Original Article

Phytochemical, Antioxidant Property And Free Radical Scavenging Activity Of Henckelia humboldtiana (Gardner) A. Weber & B.L. Burtt (Gesneriaceae)

R. Senthil kumar[1], A. Rajesh[2], L. Mullai Nathan[3], P.S. Tresina[4], V.R. Mohan[5*]

[1,3]Department of Botany, Annamalai University, Chidambaram, Tamil Nadu
[2]Department of Medicinal Botany, Govt. Siddha Medical College, Palayamkottai, Tamil Nadu
[4,5*]Ethnopharmacology unit, Research Department of Botany, V.O. Chindambaram College, Thoothukudi, Tamil Nadu

***Corresponding Author:-** V.R. Mohan

*Ethnopharmacology unit, Research Department of Botany, V.O. Chindambaram College, Thoothukudi, Tamil Nadu, email: vrmohanvoc@gmail.com

DOI: 10.47750/pnr.2023.14.03.269

Abstract

The present study was carried out to investigate the phytochemical and *in vitro* antioxidant activity of whole plant of *Henckelia humboldtiana*. Qualitative phytochemical screening of methanol and ethanol extracts of *Henckelia humboldtiana* revealed the presence of alkaloids, catechins, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, sugars, glycosides and xanthoproteins. Total phenolic and flavonoid contents were determined. Antioxidant activity have been tested using various antioxidant model systems viz. DPPH, hydroxyl, superoxide, ABTS and reducing power. Ethanol extract of *H. humboldtiana* is found to possess higher DPPH, superoxide and ABTS radical scavenging activity while methanol extracts is found to possess higher hydroxyl radical scavenging activity. Ethanol extract of whole plant of *Henckelia humboldtiana* shows the highest reducing ability. This study indicates the significant free radical scavenging potential of *Henckelia humboldtiana* whole plant. This is the first report on the antioxidant property of this plant.

Keywords: *Henckelia humboldtiana*, Phytochemical, Total phenolic, Antioxidant, Reducing power

INTRODUCTION

Recently, an increasing number of studies are being conducted to explore natural compounds rich in antioxidants properties because of their significance in treating various chronic disorders, such as cancer and cardiovascular disease.

Numerous studies hold up the fact that many diseases are caused by oxidative stress consequential from a discrepancy in the neutralization and arrangement of pro-oxidants. Human bodies naturally produce free radicals. These overloaded free radicals contradict with biological macromolecules, such as lipids, proteins and DNA, in healthy human cells. This results in the stimulation of carcinogenesis, cardiovascular disease, atherosclerosis, aging and inflammatory diseases (Brahma *et al*., 2016; Banother *et al*., 2017). It has been confirmed that antioxidant substances may be defensive against above mentioned diseases.

Many people consume antioxidants as defense against oxidative stress. The synthetic antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole and tertiary butyl hydroquinone are associated with the toxic due to presence of higher amount of preservatives. Hence, ascertaining potential natural antioxidant sources can be useful alternative to ensure better health. (Tanvir *et al*., 2017). Worldwide currently, there has been an augmented interest to find antioxidant compounds which are pharmacologically active and have minor or no side effects (Ntie-Kang *et al*., 2013). One of the special sources of antioxidant is plant based natural phenolic compounds. Phenolic compounds are found to be one of the most essential groups of plant secondary metabolites. The plant containing higher amount of phenolic component as antioxidant constituents has always detected researchers to search for novel medications to develop healthy life for humans.

Genus *Henckelia* comprises approximately 180 species, belongs to the family Gesneriaceae. A few uses of traditional medicines are known for roots and leaves of *Henckelia* in South-East Asia as a protective medicine after childbirth, as a poultice on wounds, to treat itch and rash and to treat cough, dysentery and colic. Research on phytochemistry and

pharmacological properties is needed to establish value of genus *Henckelia* as a medicinal plant, which appears to be marginally as yet (Rachman and Kiew, 2003). Hence, the current study planned to investigate the phytochemical and antioxidant activity of the whole plant of *Henckelia humboldtiana*.

