

Phytochemical Screening and Anti-oxidant Effects of Ethanolic Extracts of Leaves and Flowers of *Euphorbia hirta* L.

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ABSTRACT: *The study was designed to evaluate total flavonoid and phenolic content, and also to observe anti-oxidant activities of ethanolic extracts of leaves and flowers of Euphorbia hirta L. Preliminary 16 qualitative screening tests for 15 different phytochemicals were performed with the extracts. The total flavonoid content of the leaf extract was 297.27 and 98.49 quercetin equivalents (QE mg/g of extract) in the flower extract. The total phenolic content of the leaf extract was 191.816 gallic acid equivalents (GAE mg/g of extract), which was much greater than that of the flower extract. The total vitamin C content in the leaf and flower extracts were 274.49 and 263.74 ascorbic acid equivalents (AAE mg/g of extract), respectively. The IC50 values for DPPH and hydrogen peroxide anti-oxidant activity of the leaf extract were 43.13 and 165.11 μg/mL, respectively. The flower extract had anti-oxidant activity against DPPH and hydrogen peroxide, with IC50 values of 35.62 and 122.05 μg/mL, respectively. The total reducing power contents of leaf and flower extracts were 151.37 and 633.84 AAE mg/g of extract.*

Keywords: *Phytochemical screening, Anti-oxidant effect, Ethanolic extract, Leaves and Flowers, Euphorbia hirta L.*

1. INTRODUCTION

Euphorbia hirta L. (*E. hirta*) is a plant species native to all tropical nations, including Cameroon. *E. hirta* is a member of the Euphorbiaceae spurge family. Although it is sometimes observed lying down, it is normally standing and slender-stemmed, spreading up to 80 cm tall [1].

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In the highlands of Bangladesh, *E. hirta* is known as ‘Dudiyaful’. It has traditionally been used to treat respiratory and gastrointestinal diseases. *E. hirta* possesses antibacterial, anti-inflammatory, galactogenic, antidiarrheal, anti-oxidant, hypoglycaemic, anti-asthmatic, anti-amoebic, antifungal, and antimalarial properties [2]. It is also consumed in the Philippines (where it is known as tawa-tawa) as a folk medicine for fevers, notably dengue fever and malaria [3]. It is also being studied in the Philippines for its potential against coronavirus (CoV) disease 2019 (COVID-19) [4]. The focus of this study was to identify the phytochemicals and quantify the flavonoid and phenolic content, and anti-oxidant profile of ethanolic extracts of *E. hirta* leaves and flowers.

2. METHODS AND MATERIALS

2.1. Plant material and preparation of the extract

In August 2022, the *E. hirta* plant was collected from the UITS campus as well as from a 100-foot roadside in Dhaka, Bangladesh. After gathering the plant, the flowers were separated from the leaves and dried for a week at room temperature. The flower and leaves were then crushed into coarse powder using a suitable grinder. The powder was properly stored in an airtight container and kept in a dry area for subsequent study processing. The ethanol was used to extract the dry powder. In different beakers, dried leaf and flower powder were placed and shaken constantly for 15 days at room temperature. After 15 days of shaking, the mixture was filtered using a clean, white, pure cotton plug. The filtrate was then passed via Whatman filter paper and was evaporated using a rotary evaporator. Table 01 shows the amount of extract obtained from the powder.

Table 01: Extract obtained from the powder.

Plant parts	Powder weight	Solvent volume	Extract Amount
Leaves	236 gm	750 m	49.5 gm
Flower	105 gm	350 m	53.4 gm

2.2. Qualitative Phytochemical Screening

The initial qualitative screening included the identification of 15 different phytochemicals [5-6].

2.2.1. Molisch's test (For carbohydrate)

1 mL of plant extract was mixed with 1 mL of Molisch reagent and 0.5 mL of conc. H_2SO_4 was added along the side of the test tube. The formation of a reddish violet or purple ring at the point of contact of two liquids confirmed the test.

2.2.2. Venitic test (For reducing sugar)

In a test tube, 1 mL of plant extract was mixed with Benedict solution and distilled water, and the greenish-to-red color indicated that the test was positive.

2.2.3. Ferric chloride test (For tannin)

2 mL of 5% $FeCl_3$ solution was mixed with 2 mL of plant extract. The presence of tannins was suggested by the presence of dark blue or greenish-black coloration.

2.2.4. Lead acetate test (For flavonoid)

The presence of flavonoids was confirmed by the production of yellow precipitate when a few drops of 10% lead acetate were added to 1 mL of the plant extract.

2.2.5. Foam test (For saponin)

For this test, 1 mL of plant extract and 1 mL of water were shaken in a test tube for five minutes. The presence of stable foam proved that the test was positive.

2.2.6. Anthraquinone test

1 mL plant extract was heated with 6 mL of 1% HCl before being filtered and shaken with 5 mL of benzene. 2 mL of 10% ammonia solution was added. The existence of anthraquinones was verified by the presence of pink and violet colors.

2.2.7. Salkowski's Test (For glycoside)

2 mL of chloroform was added to 1 mL of plant extract. Conc. H_2SO_4 was mixed. The presence of glycoside was confirmed by the formation of a reddish-brown color.

