

Evaluation of antioxidant activity and estimation of phenolics and flavonoids of *Euodia hortensis* leaves

Mohammad Abbas Gani*, Naznin Akhter¹, K M Khairul Alam,
M Salahuddin Bhuiya and Naima Nusrat Jahan²

Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Abstract

Antioxidants, which can be obtained from natural sources like plants, play a crucial role in combating free radicals that are directly connected to inducing oxidative stress in the body. The present study assessed the antioxidant potential, along with the phenolics and flavonoids estimation, of the methanol extract of *E. hortensis* leaves, a traditional medicinal plant. The antioxidant property was assessed through DPPH radical scavenging assay, FRAP (ferric reducing antioxidant power) assay, and TAC (total antioxidant capacity) determination. Moreover, total phenolics and flavonoids were measured spectrophotometrically. The plant extract displayed an IC₅₀ of 162.30±2.86 µg/mL, dose-response reductive ability, and 51.1±6.9 mg AAE per g of dry extract in DPPH, FRAP, and TAC assays, respectively. Total phenolics and flavonoids were noted as 149.2±12.0 mg GAE per g of dry extract and 142.0±9.1 mg QE per g of dry extract, respectively. These findings suggest that *E. hortensis* is a probable natural source of antioxidants to help fight free radicals associated with various disorders.

Keywords: Antioxidant, total phenolic content, total flavonoid content, free radical, *E. hortensis*.

INTRODUCTION

Oxidative stress is a harmful biological condition for life resulting from the overproduction of free radicals in the body through normal cellular redox processes (Pham-Huy *et al.*, 2008; Tumilaar *et al.*, 2024). Other external sources, like radiation, chemicals, ozone, heat, etc., promote oxidative destruction by giving rise to free radicals. Free radicals don't have paired electrons, and these unstable species are prone to being very reactive (Tumilaar *et al.*, 2024). Free radicals play a positive role at certain levels, whereas an excess can be dangerous in the human body, causing oxidative stress, and this stress is attributed to the development of a number of neurodegenerative and chronic disorders, including cancer. Antioxidants produced in the body or provided externally play an important role in preventing these diseases by scavenging free radicals (Pham-Huy *et al.*, 2008). Plants have been well recognized since ancient times to leave their beneficial footprints in folk medicines, and these medicinal plants might offer a natural and safe source of antioxidants to neutralize the destructive free radicals (Saeed *et al.*, 2012).

E. hortensis (Synonym: *E. longifolia*, *E. suaveolens*; Family: Rutaceae; Common name: Lacy Lady Aralia, Zodia) is a shrub or tree commonly planted as an ornamental. Leaves,

¹International Regulatory Affairs, Beximco Pharmaceuticals PLC., Dhaka-1205, Bangladesh.

²Department of Pharmacy, Independent University, Bangladesh, Dhaka-1229, Bangladesh.

* Corresponding author. Email: abg@juniv.edu

roots, and barks of *E. hortensis* are used to treat a number of disorders such as fever, toothache, stomachache, stomach ulcer, menstrual pain, pain during childbirth, cold, boils, malaria, etc. Scientific study on the pharmacological properties of *E. hortensis* is still inadequate. Traditional medicinal use of this plant needs to be validated through a modern research approach (Djamal, 2003; NParks, 1999). In this study, *E. hortensis* leaves were investigated through well-established procedures to unveil the probable antioxidant activity, along with total phenolics and flavonoids estimation.

MATERIALS AND METHODS

Chemicals: Methanol, DPPH, ascorbic acid, monosodium phosphate (dihydrate), disodium phosphate (dihydrate), concentrated sulfuric acid (98%), sodium phosphate, aluminum chloride, ammonium molybdate, folin-ciocalteu reagent, ferric chloride, sodium carbonate, gallic acid, and potassium acetate were purchased from Merck Co. (Darmstadt, Germany), whereas trichloroacetic acid (TCA), potassium ferricyanide [$K_3Fe(CN)_6$], and quercetin were purchased from Loba Chemie (Mumbai, India).