MATERIALS AND METHODS

Collection of plant sample

The whole plant of *Henckelia humboldtiana* (Gardner) A. Weber & B.L. Burtt were collected from Valparai, Anaimalai Tiger Reserve, Coimbatore District, Tamil Nadu. The specimens collected were identified with the local flora and authenticated by Botanical survey of India, Southern Circle, Coimbatore.

Preliminary phytochemical screening

The qualitative tests to categorize the numerous chemical ingredients were carried out in different (petroleum ether, benzene, ethyl acetate, methanol, ethanol and aqueous) solvent extracts of *H. humboldtiana* whole plant using the procedures suggested by Brinda *et al.* (1981). They were tested for alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, glycosides, carbohydrates, xanthoproteins and fixed oil.

Estimation of total phenolics

Total phenolic content was estimated using Folin-Ciocalteau reagent based assay as previously described (Mc Donald *et al*., 2001) with little modification. To 1 ml of each extract (100 µg/ml), 5ml of Folin-Ciocalteau reagent (diluted ten- fold) and 4 ml (75 g/L) of Na2CO3 were added. The mixture was allowed to stand at 20^{o}C for 30 min and the absorbance of the developed colour was recorded at 765 nm 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/100 g dry weight of extract).

Estimation of flavonoids

Total flavonoid content was determined according to Eom *et al*. (2007). An aliquot of 0.5 ml of samples were mixed with 0.1 ml of 10% aluminium chloride and 0.1 ml of potassium acetate (1M). In this mixture, 4.3 ml of 80% methanol was added to make 5 ml volume. This mixture was vortexed and the absorbance was calculated spectrophotometrically at 415 nm. The value of optical density was used to calculate the total flavonoid content there in the sample.

Antioxidant activity

Benzene, Petroleum ether, methanol, ethyl acetate and ethanol extracts of *H. humboldtiana* whole plant were used to determine the *in vitro* antioxidant activity.

DPPH radical scavenging assay

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging ability of different solvent extracts of *T. seylanicus* ssp *travancoricus* leaf was determined by the method of Shen *et al*. (2010). DPPH solution of 0.1 mM was prepared using methanol. 1 mL of this prepared solution was added to 3 mL of different concentration (50, 100, 200, 400 and 800 µg/ml) of extracts. Then it was shaken forcefully and allowed to stand. After 30 min, absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation) using Ascorbic acid as standard. Solution with lower absorbance values indicates more free radical scavenging activity. It was calculated by using the following formula.

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

where, A_1 is the absorbance in presence of all of the extract samples and reference A_0 is the absorbance of the control reaction

Hydroxyl radical scavenging assay

The Hydroxyl radical scavenging assay was measured using the modified method of Halliwell *et al*. (1987). Various stock solutions used in this method are EDTA (1 mM), $FeCl_3$ (10 mM), Ascorbic Acid (1 mM), H_2O_2 (10 mM) and Deoxyribose (10 mM). All the solutions were prepared in distilled deionized water.

Hydroxyl radical scavenging activity was carried out by adding 0.1 ml EDTA, 0.01 ml of $FeCl_3$, 0.1 ml H_2O_2, 0.36 ml of deoxyribose, 1.0 ml of the extract of different concentration, 0.33 ml of phosphate buffer (50 mM, pH 7.9), 1.0 ml of ascorbic acid in sequence. This mixture was kept at 37 °C for 1 h. After incubation period, 1.0 ml from the mixture was added to 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to produce the pink chromogen. It was measured at 532 nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging assay

Superoxide anion scavenging activity was measured by the method of Srinivasan *et al*. (2007). The superoxide anion radicals were generated in 3.0 ml of Tris-HCL buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract of different concentration (50, 100, 200, 400 and 800 μg/ml), and 0.5 ml Tris-HCl buffer (16 mM, pH 8.0). An addition of 0.5 ml PMS solution (0.12 mM) was carried to start the reaction. This mixture is 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+)

It was carried out by using slightly modified method of Huang *et al*. (2011). In this method, 7 mM ABTS solution and 2.45 mM potassium per sulphate were used to produce ABTS radical cation (ABTS+). This reaction mixture is kept in dark for 12-16 h at room temperature. Then this solution was diluted with ethanol to get an absorbance value of 0.70 + 0.02 at 734 nm. Then 3.9 ml of diluted ABTS+ solution is added with sample extract and is used for measuring absorbance at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Trolox used a standard. Resultant data were expressed as trolox equivalent antioxidant capacity (TEAC).

