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INVITRO EVALUATION OF FREE RADICAL SCAVENGING POTENTIAL OF VARIOUS EXTRACTS OF WHOLE PLANT OF *Borreria hispida* (Linn)

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ABSTRACT

The objective of this study was to examine the *in vitro* free radical scavenging potential of various extracts of whole plant of *Borreria hispida* (Linn). The antioxidant activity was evaluated by 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. An IC_{50} value was found that methanolic extract of *Borreria hispida* is more effective in DPPH radical scavenging activity than that of ethyl acetate and petroleum ether extract. But when compare to the all the three extracts with Rutin (standard), the methanolic extract of the *Borreria hispida* showed the moderate result. The methanolic extract of *Borreria hispida* was found to more antioxidant activity in the scavenging superoxide radical activity. The IC_{50} value of the methanolic extract of *Borreria hispida* and Quercetin were found to be 65µg/ml and 60µg/ml respectively. The Iron chelating activity of the methanolic extract of *Borreria hispida* was found to most effective than that of standard EDTA. It is concluded that a whole plant of the methanolic extract of *Borreria hispida* was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. The results of this study show that the methanolic extract of *Borreria hispida* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.

KEY WORDS:

Whole plant of *Borreria hispida*, *Invitro* antioxidant, DPPH assay, Superoxide anion, Iron chelating activity.

INTRODUCTION

Recently, there has been a surge in research on the potential role of antioxidants in the treatment of atherosclerosis, heart failure, liver dysfunction. Neurogenetic disorders, cancer, and diabetes mellitus[1].Oxidative damage in the human body plays an important causative role in disease initiation and progression [2, 3].

Free radicals are highly reactive and very short lived molecules. Although free radicals perform some useful functions, they are toxic when generated excess. Damage from free radicals and

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reactive oxygen species has been linked to some neurodegenerative disorders[4, 5] and cancers[6], and oxidation of low-density lipoprotein is a major factor in the promotion of coronary heart disease (CHD) and atherosclerosis[7, 8]. Diets high in fruits and vegetables and low in cholesterol and fats are inversely correlated with the incidence of CHD and cancer [9, 10 and 11].

Natural antioxidants from fruits and vegetables provide a measure of protection that slows the process of oxidative damage[2]. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the total antioxidant activity of many fruits and vegetables[12, 13]. Fruits and vegetables are high in flavonoid content; it is estimated that humans consume between a few hundred milligrams and one gram of flavonoids every day[14, 15]. Human studies have found that flavonoids appear in blood plasma, at pharmacologically active levels, after eating certain foods but do not accumulate in the plasma[16]. Regular consumption of flavonoids may increase longevity by reducing inflammation and contributing to a reduction in CHD[7].

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 (Vasanthi HR, 2009)[17]. 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 (Petroleum ether, Ethyl acetate and Methanol) of this plant by various *in vitro* models.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The whole plant of *Borreria hispida* (Linn), were collected from Naserath, 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[18] for 24 hrs. And the 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

DPPH photometric assay[19]

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[19]. A methanolic solution of 0.5ml of DPPH (0.4mM) 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

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served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

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

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

Superoxide radical scavenging activity[20]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted 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 (1975)[20]. The assay mixture contained sample with 0.1ml 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 where in 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 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 (1996)[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 (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg 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

Oxidative stress, in which large quantities of reactive oxygen species (ROS) like hydrogen peroxide, superoxide ($^*O^-_2$), hydrogen radical (OH^-), singlet oxygen and nitrogen species are generated, is one of the earliest responses to stress. These ROS have a role in disease and aging in animals[22]. The antioxidant system protects the organism against ROS-induced oxidative damage. There are restrictions on the use of synthetic antioxidant such as BHT, as they suspected to be carcinogenic[23]. Natural antioxidants, therefore, have gained importance.

DPPH scavenging activity

The DPPH radical scavenging activity of petroleum ether extract of *Borreria hispida* presented in Table 1. The IC_{50} values of the petroleum ether extract of *Borreria hispida* and

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Rutin were found to be 1490µg/ml and 480µg/ml respectively.

Table 1

Effect of Petroleum ether extract of Borreria hispida (Linn) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	10.44 ± 0.070	18.85 ± 0.076
2	250	14.93 ± 0.010	22.08 ± 0.054
3	500	24.51 ± 0.015	52.21 ± 0.022
4	1000	34.88 ± 0.091	69.83 ± 0.014
		IC_{50} = 1490 µg/ml	**IC_{50} = 480 µg/ml**

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

The DPPH radical scavenging activity of ethyl acetate extract of *Borreria hispida* presented in Table 2. The IC_{50} values of the ethyl acetate extract of *Borreria hispida* and Rutin were found to be 1430µg/ml and 480µg/m respectively

Table 2

Effect of Ethyl acetate extract of Borreria hispida (Linn) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Rutin)
1	125	17.59 ± 0.081	18.85 ± 0.076
2	250	18.57 ± 0.067	22.08 ± 0.054
3	500	30.77 ± 0.042	52.21 ± 0.022
4	1000	34.75 ± 0.039	69.83 ± 0.014
		IC_{50} = 1430 µg/ml	**IC_{50} = 480 µg/ml**

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

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The percentage of DPPH radical scavenging activity of methanolic extract of *Borreria hispida* depicted in Table 3. The IC_{50} values of the methanolic extract of *Borreria hispida* and Rutin was found to be 1130µg/ml and 480µg/m respectively.