Plant Extract: The fresh plant, *E. hortensis*, was collected from Jahangirnagar University campus, and a voucher specimen in favor of botanical authentication (accession number: DACB 88264) was filed in the Bangladesh National Herbarium. The leaves of *E. hortensis* were washed adequately with normal clean water, dried in the sun, and finally ground into powder. 1200 mL of methanol was mixed with 300 g of powder in a glass jar, which was then placed in a mechanical shaker for a period of 3 days. Filter paper known as Whatman No. 1 was used to filter the mixture, and the residue was mixed with methanol (1200 mL) twice more following the same conditions. The filtrates were put together, and a rotary evaporator, maintaining reduced pressure and low temperature, was utilized to evaporate the solvent (Saeed *et al.*, 2012). A concentrated extract was obtained with a 10.7% yield.

Antioxidant Activity Study: The capability of *E. hortensis* leaves to hinder the oxidation was scrutinized through the following three different well-established methods.

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Test: This test, as narrated by Braca *et al.* (2001) with minor changes, was utilized to judge the scavenging power of test samples against free radicals. 1 mL sample of different concentrations was combined with 3 mL of methanolic DPPH solution (4 mg/100 mL). The mixed solution was kept in a dark place for 30 minutes, and then the absorbance at 517 nm was measured. The inhibition in percentage (%) was calculated, and the IC_{50} of the samples was determined. As a standard, ascorbic acid was used to compare the results.

Reducing Power Assay: The Oyaizu (1986) method, with slight modifications, was employed to check the reduction capacity of test samples from ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) (Fejes *et al.*, 2000). 2.5 mL of each potassium ferricyanide (1%), phosphate buffer (0.2 M, pH 6.6), and a sample of different concentrations were mixed adequately. An incubation at 50°C for a period of 20 minutes was completed, and later cooling of the mixture was done at normal room temperature. 2.5 mL trichloroacetic acid (10%) was

added to the solution mixture and centrifuged for 10 min at 3000 rpm. 2.5 mL of distilled water and an equal amount of supernatant from the centrifuged mixture were mixed with 1% FeCl_3 (0.5 mL) solution prepared immediately. After 10 minutes of reaction, the samples were used for absorbance measurement at 700 nm using a spectrophotometer. The absorbance of a typical blank solution containing water instead of the samples was also measured as above.

Total Antioxidant Capacity (TAC): TAC of *E. hortensis* leaves was investigated through the phosphomolybdenum method (Prieto *et al.*, 1999; Renugadevi *et al.*, 2018). A reagent solution was obtained by mixing ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulphuric acid (0.6 M). 3 mL of the prepared reagent solution was assembled with 0.3 mL of the sample solution of various concentrations kept in different test tubes. The reaction mixtures were held for incubation at 95 °C for a period of 90 minutes to complete the reaction. Upon cooling to room temperature, the absorbance of each sample was taken at 695 nm.

Total Phenolic Content: Phenolic contents of the targeted extract were measured by a slightly modified colorimetric procedure as stated by Škerget *et al.* (2005). The oxidizing agent employed in this method was Folin–Ciocalteu reagent (FCR), which was diluted in water at a ratio of 1:10. The diluted FCR (5 mL) was added to the sample solution (1 mL), and the mixture was placed for 5 minutes to complete the reaction. 4 mL sodium carbonate (7.5%) was blended with the previous mixture, and the whole solution was put at room temperature for 30 minutes. After the incubation period, a UV-visible spectrophotometer was employed for the measurement of the samples' absorbance.

Total Flavonoid Content: The method, as narrated by Hayat *et al.* (2020) with slight alterations, was applied to estimate the total flavonoid content. The sample solution (1 mL) was gently mixed with methanol, AlCl_3 (10%), and potassium acetate (1 mole), each of which was taken as 3 mL, 200 μL , and 200 μL , respectively. The final volume of the mixed solution was adjusted to 10 mL by adding distilled water. Proper mixing was ensured, and an incubation at 25 °C was completed for a period of one hour in the absence of light. A spectrophotometer employing a wavelength of 415 nm was used for absorbance measurement.

RESULTS AND DISCUSSION

Antioxidant Activity: Antioxidants act as a defense system in the human body to fight against the free radicals, which are well recognized to exert a crucial role in developing a variety of diseases, including Alzheimer's disease, cardiovascular disorder, cancer, and Parkinson's disease (Alam *et al.*, 2013). Medicinal plants, as a natural, safe, and competent source of antioxidants, have become desirable due to the unwanted and/or harmful effects of many synthetic antioxidants (Atki *et al.*, 2019; Fejes *et al.*, 2000; Saeed *et al.*, 2012). The antioxidant potential of the medicinal plant *E. hortensis* was studied through three different methods. Methanol was used as the extraction solvent, as it is well recognized for extracting antioxidant compounds more effectively (Do *et al.*, 2014; Muhamad *et al.*,

2014). Efficient extraction and greater solubility of this polar solvent make methanol suitable for extracting various compounds, such as phenolics, terpenoids, anthocyanins, lipids, proteins, and polysaccharides (Lee *et al.*, 2024).