Reducing power

Reducing power was determined by the method of Kumar and Hemalatha (2011). In this sodium phosphate buffer (5.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 ml, 1.0%) is mixed with 1.0 ml of the five studied concentration of extract respectively. This mixture was incubated at 50 °C. After 20 min, 5 ml of 10% trichloroacetic acid was added and centrifuged at 5000 rpm (10 min at 5°C) in a refrigerator centrifuge. After centrifugation, the upper layer (5.0 ml) was diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700 nm.

Statistical analysis

All the experiments were estimated in triplicate determinations. The statistical analysis system SPSS was used to analyze the data.

RESULTS AND DISCUSSION

The qualitative phytochemical screening of methanol and ethanol extracts of *H.humboldtiana* whole plant showed the presence of alkaloids, catechins, flavonoids, phenols, quinones, saponins, steroids, tannins, terpenoids, glycosides, sugars and xanthoproteins (Table 1). The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and this plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Total phenolic and flavonoids contents

The total phenolic and flavonoid contents of the ethanol extract of *H. humboldtiana* were found to be 1.14g 100 g^{-1} and 1.24g 100 g^{-1} respectively. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as antiapoptosis, antiaging, anticarcinogenic, antiinflammatory, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. Numerous medicinal herbs were reported to have antioxidant activity and possess different phenolic compounds and flavonoids (Chanda and Dave, 2009) These compounds are gaining extensive attention in part few years because of their physiological role viz., free radical quenching, antidiabetic, anticarcinogenic and antiinflammatory effects (Manthey 2000). In the present study, a substantial level of phenols in the whole plant extract of *H. humboldtiana* is correlated with their free radical scavenging property. The results of present investigation is consistent with earlier reports and it also revealed the relationship between total phenolic contents and antioxidant capacity of extracts (Biju *et al*., 2014; Baba and Malik, 2015).

Table 1. Preliminary phytochemical screening of *H. humboldtiana*

Phytochemical constituents	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol	Aqueous
Alkaloids	–	–	+	+	+	–
Anthraquinones	–	–	–	–	–	–
Catechins	+	–	+	+	+	+
Coumarins	–	+	–	–	–	–
Flavonoids	–	+	+	+	+	+
Phenols	+	+	+	+	+	+
Quinones	–	–	+	+	+	–
Saponins	–	+	+	+	+	+
Steroids	+		+	+	+	–
Tannins	–	+	+	+	+	+
Terpenoids	–	+	+	+	+	+
Sugars	+	+	+	+	+	+
Glycosides	+	+	+	+	+	–

Xanthoproteins	+	+	+	+	+	+
Fixed oil	+	–	+	–	–	–

+ present – absent

Antioxidant activity

DPPH radical scavenging activity

DPPH test, which is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants is a direct and reliable method for determining radical scavenging action. Ascorbic acid was selected as the reference antioxidant for this experiment. DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance (Hasan *et al*., 2009) Figure 1 summarizes the antioxidant activity of different solvent extracts of *H. humboldtiana* using DPPH radical scavenging assay and expressed as percentage reduction of the initial DPPH absorption by the tested compound. The DPPH radical scavenging effect increased with the concentration of standard and extracts. Ethanol extract was effective (118.20% and IC_{50} value 31.26 µg/ml) at quenching the DPPH radicals followed by methanol (110.06% and IC_{50} value 30.16 µg/ml) extract. The percentage of inhibition and IC_{50} value of standard ascorbic acid were 118.50% and 33.52 µg/ml respectively (Table 2). In general the radical scavenging activity of extracts could be related to the nature of phenolics and their hydrogen donating ability (Anusaya *et al*., 2009).

