Research Article

Evaluation of *In vitro* Antioxidant Properties of *Hedyotis leschenaultiana* DC (Rubiaceae)

Vallinayagam Sornalakshmi, Pious Soris Tresina, Maria Louis Packia Lincy, Koilpitchai Paulpriya, Veerabahu Ramasamy Mohan*
Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu, India.
***Corresponding author's E-mail:** vrmohanvoc@gmail.com

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ABSTRACT

The *in vitro* antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extract of whole plant of *Hedyotis leschenaultiana* have been tested using various antioxidant model systems viz; DPPH, hydroxyl, superoxide, ABTS and reducing power. Methanol extract of *H. leschenaultiana* whole plant is found to possess higher DPPH, superoxide and ABTS radical scavenging activity, while petroleum ether extract is found to possess higher hydroxyl radical scavenging activity. Methanol extract of whole plant of *H. leschenaultiana* showed the highest reducing ability. The study indicates the significant free radical scavenging potential of *H. leschenaultiana* whole plant which can be experimented for the treatment of various free radical mediated ailments.

Keywords: *Hedyotis leschenaultiana*, DPPH, free radical, reducing power.

INTRODUCTION

Free radicals are compounds generated from normal body processes and also from environmental pollutants. They tend to attack the cells of our body causing them to deteriorate. Antioxidants quench free radicals, so that they will not attack living cells. Antioxidant properties have been extensively studied and are among the first link between chemical reactions and biological activities.[1] It has been shown that antioxidants and free radical scavengers are relevant in the prevention of pathologies such as arteriosclerosis, heart diseases, cancer and arthritis, in which reactive oxygen species or free radicals are implicated.[2] The two most commonly used synthetic antioxidants; butylated hydroxyanisole (BHA) and butylated hydroxytolune (BHT) have begun to be restricted because of their toxicity and DNA damage induction.[3,4]

Several types of plant materials such as vegetables, fruits, leaves, oilseeds, cereals, crops, bark and roots, spices and herbs and crude plant drugs are potential sources of antioxidant compounds. Most of the isolated constituents with antioxidant activity are phenolic compounds.[5] A systematic search for useful bioactive compounds from medicinal plants is now considered to be a rational approach in nutraceutical and drug research. There is a worldwide trend towards the use of natural antioxidants. For this reason, an extreme search for different types of antioxidants in various medicinal plants has been undertaken.

The genus *Hedyotis* finds a prominent place in different Indian system of medicine. The different ethnic communities in India have used different species of *Hedyotis* in the treatment of various ailments.[6] Taking into the consideration of the medicinal importance of *Hedyotis*, the different solvent extracts of whole plant of *Hedyotis leschenaultiana* DC have undertaken to evaluate the *in vitro* antioxidant activities using different models. However, to the best of our knowledge, there is no information in the literature about *in vitro* antioxidant activities of different solvent extracts of this plant.

MATERIALS AND METHODS

The entire fresh plant materials of *Hedyotis leschenaultiana* DC were collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. The plant samples were identified with the help of local flora and authenticated by Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen of collected plants were deposited in the Ethnopharmacological Unit, PG & Research Department of Botany, V.O. Chidambaram College, Thoothukudi District, Tamil Nadu. The whole plant of *H. leschenaultiana* was cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant materials were granulated or powdered by using a blender and sieved to get uniform particles by using sieve No. 60. The final uniform powder of the plant was used for the extraction of active constituents of the plant materials.

Chemicals

All the chemicals and reagents used in the experiments were of analytical grade and were obtained from BDH (England and India), E. Merck (Germany), Sigma Chemical Company (U.S.A.), Sarabhai, M. Chemicals (India) and LOBA–Chemie Indo Austranol Co., (India). Whenever necessary, the solvents were redistilled before use.