DPPH Radical Scavenging Assay: The stable DPPH free radical can be reduced by receiving an electron or hydrogen from the antioxidant, and this reduction is characterized by a shift in the color from purple to yellow. Upon completion of the reaction, a decrease in the measured absorbance of the solution denotes antioxidant activity (Renugadevi *et al.*, 2018). Moreover, the lower the IC₅₀ value of the solution, the higher the antioxidant potential. An agent with an IC₅₀ of less than 5000 µg/mL is predicted to be an active antioxidant (Atki *et al.*, 2019). The extract of this study presented an IC₅₀ of 162.30±2.86 µg/mL, indicating the active potential of free radical scavenging ability. Though the extract showed lower antioxidant capability than the standard drug (ascorbic acid), the capacity to scavenge the free radicals was augmented with an increase in concentration (Table 1 and Figs. 1 & 2). This suggests the therapeutic potential of *E. hortensis* as a nature-derived source of potential antioxidants against oxidative stress-related disorders. A previous study conducted by Ravi *et al.* (2016) on the aqueous extract of *E. ridleyi* leaves, a medicinal plant of the same family (Rutaceae), showed much lower antioxidant activity (IC₅₀ of 5.04 ± 2.17 mg/mL) than the present extract. Another previous study conducted by Gali & Bedjou (2019) on the ethanol aerial part extract of *R. chalepensis*, a different member of the Rutaceae family, exhibited stronger radical scavenging power (IC₅₀ of 151.10 ± 0.49 µg/mL) than the *E. hortensis*. This discrepancy might be due to the presence of phytochemicals, which can act as hydrogen donors in the respective extract (Saeed *et al.*, 2012). In addition, the solvent and the extraction method used influence the scavenging strength of the medicinal plant extract. Differences in polarity and chemical properties of plant components determine their solubility in a specific solvent (Do *et al.*, 2014).

Table 1. Percentage inhibition and IC₅₀ of ascorbic acid (standard) and EH methanol extract

Ascorbic Acid (Standard)			EH Methanol Extract		
Concentration (µg/mL)	% Inhibition (Mean±SD)	IC ₅₀ (µg/mL) (Mean±SD)	Concentration (µg/mL)	% Inhibition (Mean±SD)	IC ₅₀ (µg/mL) (Mean±SD)
0.781	3.0±0.4	13.39±0.05	12.5	19.6±2.1	162.30±2.86
1.563	5.8±1.3		25	26.8±3.3	
3.125	11.7±0.3		50	33.9±1.0	
6.25	25.7±0.6		100	45.9±0.5	
12.5	50.6±0.3		200	60.0±3.1	
25	90.1±0.4		400	84.3±0.2	

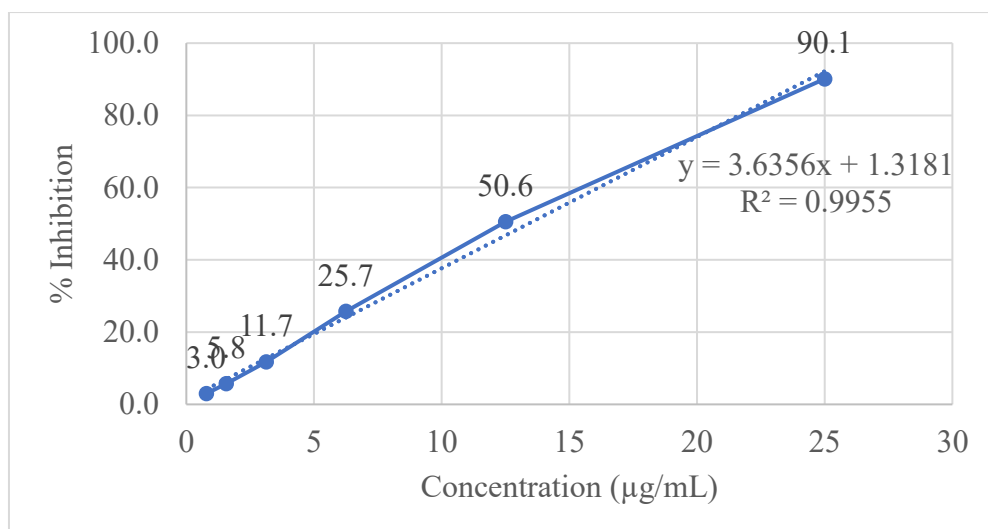


Fig. 1. Percentage inhibition of DPPH by ascorbic acid (standard). The equation, $y = 3.6356x + 1.3181$, obtained from this graph, was used to calculate the IC_{50} of the standard