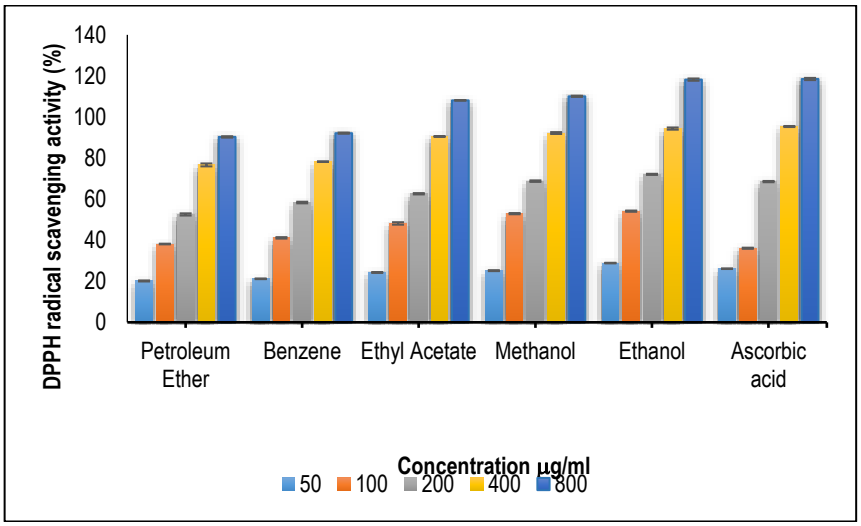

Figure 1: DPPH radical scavenging activity of *H. humboldtiana*

Hydroxyl radical scavenging activity

Hydroxyl radical are highly reactive oxygen species which target the cell membrane phospholipids and damage the cell. These hydroxyl radicals are capable of damaging bio molecules. In this method hydroxyl radical were generated by the H_2O_2 and the ferrous. These hydroxyl radicals reacted with 2-deoxyribose and formed a malonaldehyde which gave the red colour with TBA (Jayasundara *et al*., 2018). The low intensity of red colour depicted the highest hydroxyl radical scavenging capacity of the plant extract. In the present study, hydroxyl radical scavenging activity of different solvent extract of *H. humboldtiana* is shown in Fig. 2. The percentage of inhibition was highest (121.28% and IC_{50} value 30.32 µg/ml) for methanol extract followed by ethanol (112.30% and IC_{50} value 27.16 µg/ml). The percentage of inhibition and IC_{50} value of standard ascorbic acid were 106.32% and 30.26 µg/ml respectively (Table 2).