Preparation of plant extract

The coarse powder (100g) of aerial part of *Hedyotis leschenaultiana* was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All

the extracts were filtered though Whatman No.41 filter paper. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

Estimation of total phenolic content

Total phenolic contents were estimated using Folin-Ciocalteau reagent based assay as previously described[7] with little modification. To 1mL of each extract (100µg/mL) in methanol, 5mL of Folin-Ciocalteau reagent (diluted ten-fold) and 4mL (75g/L) of Na_2CO_3 were added. The mixture was allowed to stand at 20°C for 30min and the absorbance of the developed colour was recorded at 765nm using UV-VIS spectrophotometer. 1mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of flavonoids

The flavonoids content was determined according to Eom et al[8]. An aliquot of 0.5ml of sample (1mg/mL) was mixed with 0.1mL of 10% aluminium chloride and 0.1mL of potassium acetate (1M). In this mixture, 4.3mL of 80% methanol was added to make 5mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H.

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method.[9] Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts at different concentration (50,100,200,400 & 800µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Hydroxyl radical scavenging activity

The scavenging capacity of hydroxyl radical was measured according to the modified method of Halliwell et al.[10] Stock solutions of EDTA (1mM), $FeCl_3$ (10 mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM) were prepared in distilled deionized water.

The assay was performed by adding 0.1mL EDTA , 0.01mL of $FeCl_3$, 0.1mL H_2O_2, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (50, 100, 200, 400 & 800µg/mL) dissolved in distilled water, 0.33mL of phosphate buffer (50mM, pH 7.9), 0.1mL of ascorbic acid in sequence . The mixture was then incubated at 37°C for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan.[11] The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, P^H 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 mL NADH (0.936mM) solution, 1.0 mL extract of different concentration (50, 100, 200, 400 & 800µg/mL), and 0.5 mL Tris – HCl buffer (16mM, P^H 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Antioxidant activity by radical cation (ABTS +)

ABTS assay was based on the slightly modified method of Huang et al.[12] ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Reducing power

The reducing power of the extract was determined by the method of Kumar and Hemalatha.[13] 1.0 mL of solution

containing 50, 100, 200, 400 & 800µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH6.6) and potassium ferricyanide (5.0 mL, 1.0%): The mixture was incubated at 50°C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

RESULTS

Total phenolic and total flavonoid contents

The total phenolic and total flavonoid contents of the methanol extract of *H. leschenaultiana* whole plant was found to be 0.68g $100g^{-1}$ and 0.92g $100g^{-1}$ respectively.

DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *H. leschenaultiana* whole plant is shown in Fig. 1. The scavenging effect increases with the concentration of standard and samples. Among the solvents tested, methanol extract exhibited highest DPPH radical scavenging activity. At 800 µg/ml concentration methanol extract of *H. leschenaultiana* possessed 129.22% scavenging activity on DPPH. IC_{50} values of methanol extract of whole plant of *H. leschenaultiana* and standard ascorbic acid were 54.98µg/ml and 34.59µg/ml respectively (Table 1).

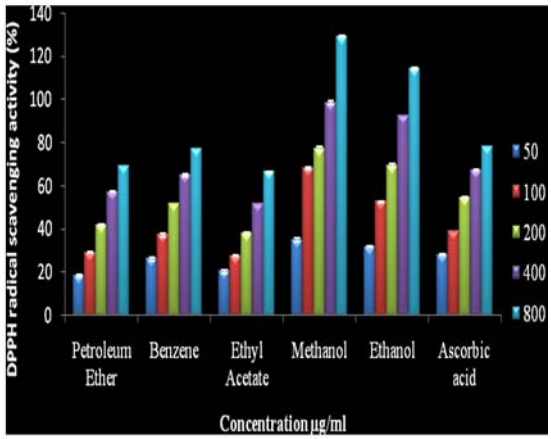

Figure 1: DPPH radical scavenging activity of different solvent extracts of whole plant of *H. leschenaultiana*

Table 1: IC_{50} values of different solvent extracts of whole plant of *H. leschenaultiana* (µg/ml)

Different solvent extract	DPPH assay	Hydroxyl assay	Superoxide assay	ABTS assay
Petroleum ether	32.82	34.26	29.83	30.56
Benzene	36.59	30.34	30.86	31.92
Ethylacetate	33.24	28.19	34.80	18.46
Methanol	54.28	24.15	39.22	34.68
Ethanol	49.36	29.22	36.87	23.14
Standard (Ascorbic acid)	34.59	33.29	32.56	-
Standard (Trolox)	-	-	-	24.66