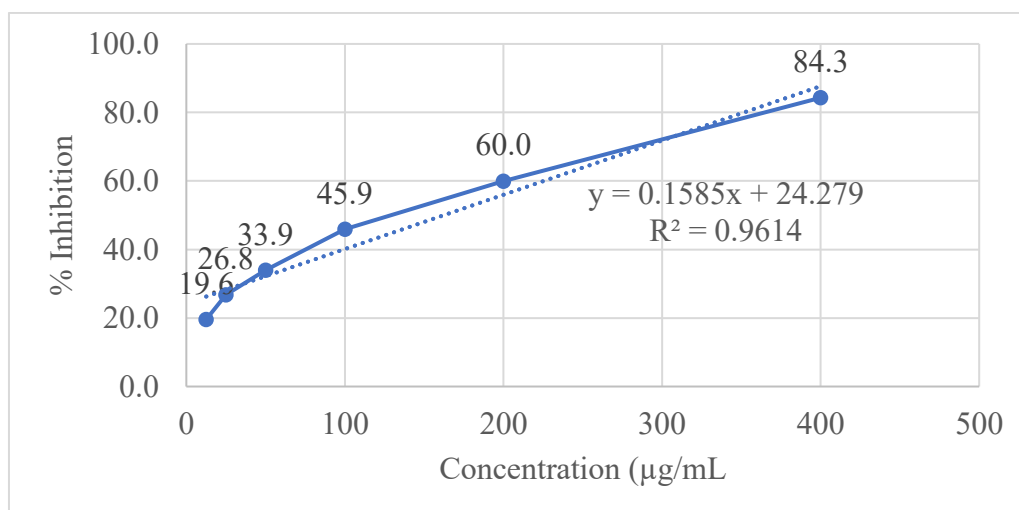


Fig. 2. DPPH inhibition by EH methanol extract. The IC_{50} of the plant extract was calculated using the equation $y = 0.1585x + 24.279$, generated from this graph

Reducing Power Assay: The FRAP (ferric reducing antioxidant power) test was used to study the reducing caliber of *E. hortensis*. In this assay, the candidate with antioxidant properties donates a hydrogen atom to destroy the chains of free radicals (Atki *et al.*, 2019). The Fe^{3+} /ferricyanide complex is transformed to the ferrous form, showing a shift in the color of the samples from yellow to green. The reducing caliber is represented by an increase in measured absorbance (Saeed *et al.*, 2012). The reducing potential of *E. hortensis* and ascorbic acid (standard) has been presented in Table 2 and Fig. 3. The result indicates the reductive ability of *E. hortensis* and serves as an indicator of antioxidant candidacy.

However, the methanol extract (root) of *R. graveolens* and the aqueous extract (leaves) of *A. ceylanica*, both of which belong to the same family (Rutaceae), exhibited more reducing power compared to *E. hortensis* (Diwan *et al.*, 2012; Fernando & Soysa, 2014). Differences in the content and the strength of reductants in respective plants might be responsible for this variation. Various factors, including plant variety, genotype, and environmental conditions, govern the presence and quality of plant antioxidants (Skrovankova & Mlcek, 2025).

Table 2. Absorbance of ascorbic acid (standard) and EH methanol extract.

Concentration ($\mu\text{g/mL}$)	Absorbance (Mean $\pm$ SD)	
	Ascorbic Acid (Standard)	EH Methanol Extract
0	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000
12.5	0.306 $\pm$ 0.006	0.030 $\pm$ 0.002
25	0.682 $\pm$ 0.015	0.043 $\pm$ 0.001
50	1.388 $\pm$ 0.078	0.071 $\pm$ 0.003
100	6.262 $\pm$ 0.066	0.130 $\pm$ 0.003
200	6.551 $\pm$ 0.140	0.239 $\pm$ 0.015