Table 3

Effect of Methanolic extract of Borreria hispida (Linn) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	12.37 ± 0.012	18.85 ± 0.076
2	250	19.68 ± 0.046	22.08 ± 0.054
3	500	23.20 ± 0.031	52.21 ± 0.022
4	1000	48.29 ± 0.023	69.83 ± 0.014
		IC_{50} = 1130 µg/ml	**IC_{50} = 480 µg/ml**

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

From the result the IC_{50} values of methanolic extract of *Borreria hispida* showed more antioxidant activity when compared to that of other two extracts. Among the three extracts showed moderate activity with standard Rutin.

Superoxide anion scavenging activity

Percentage scavenging of superoxide anion activity of petroleum ether extract of *Borreria hispida* was presented in table 4. The IC_{50} value of plant extract and Quercetin was recorded as 170µg/ml and 60µg/ml respectively.

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Table 4

Effect of Petroleum ether extract of Borreria hispida (Linn) on Superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Quercetin)
1	125	44.45 ±0 .015	73.81 ± 0.006
2	250	81.18 ± 0.049	91.31 ± 0.011
3	500	86.74 ± 0.030	92.99 ± 0.024
4	1000	97.86 ± 0.027	98.01 ± 0.012
		IC_{50} = 170 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 *Borreria hispida* was presented in table 5. The IC_{50} value of plant extract and Quercetin was recorded as 80µg/ml and 60µg/ml respectively.

Table 5

Effect of Ethyl acetate extract of Borreria hispida (Linn) on Superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	66.09 ± 0.051	73.81 ± 0.006
2	250	80.38 ± 0.029	91.31 ± 0.011
3	500	91.87 ± 0.031	92.99 ± 0.024
4	1000	93.27 ± 0.019	98.01 ± 0.012
		IC_{50} = 80 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 *Borreria hispida* was presented in table 6. The IC_{50} value of plant extract and Quercetin was recorded as 65µg/ml and 60µg/ml respectively.

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Table 6

Effect of Methanolic extract of Borreria hispida (Linn) on Superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	64.43 ± 0.015	73.81 ± 0.006
2	250	68.72 ± 0.031	91.31 ± 0.011
3	500	90.68 ± 0.032	92.99 ± 0.024
4	1000	94.30 ± 0.025	98.01 ± 0.012
		IC_{50} = 65 µg/ml	**IC_{50} = 60 µg/ml**

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

Based on the above results the IC_{50} values of methanolic extract of *Borreria hispida* was found strong superoxide radical scavenging activity; whereas ethyl acetate showed moderate activity and petroleum ether extract showed weak activity when compared to that of standard Quercetin.

Iron chelating activity

Iron binding capacity of the petroleum ether extract of *Borreria hispida* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 7. The IC_{50} value of plant extract and EDTA was recorded as 190µg/ml and 65µg/ml respectively.

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Table 7

Effect of Petroleum ether extract of Borreria hispida (Linn) on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (EDTA)
1	125	31.64 ± 0.020	58.68 ± 0.007
2	250	55.45 ± 0.037	65.87 ± 0.018
3	500	85.34 ± 0.029	83.83 ± 0.012
4	1000	93.90 ± 0.022	97.90 ± 0.019
		IC_{50} = 190 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 *Borreria hispida* 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 was recorded as 180µg/ml and 65µg/ml respectively.

Table 8

Effect of Ethyl acetate extract of Borreria hispida (Linn) on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethylacetate extract)	Standard (EDTA)
1	125	31.64 ± 0.016	58.68 ± 0.007
2	250	54.87 ± 0.011	65.87 ± 0.018
3	500	93.90 ± 0.029	83.83 ± 0.012
4	1000	94.43 ± 0.021	97.90 ± 0.019
		IC_{50} =180 µg/ml	**IC_{50} = 65 µg/ml**

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

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Iron binding capacity of the methanolic extract of *Borreria hispida* 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 was recorded as 140µg/ml and 65µg/ml respectively.

Table 9

Effect of Methanolic extract of Borreria hispida (Linn) on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (EDTA)
1	125	35.89 ± 0.044	58.68 ± 0.007
2	250	59.47 ± 0.029	65.87 ± 0.018
3	500	72.74 ± 0.036	83.83 ± 0.012
4	1000	94.97 ± 0.013	97.90 ± 0.019
		IC_{50} = 140 µg/ml	**IC_{50} = 65 µg/ml**

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

Based on the above results indicated, the methanolic extract of *Borreria hispida* was found more antioxidant activity when comparable to that of other two extracts. Among the three extracts showed moderate activity with standard EDTA respectively.