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *H. leschenaultiana* whole plant is shown in Fig. 2. Petroleum ether extract showed very potent activity. At 800 µg/ml concentration, *H. leschenaultiana* possessed 87.53% scavenging activity on hydroxyl radical. IC_{50} values of petroleum ether extract of whole plant of *H. leschenaultiana* and standard ascorbic acid were 34.26µg/ml and 33.29µg/ml respectively (Table 1).

Superoxide radical scavenging activity

H. leschenaultiana whole plant extracts were subjected to superoxide scavenging assay and the results were shown in Fig. 3. It indicates that, methanol extract of *H. leschenaultiana* whole plant (800µg/ml) exhibited the maximum superoxide scavenging activity of 116.33% which is higher than the standard ascorbic acid whose scavenging effect is 81.62 %. IC_{50} values of methanol extract of whole plant of *H. leschenaultiana* and standard ascorbic acid were 39.22µg/ml and 32.56µg/ml respectively (Table 1).

ABTS radical cation scavenging activity

H. leschenaultiana whole plant extracts were subjected to be ABTS radical cation scavenging activity and the results were shown in Fig. 4. The methanol extract exhibited potent ABTS radical cation scavenging activity in concentration dependent manner.

At 800 µg/ml concentration, *H. leschenaultiana* whole plant possessed 91.50 % scavenging activity on ABTS which is higher than the standard trolox whose scavenging activity is 66.99%. IC_{50} values of methanol extract of whole plant of *H. leschenaultiana* and standard trolox were 34.68µg/ml and 24.66µg/ml respectively (Table 1).

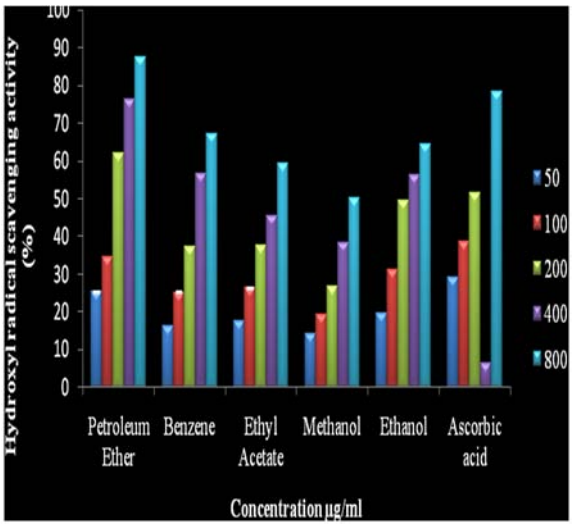

Figure 2: Hydroxyl radical scavenging activity of different solvent extracts of whole plant of *H. leschenaultiana*

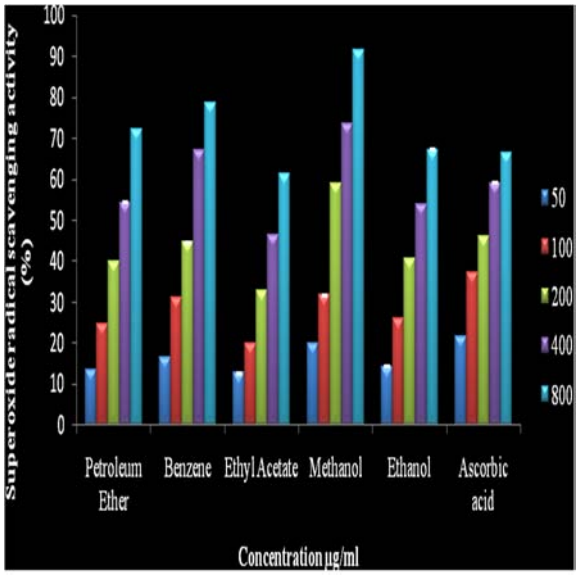

Figure 3: Superoxide radical scavenging activity of different solvent extracts of whole plant of *H. leschenaultiana*