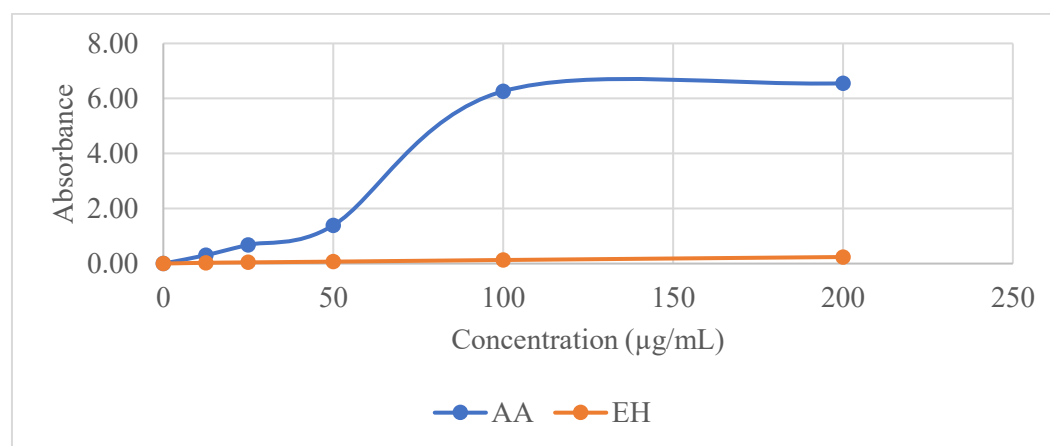


Fig. 3. Comparison of reducing power between ascorbic acid (AA) and EH methanol extract. Though AA showed a stronger response, both samples displayed a rising response with increasing dose

Total Antioxidant Capacity (TAC): The test sample exhibits antioxidant attributes in the phosphomolybdenum assay by scavenging free radicals through an electron transfer, where reduction of molybdate (VI) occurs, leading to a green molybdate (V) complex formation with phosphate at acidic pH (Hussen & Endalew, 2023). Flavonoids and related polyphenols of medicinal plants play prominent roles in reducing phosphomolybdate (Saeed *et al.*, 2012). Calibration curve (Fig. 4) of ascorbic acid was used to calculate TAC of the *E. hortensis* extract. The result obtained has been presented in Table 3, which proves the antioxidant properties of the plant, *E. hortensis*, via the phosphomolybdenum method.

However, the extract displayed a comparatively lower TAC than *R. chalepensis* (TAC of 159.03 ± 1.62 $\mu\text{g AAE/mg}$) and *A. monophylla* (TAC of 686 mg AAE/g), two medicinal plants of the same family (Rutaceae) (Gali & Bedjou, 2019; Das & Swamy, 2016). This variation might be due to plant variety, genotype, and environmental conditions, which could affect the presence and strength of flavonoids and related polyphenols (Skrovankova & Mlcek, 2025).

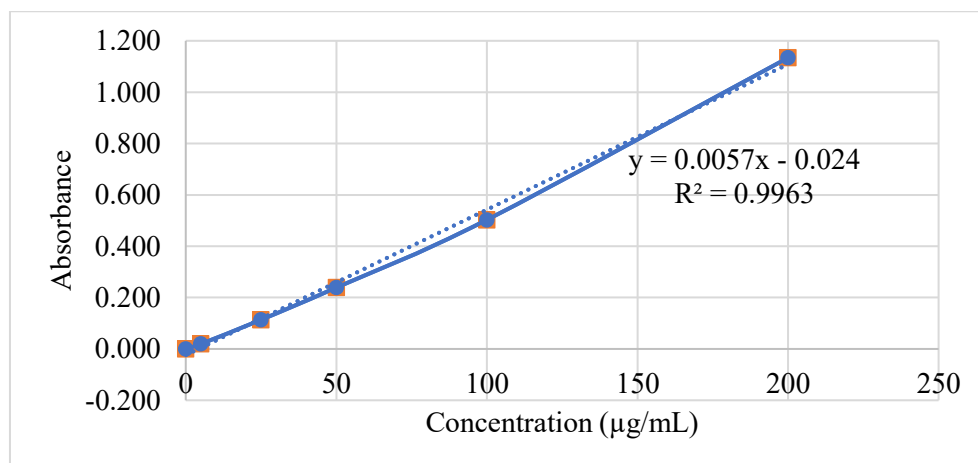


Fig. 4. Calibration curve of ascorbic acid using phosphomolybdenum assay. The TAC of the extract was determined using the equation, $y = 0.0057x - 0.024$, obtained from this curve