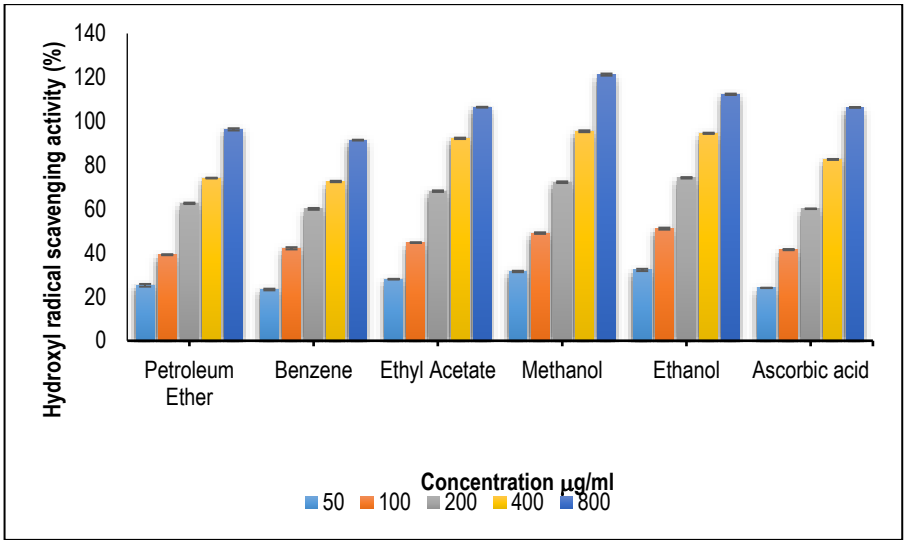

Figure 2: Hydroxyl radical scavenging activity of *H. humboldtiana*

Superoxide radical scavenging activity

Superoxide possesses few detrimental actions which inflicts upon the cells in the body and later leads to many ailments. Thus, a proposal has been entrenched to save the comparative interceptive capability of the antioxidant extracts to scavenge the superoxide radical (Vani *et al*., 1997; Sowmya and Ananthi, 2021). Results of superoxide scavenging activity of *H. humboldtiana* were shown in Fig. 3 and it clearly indicate that ethanol extract of the *H. humboldtiana* exhibited enhanced scavenging activity of 121.46% with IC_{50} value 33.24 µg/ml. The percentage of inhibition and IC_{50} value of standard ascorbic acid were 112.12% and 30.18 µg/ml respectively (Table 2). The radical scavenging activity of *H. humboldtiana* extract is possibly dependent on the number and the location of OH groups in the phenolic compounds present in the extract (Khanduja *et al*., 2006).

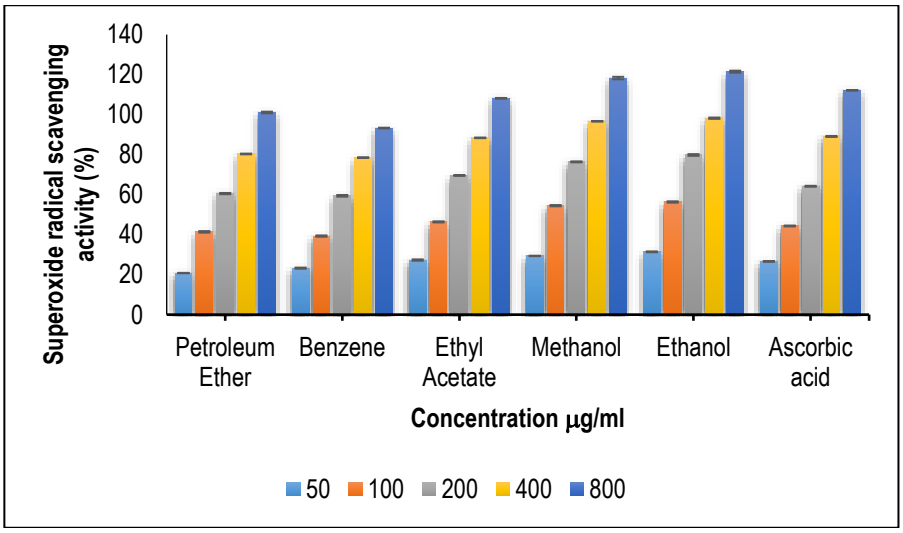

Figure 3: Superoxide anion radical scavenging activity of *H. humboldtiana*

ABTS radical cation scavenging activity

Another screening method for antioxidant activity is the ABTS radical cation decolouration assay. The ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^{+}$, which has a characteristic long wavelength absorption spectrum (Mbaebie *et al*., 2012). The ABTS chemistry involves direct generation of ABTS radical mono cation with no involvement of any intermediary radical. Figure 4 shows the results of scavenging activity for the ABTS radical assay. Ethanol extract of *H. humboldtiana* had the antioxidant activity with 116.04% of inhibition and IC_{50} value 30.14 µg/ml. It is followed by methanol, (109.62% and IC_{50} value 28.16 µg/ml) extract. The percentage of inhibition and IC_{50} value of standard trolox were 112.60% and 31.86 µg/ml respectively (Table 2). The scavenging of ABTS can be attributed to the abundance of aromatic rings, types of hydroxyl group substitution, molecular weight of phenolic compounds (Ngamlai *et al*., 2022).