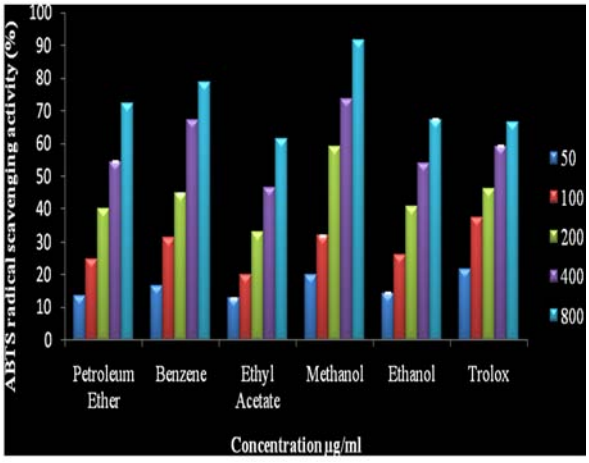

Figure 4: ABTS radical cation scavenging activity of different solvent extracts of whole plant of *H. leschenaultiana*

Reducing power

Fig. 5 showed the reducing ability of different solvent extracts of *H. leschenaultiana* whole plant when compared to ascorbic acid. Absorbance of the solution was increased when the concentration increased. A higher absorbance indicates a higher reducing power. Among the solvent tested, methanol extract exhibited higher reducing activity.

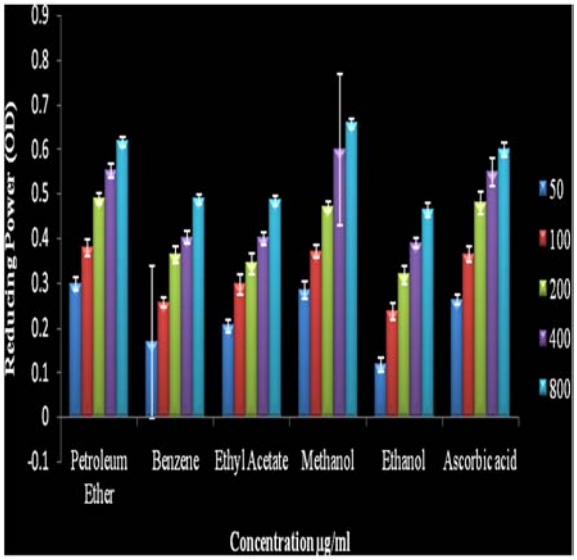

Figure 5: Reducing power activity of different solvent extracts of whole plant of *H. leschenaultiana*

DISCUSSION

The systematic literature collection, pertaining to this investigation indicates that, the plant phenolics constitute one of the major groups of compounds using as primary antioxidants or free radical scavengers. Therefore, it is necessary to determine the total amount of phenols and flavonoids in the plant extract chosen for the study. Flavonoids are the most diverse and widespread group of natural compounds and are likely to be the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging activity.

Flavonoids are important secondary metabolites of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effects of flavonoids is correlated with their antioxidant activity.[14] Phenolic compounds are considered to be the most important antioxidants of plant materials. They contribute one of the major groups and compounds acting as primary antioxidants or free radical terminators. Antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties.[15] The presence of these compounds such as total phenolics and flavonoids in *H. leschenaultiana* extract may give credence to its local usage for the management of oxidative stress induced ailments.

Free radicals and other reactive species are thought to play an important role in many human diseases. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. Many secondary metabolites which include flavonoids, phenolic compounds, etc. serve as sources of antioxidants and do scavenging activity.[16,17] In this study, it is evident that, the extract of the study species, *H. leschenaultiana* possess effective antioxidant activity. The antioxidant activity may be due to the presence of respective phytochemicals like flavonoids, phenolics, etc.

In vitro antioxidant activity of the petroleum ether, benzene, ethyl acetate, methanol and ethanol extract of *H. leschenaultiana* were investigated in the present study by DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activities. These methods have proven the effectiveness of the extracts in comparison to that of the reference standard antioxidants, ascorbic acid and trolox.