Table 3. Calculated TAC of EH leaves methanol extract

Concentration ($\mu\text{g/mL}$)	Total Antioxidant Activity (mg ascorbic acid equivalent/g of dry extract)	
	Individual Value	Mean $\pm$ SD
50	57.8	51.1 $\pm$ 6.9
100	51.6	
200	44.0	

Total Phenolic Content (TPC): Phenolics, secondary metabolites of plants with diverse biological activities, contain hydroxyl groups in their structure, which are able to donate electrons, leading to the inhibition of new radical formation. Phenolic compounds can donate hydrogen, quench singlet oxygen, reduce other substances, and chelate metals, all of which exhibit their antioxidant activity (Atki *et al.*, 2019; Mehmood *et al.*, 2022). Calibration curve (Fig. 5) of gallic acid was utilized to estimate TPC of *E. hortensis*, and the calculated TPC has been presented in Table 4. The results of this study indicate that phenolics are among the substances in *E. hortensis* contributing to the antioxidant capacity shown in the above DPPH scavenging assay, TAC assay, and FRAP assay. Previous studies (Ravi *et al.*, 2016; Gali & Bedjou, 2019) on *E. ridleyi* and *R. chalepensis* (both of which belong to the Rutaceae family) reported TPC of 44.56 ± 0.35 mg GAE/g and 61.61 ± 0.70 $\mu\text{g GAE/mg}$, respectively, both of which are lower than that of *E. hortensis* found

(149.2±12.0 mg GAE/g) in this study. The observed variation may be due to plant variety, genotype, climate, plant nutrition, solvent polarity, and extraction conditions (Ayele *et al.*, 2022; Skrovankova & Mlcek, 2025).

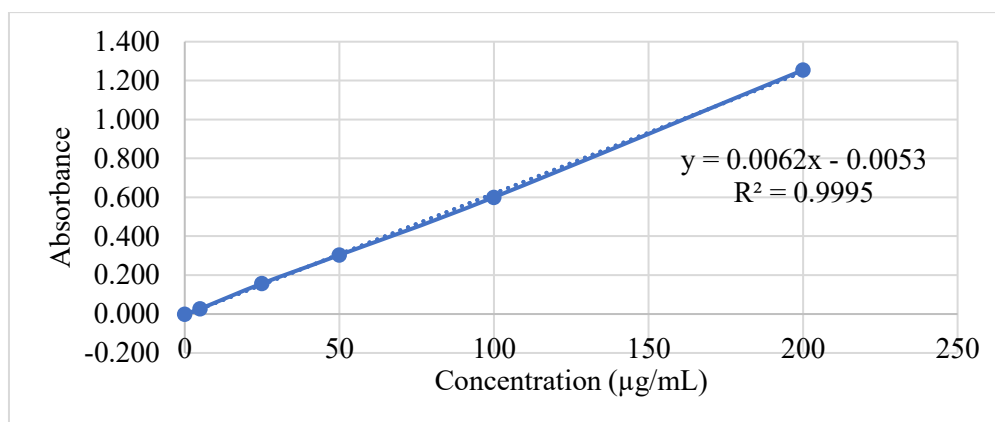


Fig. 5. Gallic acid calibration curve for phenolic content determination. The TPC of the extract was determined using the equation, $y = 0.0062x - 0.0053$, generated from this graph

Table 4. Calculated TPC of EH methanol extract

Concentration (µg/mL)	Total Phenolic Content (mg gallic acid equivalent/g of dry extract)	
	Individual Value	Mean±SD
50	156.7	149.2±12.0
100	155.6	
200	135.3	

Total Flavonoid Content (TFC): Flavonoids, a major class of phenolic substances, are natural plant constituents that are well known for their potent radical scavenging capacity. In addition, the anticancer, anti-inflammatory, antiviral, antibacterial, and anti-allergic activities of flavonoid derivatives have been well reported (Ayele *et al.*, 2022; Saeed *et al.*, 2012). The flavonoids can inhibit oxidation due to the presence of free hydroxyl groups that react effectively with most oxidizing agents (Mehmood *et al.*, 2022; Saeed *et al.*, 2012). The flavonoid contents of *E. hortensis* extract presented a similar trend to that of the phenolic contents. The total flavonoid contents shown in Table 5 were calculated using the calibration curve of quercetin (Fig. 6). The study results imply that flavonoids are among the chief contributors to the scavenging activity demonstrated in the above assays. In this study, a higher TFC (142.0±9.1 mg QE/g) was observed than in *E. ridleyi* and *R. chalepensis* (two medicinal plants of the Rutaceae family), for which TFC was recorded as 0.14±0.03 mg QE/g and 25.68 ± 1.19 µg QE/mg, respectively (Ravi *et al.*, 2016; Gali & Bedjou, 2019). Factors such as plant genotype, variety, climate, plant nutrition, and the extraction efficiency of the respective solvent may contribute to these differences (Ayele *et al.*, 2022; Skrovankova & Mlcek, 2025).