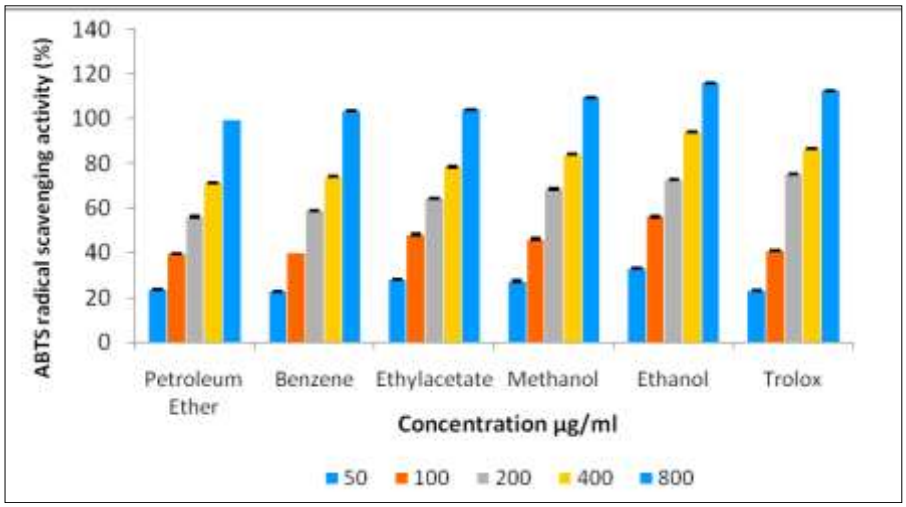

Figure 4: ABTS radical anion scavenging activity of *H. humboldtiana*

Reducing power

The reducing properties are associated with the presence of reductants, which have been shown to exert antioxidant action by breaking free radical chain through donation of hydrogen atom (Gordon, 1990). Figure 6 summarizes the reducing potential of various solvent extracts of *H. humboldtiana* and ascorbic acid. Though the increased concentration of the extracts shows higher reducing activity, the relationship was not found to be linear. This might be attributed to the uneven distribution of the bioactive constituents in the crude extract. Among the solvent tested, ethanol extract of *H. humboldtiana* exhibited strong reducing ability. The antioxidant activity might be directly correlated to the phenolic content of *H. humboldtiana* extracts. Therefore the plant extracts are electron or hydrogen atom donors and can react with free radicals to convert them to more stable products and terminate radical chain reaction. Similar observations between the total phenolic constituents and dose dependent reducing power activity have been reported for several plant extracts (Rout *et al*., 2022; Ngamlai *et al*., 2022).

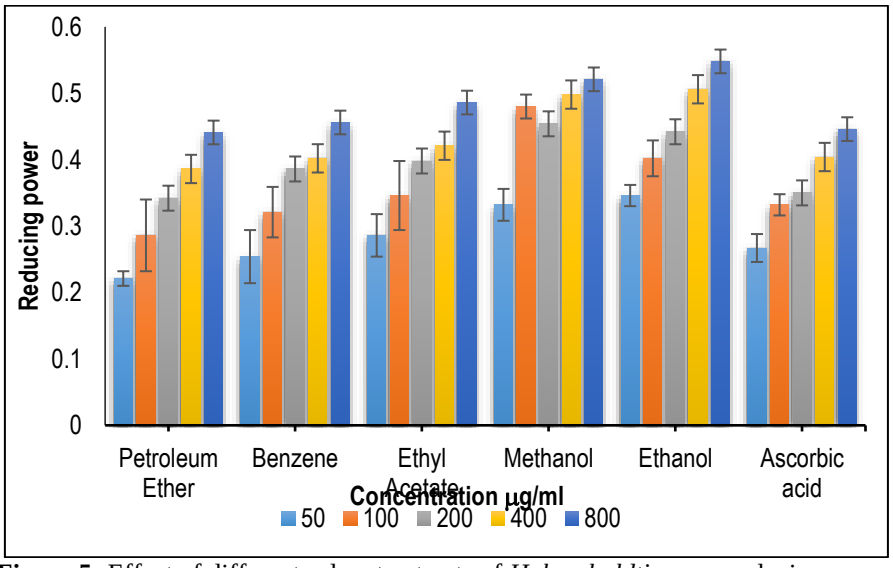

Figure 5: Effect of different solvent extracts of *H. humboldtiana* on reducing power