DPPH assay is the most widely reported method for screening antioxidant activity of many plant drugs, based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. This procedure involves measurement of decrease in absorbance of DPPH at its absorbance maxima of 516nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. DPPH is a stable, nitrogen-centered free radical which produces violet color in ethanol solution. It was reduced to a yellow colored product, diphenylpicrylhydrazine, with the addition of *H. leschenaultiana* in a concentration-dependent manner.[18] Among the solvents tested, methanol extract of *H. leschenaultiana* whole plant exhibited more DPPH radical scavenging activity.

Hydroxyl radicals are major active oxygen species causing lipid peroxidation and enormous biological damage. Hydroxyl radical scavenging capacity of *H. leschenaultiana* extract is directly related to its antioxidant activity. This method involves *in vitro* generation of hydroxyl radicals using Fe^{3+}/ascorbate/EDTA/H_2O_2 system using Fenton reaction. The oxygen derived hydroxyl radicals along with the added transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid.[19] When *H. leschenaultiana* extract was added to the reaction mixture, it removed the hyxdroxyl radicals from the sugar and prevented the reaction. Among the solvent tested, petroleum ether possessed more hydroxyl radical scavenging activity when compared with standard ascorbic acid. The hydroxyl radical scavenging activity may be due to the presence of various phytochemicals including polyphenols and flavonoids in *H. leschenaultiana* whole plant extract.

Superoxide anion is also very harmful to cellular components and produced from molecular oxygen due to oxidative enzyme of body as well as via non-enzymatic reaction such as autoxidation by catecocholamines.[20] The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560nm, *H. leschenaultiana* extract indicated ability to quench superoxide radicals in the reaction mixture. The present study showed potent superoxide radical scavenging activity for *H. leschenaultiana* whole plant extract. Methanol extract showed potent superoxide radical scavenging activity with IC_{50} value of 39.22 µg/ml compared to ascorbic acid 32.56 µg/ml.

ABTS radical scavenging activity is relatively a recent one, which involves a more drastic radical, chemically produced and is often used for screening complex antioxidant mixtures such as plant extracts, beverages and biological fluids. The ability in both organic and aqueous media and the stability in a wide pH range raised the interest in the use of $ABTS^{+}$ for the estimation of antioxidant activity.[12] In the present study, methanol extract of whole plant of *H. leschenaultiana* were fast and effective scavengers of ABTS radical and this activity was higher than that of trolox standard. Proton radical scavenging is an important attribute of antioxidants. ABTS a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals.[21]

Several reports indicated that the reducing power of bioactive compounds was associated with antioxidant activity.[22] Therefore, it is necessary to determine the reducing power of phenolic constituents contained in the plant extracts to elucidate the relationship between their antioxidant effect and their reducing power.

In the present study, increase in absorbance of the reaction mixture indicates the reductive capabilities of *H. leschenaultiana* extract in concentration dependent manner when compared to the standard ascorbic acid.

CONCLUSION

The present study reveals that the whole plant of *H. leschenaultiana* exhibits satisfactory scavenging effect in all the radical scavenging assays. This is the first report on the antioxidant property of this plant. It is reported that, total phenolics and flavonoids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms.[23] Thus in the present study, the antioxidant potential of *H. leschenaultiana* may be attributed to the presence of flavonoids, phenolics and other constituents present in them.

REFERENCES

1. Trouillas P, Calliste CA, Allias DP, Simon A, Maofak A, Delage C, Duroux JL, Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin country side herbal teas, Food Chemistry, 80, 2008, 399-407.
2. Middleton EJR, Kandaswami C, Theobarides JC, The effects of plant flavonoids on mammalian cell: Implications for inflammation, heart disease cancer, Pharmacological Reviews, 52, 2000, 673-751.

