Borreria hispida* showed the significant result. The methanolic extract of *Borreria hispida* contains high amount of phenolic compounds than that of other two extracts. So, the *invitro* study clearly indicates that the methanolic extract of *Borreria hispida* has a strong antioxidant activity. This study revealed that methanolic extract of whole plant of *Borreria hispida* comprise effective potential source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: Whole plant of *Borreria hispida*, *Invitro* antioxidant, Total antioxidant activity, FRAP assay, Total Phenol.

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INTRODUCTION

Oxidative stress induced by reactive Oxygen species (ROS) is implicated in the pathogenesis of a variety of vascular diseases, including atherosclerosis, hypertension and coronary artery diseases [1]. 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) [2,3]. There is extensive evidence to implicate free radicals in the development of degenerative diseases [4]. It is suggested that free radical damage to cells leads to the pathological changes associated with aging [5]. Radical reactions are also important in the development of chronic diseases that are life limiting like cancers, hypertension and cardiac infarction, atherosclerosis, rheumatism and also in cataract [6].

Free radical induced oxidative stress, which involve preventive mechanisms, repair mechanism, physical defenses and antioxidant defenses [7]. It is commonly recognized that antioxidants radicals can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutation and therefore, help prevent cancer or heart diseases [8]. 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 [9]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases [10].

Borreria hispida is belongs to the family Rubiaceae. It is widely distributed in throughout India, up to 900m in hills and on all dry lands as a weed. The seed of *Borreria hispida* is used as PPAR-alpha gene expression, antioxidant redox status, protein metabolism in STZ diabetic rats .Potential role of *Borreria hispida* in ameliorating cardiovascular risk factor [11]. The literature survey showed that no study has been done on antioxidative stress activity of *Borreria hispida.* Therefore, we were interested in studying free radical scavenging potential of various extracts of this plant by different *in- vitro* models.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The whole plant of *Borreria hispida* (Linn), were collected from Nasereth, Tuticorin 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 *Borreria hispida* (Linn) 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 [12] for 24 hrs. Then 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)

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex [13]. 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

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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

A modified method of Benzie and Strain [14] 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 phenol

The measurement of total phenol is based on Mallick and Singh [15]. 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.

RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases [16].They are also involved in autoimmune disorders like rheumatoid arthritis etc [17].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 *Borreria hispida* was estimated and the results are presented in Table 1. The petroleum ether extract of *Borreria hispida* exhibited a maximum total antioxidant activity of 48.83% 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 *Borreria hispida* and ascorbate were found to be 1150µg/ml and 410µg/ml respectively.

Table 1: Total antioxidant activity of Petroleum ether extract of *Borreria hispida* (Linn) by Phosphomolybdic acid method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	13.69 ± 0.070	26.87 ± 0.076
2	250	25.61 ± 0.014	30.30 ± 0.054
3	500	41.09 ± 0.096	60.64 ± 0.022
4	1000	48.83 ± 0.043	55.23 ± 0.014
		IC_{50} = 1150 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 *Borreria hispida* was estimated and the results are presented in Table 2. The ethyl acetate extract of *Borreria hispida* exhibited a maximum total

antioxidant activity of 80.01 % 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 *Borreria hispida* and ascorbate were found to be 260µg/ml and 410µg/ml respectively.

Table 2: Total antioxidant activity of Ethyl acetate extract of *Borreria hispida* (Linn) by Phosphomolybdic acid method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	39.72 ± 0.066	26.87 ± 0.076
2	250	49.68 ± 0.047	30.30 ± 0.054
3	500	58.08 ± 0.072	60.64 ± 0.022
4	1000	80.01 ± 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 *Borreria hispida* was estimated and the results are presented in Table 3. The methanolic extract of *Borreria hispida* exhibited a maximum total antioxidant activity of 84.75 % 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 *Borreria hispida* and ascorbate were found to be 160µg/ml and 410µg/ml respectively.

Table 3: Total antioxidant activity of Methanolic extract of *Borreria hispida* (Linn) by Phosphomolybdic acid method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	41.91 ± 0.012	26.87 ± 0.076
2	250	69.17 ± 0.049	30.30 ± 0.054
3	500	75.47 ± 0.036	60.64 ± 0.022
4	1000	84.75 ± 0.024	55.23 ± 0.014
		IC_{50} = 160 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 *Borreria hispida* was most effective among the extracts. But when compare all the extracts with standard the methanolic extract of *Borreria hispida* was found strong antioxidant activity. The IC_{50} of the methanolic extract of *Borreria hispida* and Ascorbate were found to be 160µg/ml and 410µg/ml respectively.

FRAP assay

The antioxidant potential of *Borreria hispida* 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 *Borreria hispida* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values are presented in Table 4. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 51.02% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 970µg/ml and 50µg/ml respectively.

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Table 4: Reducing ability of Pet. ether extract of *Borreria hispida* (Linn) on FRAP assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	15.49 ± 0.077	72.04 ± 0.014
2	250	24.21 ± 0.027	82.05 ± 0.034
3	500	39.97 ± 0.022	86.04 ± 0.026
4	1000	51.02 ± 0.041	98.07 ± 0.041
		IC_{50} = 970 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 *Borreria hispida* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values are presented in Table 5. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 76.46% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 180µg/ml and 50µg/ml respectively.

Table 5: Reducing ability of Ethyl acetate extract of *Borreria hispida* (Linn) on FRAP assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	40.20 ± 0.016	72.04 ± 0.014
2	250	58.28 ± 0.011	82.05 ± 0.034
3	500	71.68 ± 0.029	86.04 ± 0.026
4	1000	76.46 ± 0.021	98.07 ± 0.041
		IC_{50} = 180 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 *Borreria hispida* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values are presented in Table 6. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 80.87% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 65µg/ml and 50µg/ml respectively.

Table 6: Reducing ability of Methanolic extract of *Borreria hispida* (Linn) on FRAP assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	60.42 ± 0.044	72.04 ± 0.014
2	250	67.65 ± 0.029	82.05 ± 0.034
3	500	75.81 ± 0.036	86.04 ± 0.026
4	1000	80.87 ± 0.013	98.07 ± 0.041
		IC_{50} = 65 µg/ml	IC_{50} = 50 µg/ml

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

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Based on the above results indicated, the methanolic extract of *Borreria hispida* 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 *Borreria hispida* showed the similar result.

Total phenol

Phenolic compounds are known as powerful chain breaking antioxidants [18]. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups [19]. The phenolic compounds may contribute directly to antioxidative action [20]. The total amount of phenolic content of various extract of whole plant of *Borreria hispida* was estimated and the results are present in Table 7.

Table 7: The total Phenolic content of various extracts of whole plant of *Borreria hispida* (Linn)

S.No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1	Petroleum ether extract of *Borreria hispida*	2.8 ± 0.087
2	Ethyl acetate extract of *Borreria hispida*	3.2 ± 0.045
3	Methanolic extract of *Borreria hispida*	4.8 ± 0.073

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

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

CONCLUSION

In conclusion, the results of the present study show that the methanolic extract of *Borreria hispida* contains the high amount of flavonoids. So, the present study suggests that the whole plant of *Borreria hispida* might be a potential source of natural antioxidant. Therefore the plant can be further harnessed for novel antioxidant/ bioactive compound which is very well evidence by the present work.

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