Table 2: IC_{50} values of different solvent extracts of *H. humboldtiana*

Solvent	IC_{50} (µg/ml)			
	DPPH	Hydroxyl radical	Superoxide	ABTS
Petroleum ether	22.42	23.14	24.38	23.16
Benzene	24.26	22.82	23.14	25.86
Ethylacetate	28.32	26.18	27.42	26.12
Methanol	30.16	30.32	28.26	28.16
Ethanol	31.26	27.16	33.24	30.14
Standard (Ascorbic acid)	33.52	30.26	30.18	-
Standard (Trolox)	-	-	--	31.86

The exact mechanism of free radical scavenging by different solvent extracts of *H. humboldtiana* whole plant is not known. However, the phytochemical analysis of *H. humboldtiana* whole plant has shown the presence of phenols and flavonoids and their concentration increased with the increase in the amount of extracts. Therefore the free radical scavenging and antioxidant behavior of *H. humboldtiana* whole plant may be due to the presence of various polyphenols and flavonoids.

CONCLUSION

The results of the present study showed that the methanol and ethanol extracts of *H. humboltiana* whole plant, which contains phenolic and flavonoids compound exhibited the great antioxidant activity. This is the first report on the antioxidant properties of this plant. The ethanol extract of *H. humboldtiana* whole plant showed strong antioxidant activity of inhibiting DPPH, superoxide and ABTS radical scavenging activity. The scavenging potential and reducing properties of the plant observed in this study indicates that it could serve as an alternative for synthetic antioxidants. However this study is not exhaustive and further analysis will be required to confirm the potential effectiveness of the plant extract.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Anusuya, N., Anusuya, S., Manian, R., Siddhuraju, P and Manian, S. 2009. Antioxidant and free radical scavenging activity of certain dietary and medicinal plant extracts. Foods. 3: 47-52.
2. Baba, S.A and Malik, A.S. 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemonti. Blume. J. Taibah Univ Sci. 9:449-454.
3. Banother, V., Neelagiri, C., Adepally, U., Lingam, J and Bommareddy, K 2017. Phytochemical screening and evaluation of in vitro antioxidant and antimicrobial activities of the indigenous medicinal plant Albizia odoratissima. Pharmaceut. Biol. 55: 1155-1161.
4. Biju, J., Sulaiman, C.T., Satheesh, G and Reddy, V.R.U.2014. Total phenolics and flavonoids in selected medicinal plants from Kerala, Int. J. Pharma. Pharm. Sci. 6: 406-408.
5. Brahma, N.P., Uddandarao, V.V., Ravindar Naik, R, Suresh, P., Meriga, B. Begum, M.S., Pandiyam, R and Saravanan, G. 2016. Ameliorative potential of gingerol: promising modulation of inflammatory factors and lipid marker enzymes expression in HFD induced obesity in rats. Mol Cell Endocrinol. 419: 139-147.
6. Brinda, P., Sasikala, B and Purushothaman, K.K. 1981. Pharmacognostic studies on Merugan kilzhangu. Medico Ethno Bot. Res. 3: 84-96.
7. Chanda, S and Dave, R 2009. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. AJMR. 3: 981-996.
8. Eom, S.H., Cheng, W.J., Hyoung, J.P., Kim, E.H., Chung, M.I., Kim, M.J., Yu, C and Cho, D.H. 2007. Far infra red ray irradiation stimulates antioxidant activity in Vitis flexuosa Thunb. Berries. Kor. J. Med. Crop. Sci. 15: 319-323.
9. Gordon, M.H. 1990. The Machanism of antioxidant action in vitro In: Food Antioxidants. Hudson, BFJ (Ed) Elsevier Applied Science, London, pp. 1-8.
10. Halliwell, B., Gutteridge, J.M.C. and Aruoma O.I. 1987. The deoxyribose method a simple best to be assay for determination of rate constants for reaction of hydroxyl radicals. Anals. Biochem. 65: 215-219.