3. Ito N, Hiroze M, Fukushima G, Tauda H, Shira T, Tatematsa M, Studies on antioxidant their carcinogenic and modifying effects on chemical carcinogenesis, Food and Chemical Toxicology, 24, 1986, 1071-1081.
4. Sasaki YF, Kawagachi S, Kamaya A, Obshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S, The correct assay with 8 mouse organs: results with 39 currently used food additives, Mutation Research – Genetic Toxicology and Environmental mutagenesis, 519, 2002, 103-119.
5. Odukoya OA, Jenkins MO, Ilori MOO, Sofidiya MO, The use of selected Nigerian natural products in management of environmentally induced slum damage, Pakistan Journal of Biological Sciences, 8, 2005, 1074-1077.
6. Sasikumar JM, Maheshu V, Aseervatham GSB, Darsini DTP, *In vitro* antioxidant activity of *Hedyotis corymbosa*(L.) Lam. aerial parts, Indian Journal of Biochemistry and Biophysics, 47, 2010, 49-52.
7. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chemistry, 73, 2001, 73-84.
8. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu C, Cho DH. Far infra red ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries, Korean Journal of Medicinal Crop Science, 15, 2007, 319-323.
9. Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity *in vitro* of selenium-contained protein from the se-enriched. *Bifodobacterium animalis* 01, Anaerobe, 16, 2010, 380-386.
10. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. Analytical Biochemistry, 65, 1987, 215-219.
11. Srinivasan R, Chandrasekar MJN, Nanjan MJ. Suresh B. Antioxidant activity of *Caesalpinia digyna* root. Journal of Ethnopharmacology, 113, 2007, 284-291.
12. Huang MH, Huang SS, Wang BS, Sheu MJ, Hou WC. Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo* and *in vivo*. Journal of Ethnopharmacology, 133, 2011, 743-750.
13. Kumar RS, Hemalatha S, *In vitro* antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites, Journal of Chemical and Pharmaceutical Research, 3, 2011, 259-267.
14. Sri J, Yu J, Pohorly J, Young C, Bryan M, Wu J, Optimization of the extraction of polyphenolics from grapes seed and by aqueous ethanol solution, Food Agriculture and Environment, 1, 2006, 42-47.
15. Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAB, Supriatno OKL, Correlation between the total phenolic and mineral contents unit antioxidant activity of eight Malaysian Bananas (Musa sp). Journal of Food Composition and Analysis. 24, 2011, 1-10.
16. Ghasemi K, Ghaseni Y, Ebrahimzadeh MA, Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pakistan Journal of Pharmaceutical Sciences, 22, 2009, 277-281.
17. Doss A, Pugalenthi M, Rajendrakumar D, Vadivel V, Phenols, flavonoids and antioxidant activity of underutilized legume seeds, Asian Journal of Experimental Biological Sciences, 1, 2010, 700-705.
18. Jothy SL, Zuraini Z, Sasidharan S, Phytochemical screening, DPPH free radical scavenging and xanthine oxidase inhibitory activities of *Cassia fistula* seed extracts, Journal of Medicinal Plant Research, 5, 2011, 1941-1947.
19. Battu GR, Ethadi SR, Veda PG, Swathi PK, Chandrika K, Rao VA, Evaluation of antioxidant and anti-inflammatory activity of *Euphorbia heyneana* Spreng. Asian Pacific Journal of Tropical Biomedicine, 2009, S191-S194.
20. Naskar S, Islam A, Mazuumder UK, Saha P, Haldar PK, Gupta M, *In vitro* antioxidant potential of hydromethanolic extract of *Phoenix dactylifera* fruits, Journal of Scientific Research, 2, 2010, 144-157.
21. Adebayo GI, Alabi OT, Owoyele BV, Soladoye AO, Anti-diabetic properties of the aqueous leaf extract of *Bougainvillea glabra* (Glory of the Garden) on alloxan induced diabetic rats, Records of Natural Products, 3, 2009, 187-192.
22. Siddhuraju P, Mohan PS, Becker K, Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, Flowers and fruit pulp, Food Chemistry, 79, 2002, 61-70.
23. Shirwaikan A, Rajendran K, Dinesh K, *In vitro* antioxidant studies of *Annona squamosa*, Indian Journal of Experimental Biology, 142, 2004, 803.

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