CONCLUSION

The present study indicated the methanolic extract of *Borreria hispida* showed strong antioxidant activity by inhibiting DPPH and superoxide anion scavenging and iron chelating activities when compared with different standards such as Rutin, Quercetin, and EDTA. In addition, the methanolic extract of *Borreria hispida* was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. The results of this study show that the methanolic extract of *Borreria hispida* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidant activity of *Borreria hispida* are currently unclear. Therefore, it is suggested that further works should be performed on the isolation and identification of the antioxidant components in *Borreria hispida*.

REFERENCE

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1. Ajitha M. and Rajnarayana K., Role of oxygen free radicals in human diseases, Indian Drugs, 2001, 38, 545-554.
2. Jacob, R. A., & Burri, B. J. (1996). Oxidative damage and defense. American Journal of Clinical Nutrition, 63, 985S–990S.
3. Kelly, F. J. (1998). Use of antioxidants in the prevention and treatment of disease. Journal of International Food Chemistry, 10, 21–23.
4. Floyd, R. A. (1999). Neuroinflammatory processes are important in neurodegenerative diseases: an hypothesis to explain the increased formation of reactive oxygen and nitrogen species as major factors involved in neurodegenerative disease development. Free Radical Biology and Medicine, 26, 1346–1355.
5. Youdim, K. A., & Joseph, J. A. (2001). A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. Free Radical Biology & Medicine, 30, 583–594.
6. Goodwin, J. S., & Brodwick, M. (1995). Diet, aging, and cancer. Clinics in Geriatric Medicine, 11, 577–589.
7. Frankel, E. N., Kanner, J., German, J. B., Parks, E., & Kinsella, J. E.(1993). Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. The Lancet, 341, 454–457.
8. Steinberg, D. (1997). A critical look at the evidence for the oxidation of LDL in atherogenesis. Atherosclerosis, 131, S5-S7.
9. Hertog, M. G. L., Feskens, E. J. M., Hollman, P., Katan, M. B., & Kromhout, D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. The Lancet, 342, 1007–1011.
10. Hertog,M. G. L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina,R., Fidanza, F., Glampaoli, S., Jansen, A., Menotti, A., Nedeljkovic,S., Pekkainen, M., Simic, B. S., Toshima, H., Fesens, E. J. M., Hollman,P. C. H., & Katan, M. B. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Archives of Internal Medicine, 55, 381–386.
11. Knekt, P., Ja¨ rvinen, R., Reunanen, A., & Maatela, J. (1996). Flavonoid intake and coronary mortality in Finland: a cohort study.British Journal of Medicine, 312, 478–481.
12. Luo, X.-D., Basile, M. J., & Kennelly, E. J. (2002). Polyphenolic antioxidants from the fruits of Chrysophyllum cainito L. (star apple).Journal of Agricultural & Food Chemistry, 50, 1379–1382.
13. Vinson, J., Jang, J., Yang, J., Dabbagh, Y., Liang, X., Serry, M., Proch, J., & Cai, S. (1999). Vitamins and especially flavonoids incommon beverages are powerful in vitro antioxidants which enrich low density lipoproteins and increase their oxidative resistance after ex vivo spikingin human plasma. Journal of Agricultural & Food Chemistry, 47, 2502–2504.
14. Hollman, P. C., & Katan, M. B. (1999). Dietary flavonoids: intake, health effects and bioavailability. Food & Chemical Toxicology, 37, 937–942.
15. Pietta, P.-G. (2000). Flavonoids as antioxidants. Journal of Natural Products, 63, 1035–1042.

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16. Cao, G., Booth, S. L., Sadowski, J. A., & Prior, R. (1998). Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruits and vegetables. American Journal of Clinical Nutrition, 68, 1081–1087.
17. Vasanthi, Hannah R PhD; Mukherjee, Subhendu MS; Lekli, Istvan MFarm; Ray, Diptarka MS; Veeraraghavan, Gayathri PhD; Das, Dipak K PhD. Potential Role of Borreria hispida in Ameliorating Cardiovascular Risk Factors. Journal of Cardiovascular Pharmacology:2009. 53 ; 6: 499-506
18. Harborne J.B. (1984) Phytochemical methods 11 Edn. In Chapman &, Hall. New York: 4-5.
19. Mensor , L.L, Meneze, F.S., Leitao, G.G., Reis, A.S., Dos santor, J.C., Coube, C.S and Leitao,S.G (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother.Res.15, 127-130.
20. Winterbourne, C.C., Hawkins, R.E., Brain, M and Carrel, R.W (1975). The estimation of red cell superoxide dismutase activity. J. Lab.chem.Med. 85, 337-341.
21. Benzie IEF and Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem.**239,**70-76.
22. Melov S. Animal models of oxidative stress, aging and therapeutic antioxidant interventions. Int J Biochem Cell Biol 2002; 34: 1395-400.
23. Singh RP, Chidamara Murthy KN, Jayaprakasha GK. Studies on the antioxidant activity of pomegranate (*Punica granatum*) Peel and seed extracts using *in vitro* models. J Agri Food Chem 2002; 50: 81-6.