11. Hasan, S.M.R., Hossain, Md.M., Akter, R., Jamila, M., Mazumder, Md. E.H. and Rahman, S. 2009. DPPH free radical scavenging activity of some Bangladesh medicinal plants. J. Med. Plants Res. 3: 875-879.
12. Huang, M.H., Huang, S.S., Wang, B.S., Shen, M.J. and Hon, W.C. 2011. Antioxidant and anti-inflammatory properties of Cardiospermum halicacaburm and its reference compounds ex vivo and in vivo. J. Ethnopharmacol. 133: 743-750.
13. Jayasundara, C.W., Deraniyagda, S.A., Hettiarachehi, C.M and Thiripuranathar, G. 2018. Antioxidant and antibacterial activities of leaves, stem, flesh and seeds of Sri Lankan variety of Cucurbita moschata. Int. J. Ayur Pharma Res. 6: 1-7.
14. Khanduja, K.L., Auti, P.K., Kumar, S., Metal, N., Soti, K.K. et al., 2006. Anti-apoptotic activity of caffeic acid, ellagic acid and ferulic acid in normal human peripheral blood mononuclear cells: Bcl-2 independent mechanism. Biochem. Biophy. Acta. 1760: 283-284.
15. Kumar, R.S. and Hemalatha, S. 2011. In vitro antioxidant activity of alcoholic leaf extract and subfractions of Alangium lamarckii Thwaites. J. Chem. Pharmaceut. Res. 3: 259-267.
16. Manthey, JA. 2000. Biological properties of flavonoids pertaining to inflammation. Microcircul. 7: 29-34.
17. Mbaebie, B.O., Edeoga, H.O and Afolayan, A.J., 2012. Phytochemical analysis and antioxidants activities of aqueous leaf bark extract of Schotia latifolia Jacq. Asian Pac. J. Trop. Biomed., 2: 118-124.
18. McDonald, S., Prenzler, P.D., Antolovich M and Robarde K 2001. Phenolic content and antioxidant activity of olive extracts. Food Chem. 73: 73-84.
19. Ngamlai, E.V., Lalthanpuii, P.B., Lalbiaknii, P.C., Ralte, V and Lalnunmawia, F. 2022. Antioxidant property and free radical scavenging activity of Hedyotis scandens (Roxb.). Rubiaceae. Curr. Tre. Biotech. Pharm. 16: 46-55.
20. Ntie – Kang, F., Lifongo, L.L., Mbaze, L.M., Ekwelle, N., Owono, L.L.O. Megnassan, E et al., 2013. Cameroonian medicinal plants: a bioactivity versus ethnobotanical survey and chemotaxonomic classification. BMC Comp. Alt. Med. 13: 1-3.
21. Rachman, E and Kiew R. 2003. Henckelia Sprengel. In. Plant Resources of South-East Asia. No. 12(3): Medicinal and poisonous plants 3. (Eds) Lemmens R.H.M.J and Bunyapraphatsara N. Prosea Foundation, Bogor, Indonesia.
22. Rout, S., Rath, B., Bhattamisra, S.K., Rath, I and Kumar, A. 2022. Antioxidant and anti-inflammatory activities of methanol and aqueous extracts of Sargassum wightii. J. Herbmed. Pharm. 11: 75-82.
23. Shen, Q., Zhang, B., Xu, R., Wang, Y., Ding, X and Li, P. 2010. Antioxidant activity in vitro of Selenium contained protein from the se-enriched. Bifodobacterium animalis 01. Anaerobe, 16: 380-386.
24. Sowmya, B.H. and Anandhi, D.U. 2021. Screening of in vitro free radical scavenging activities of the fruit extracts of Psidium guajava L. Indian J. Trad. Know. 20: 716-722.
25. Srinivasan, R., Chandrasekar, M.J.N., Nanjan, M.J. and Suresh. B 2007. Antioxidant activity of Caesalpinia digyna root. J. Ethnopharmacol. 113: 284-291.
26. Tanvir E.M., Hossan, M.S., Hossain, M.F et al. 2017. Antioxidant properties of popular turmeric (Curcuma longa) varieties from Bangladesh. J. Food. Qual., 2017: 1-8.
27. Vani, T., Rajani, M, Sarkar, S and Shishoo, C.J. 1997. Antioxidant properties of the ayurvedic formulation triphala and its constituents. Int. J. Pharmacogn. 35: 313-317.