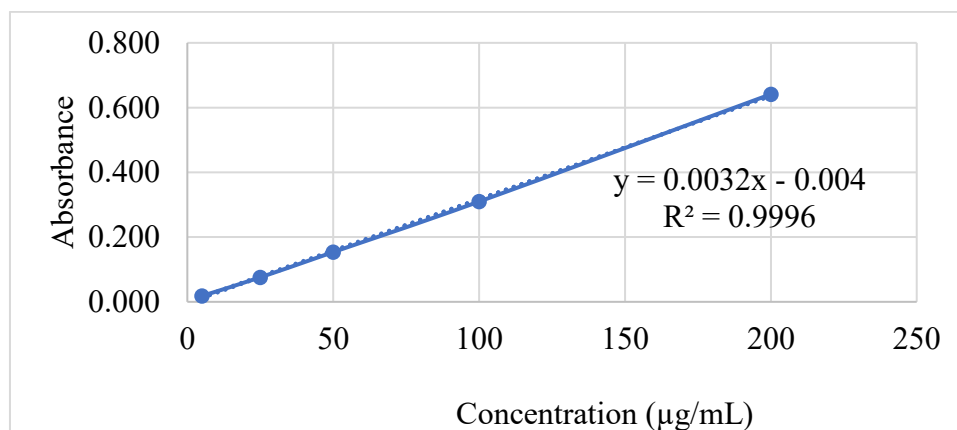


Fig. 6. Quercetin calibration curve for flavonoid content determination. This curve yielded the equation, $y = 0.0032x - 0.004$, which was used to determine the TFC of the extract

Table 5. TFC of EH Methanol Extract

Concentration (µg/mL)	Total Flavonoid Content (mg quercetin equivalent/g of dry extract)	
	Individual Value	Mean±SD
50	152.1	142.0±9.1
100	134.4	
200	139.6	

Conclusion: The present study on the traditional medicinal plant, *E. hortensis*, was conducted to check out the antioxidant capacity, along with the phenolic and flavonoid substances estimation. The IC_{50} value gathered from the DPPH scavenging assay qualifies *E. hortensis* as a promising natural source of active antioxidants. The FRAP and TAC assays further confirmed the radical scavenging power of *E. hortensis* extract. A considerable amount of TPC and TFC implies that phenolics and flavonoids are among the major constituents of *E. hortensis*, contributing to the scavenging activity. The overall study summarizes that *E. hortensis* might be capitalized as a natural source of active antioxidants to fight against oxidative stress-related disorders. Further in vivo studies are appreciated to strengthen the present findings.

Acknowledgement: The project was funded by Jahangirnagar University, Dhaka 1342, Bangladesh.

Competing Interest: The authors declare no competing interests.

REFERENCES

Alam, M. N., Bristi, N. J. and Rafiquzzaman, M. 2013. Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharm. J.*, **21**(2): 143-152.

- Atki, Y. E., Aouam, I., Taroq, A., Lyoussi, B., Taleb, M. and Abdellaoui, A. 2019. Total phenolic and flavonoid contents and antioxidant activities of extracts from *Teucrium polium* growing wild in Morocco. *Mater. Today Proc.*, **13**: 777-783.
- Ayele, D. T., Akele, M. L. and Melese, A. T. 2022. Analysis of total phenolic contents, flavonoids, antioxidant and antibacterial activities of *Croton macrostachyus* root extracts. *BMC Chem.*, **16**(1): 30.
- Braca, A., De Tommasi, N., Di Bari, L., Pizza, C., Politi, M. and Morelli, I. 2001. Antioxidant principles from *Bauhinia tarapotensis*. *J. Nat. Prod.*, **64**(7): 892-895.
- Das, A. K. and Swamy, S. 2016. Antioxidant activity and determination of bioactive compounds by GC-MS in fruit methanol extracts-a comparative analysis of three *Atalantia* species from south India. *J. Appl. Pharm. Sci.*, **6**(2), 130-134.
- Diwan, R., Shinde, A. and Malpathak, N. 2012. Phytochemical composition and antioxidant potential of *Ruta graveolens* L. in vitro culture lines. *J. Bot.*, **1**: 685427.
- Djamal, J. 2003. *Euodia hortensis* J.R. forster, J.G. Forster. In: Lemmens, R.H.M.J. & Bunyapraphatsara, N. (eds). Plant Resources of South-East Asia. 12(3). **Medicinal and Poisonous Plants 3**, pp. 196-197. Backhuys Publishers, Leiden.
- Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S. and Ju, Y. H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J. Food Drug Anal.*, **22**(3): 296-302.
- Fejes, S., Blázovics, A., Lugasi, A., Lemberkovics, É., Petri, G. and Kéry, Á. 2000. In vitro antioxidant activity of *Anthriscus cerefolium* L. (Hoffm.) extracts. *J. Ethnopharmacol.*, **69**(3): 259-265.
- Fernando, C. D. and Soysa, P. 2014. Total phenolic, flavonoid contents, in-vitro antioxidant activities and hepatoprotective effect of aqueous leaf extract of *Atalantia ceylanica*. *BMC Complement. Altern. Med.*, **14**(1): 395.
- Gali, L. and Bedjou, F. 2019. Antioxidant and anticholinesterase effects of the ethanol extract, ethanol extract fractions and total alkaloids from the cultivated *Ruta chalepensis*. *S. Afr. J. Bot.*, **120**: 163-169.
- Hayat, J., Akodad, M., Moumen, A., Baghour, M., Skalli, A., Ezrari, S. and Belmalha, S. 2020. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from 2 different localities of Northeast of Morocco. *Heliyon*, **6**(11).
- Hussen, E. M. and Endalew, S. A. 2023. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC Complement. Med. Ther.*, **23**(1): 146.
- Lee, J. E., Jayakody, J. T. M., Kim, J. I., Jeong, J. W., Choi, K. M., Kim, T. S. and Ryu, B. 2024. The influence of solvent choice on the extraction of bioactive compounds from Asteraceae: A comparative review. *Foods*, **13**(19): 3151.
- Mehmood, A., Javid, S., Khan, M. F., Ahmad, K. S. and Mustafa, A. 2022. In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. *BMC Chem.*, **16**(1): 64.
- Muhamad, N., Muhmed, S. A., Yusoff, M. M. and Gimnun, J. 2014. Influence of solvent polarity and conditions on extraction of antioxidant, flavonoids and phenolic content from *Averrhoa bilimbi*. *J. Food Sci. Eng.*, **4**: 255-260.
- NParks Flora & Fauna Web. 1999. *Euodia suaveolens* Scheff., A Singapore Government Agency Website, Information Retrieved on August 10, 2024. <https://www.nparks.gov.sg/florafaunaweb/flora/1/9/1999>.
- Oyaizu, M. 1986. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr. Diet.*, **44**(6): 307-315.
- Pham-Huy, L.A., He, H. and Pham-Huy, C. 2008. Free radicals, antioxidants in disease and health. *IJBS*, **4**(2): 89-96.

- Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.*, **269**(2): 337-341.
- Ravi, N. H., Maskam, N. A., Hassan, H. H. M. and Nor, M. M. 2016. Phytochemicals Screening and Antioxidant Activities of Aqueous Leaves Extract of *Euodia ridleyi* and *Colubrina asiatica*. *Int. J. Sci. Res.*, **5**(4): 2004-2007.
- Renugadevi, K., Nachiyar, C. V., Sowmiya, P. and Sunkar, S. 2018. Antioxidant activity of phycocyanin pigment extracted from marine filamentous cyanobacteria *Geitlerinema* sp TRV57. *Biocatal. Agric. Biotechnol.*, **16**: 237-242.
- Saeed, N., Khan, M. R. and Shabbir, M. 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement. Altern. Med.* **12**: 1-12.
- Škerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., Simonič, M. and Knez, Ž. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.*, **89**(2): 191-198.
- Skrovankova, S. and Mlcek, J. 2025. Antioxidant potential and its changes caused by various factors in lesser-known medicinal and aromatic plants. *Horticulturae*, **11**(1): 104.
- Tumilaar, S. G., Hardianto, A., Dohi, H. and Kurnia, D. 2024. A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds. *J. Chem.*, **1**: 5594386.