2.2.8. Ferric chloride test (For phenol)

1 drop of 10% $FeCl_3$ solution was added to 1 mL plant extract. The presence of phenol can be detected by the presence of a bluish-black color.

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2.2.9. Sulphuric acid test (For terpenoid)

3 mL of chloroform was added to 1 mL of plant extract. This was then evaporated before adding 2 mL of conc. H_2SO_4 and heating for around 3 minutes. Terpenoids were detected by a greyish color.

2.2.10. Sulphuric acid test (For steroid)

2 mL of chloroform was added to the plant extract. 2 mL of conc. H_2SO_4 was introduced by the test tube sides and the bottom chloroform layer was inspected for red color.

2.2.11. Phlobatannin test

1 mL of plant extract was mixed with 2-3 drops of dilute HCl before boiling. The presence of phlobatannins was verified by the red color.

2.2.12. Hydroxyanthraquinone test (For anthraquinone glycoside)

A few drops of 10% KOH solution were added to 1 mL of the plant extract. The appearance of a crimson color verified the test.

2.2.13. Cardiac glycoside test

2 mL acetic acid and 1 drop of $FeCl_3$ was added to 1 mL plant extract. The existence of cardiac glycoside was established by the presence of a greenish or violet color.

2.2.14. Dilute ammonia test (For flavonoid)

1 mL plant extract mixed with 3 mL 10% ammonia solution, then a few drops of H_2SO_4 were added. The presence was verified by the yellow precipitate.

2.2.15. Volatile oil test

2 mL of plant extract was mixed with 0.1 mL NaOH and agitated before adding a few drops of dilute HCl. The existence of volatile oil was verified by the appearance of a white precipitate.

2.2.16. Mayer's test (For alkaloid)

One or two drops of Mayer's reagent were added to 1 mL of plant extract. A white or creamy precipitate validated the test as positive.

2.3. Quantitative Phytochemical Analysis

2.3.1. Determination of Total Flavonoid Content

In a test tube, standard quercetin or 0.3 mL extract of different concentrations was well mixed with 0.9 mL of ethanol, 60 μ L of 10% aluminium chloride ($AlCl_3$) solution, 60 μ L of 1M potassium acetate (CH_3COOK) solution, and 1.68 mL of distilled water. The mixture was then incubated for 30 minutes at room temperature. The absorbance of the solution was then measured at 415 nm in comparison to the blank. The total flavonoid content was estimated as QE mg/g of extract using the calibration curve [7].

2.3.2. Determination of Total Phenol Content

To make the reaction mixture, 1.5 mL of 10% Folin-Ciocalteu's reagent was mixed with different concentrations of 0.3 mL of ethanolic extract. After 5 minutes, 1.2 mL 7.5% $NaHCO_3$ was added, and the mixture was incubated for 90 minutes. A blank containing only ethanol was utilized. The absorbance was calculated with a spectrophotometer at 765 nm, and the mean value of absorbance was obtained. The technique was repeated for the gallic acid standard solution and a calibration curve was used to measure the total phenolic content as GAE mg/g of extract [8].

2.3.3. Determination of Total Vitamin C Content

After 45 minutes, the extract was combined with 10 mL of 1% metaphosphoric acid and filtered. 5 mL of 2,6-dichlorophenol-indophenol (0.01% in distilled water) solution was added to 0.5 mL of filtrate. Within 30 minutes, the absorbance of the solution was measured at 513 nm against blank ethanol. The ascorbic acid calibration curve was used to quantify the total vitamin C content [9].

2.3.4. Anti-oxidant Assays

2.3.4.1. DPPH radical scavenging assay

The hydrogen donating and radical scavenging properties of ethanol extracts of *E. hirta* fruit and leaves and standard ascorbic acid were evaluated using the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) [10]. In this case, a 0.002% solution of DPPH in ethanol was prepared and kept from light by putting it in a dark room and folding it with aluminium foil. In each test tube, 0.5 mL of different concentrations of the extract and standard (ascorbic acid) were collected, 3 mL 0.002% DPPH solution was added, and the test tubes were left in the dark for 30 minutes.

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After 30 minutes, the absorbance was measured at 517 nm. A control sample containing ethanol was obtained, and the absorbance at 517 nm was measured. The percentage inhibition was calculated according to the following equation:

$$\% \text{ Inhibition} = (1 - A1/A0) \times 100$$

Where, A1 = Absorbance of the extract or standard, A0 = Absorbance of the control.

2.3.4.2. Hydrogen peroxide (H₂O₂) scavenging assay

The Ruch technique was used to test the plant extract's ability to scavenge hydrogen peroxide (H₂O₂) [11]. 3.4 mL of extract and standard (ascorbic acid) concentrations were combined with 0.6 mL of 40 mM H₂O₂ solution produced in phosphate buffer (0.1 M; pH 7.4) and incubated for 15 minutes. The solution's absorbance was measured at 230 nm. A control sample containing all reagents except the extract/standard was obtained and the absorbance at 230 nm was determined. The percentage inhibition was calculated according to the following equation:

$$\% \text{ Inhibition} = (1 - A1/A0) \times 100$$

Where, A1 = Absorbance of the extract or standard, A0 = Absorbance of the control

2.3.4.3. Reducing power assay

The reducing power of extracts and standards was determined using Oyaizu's approach [12]. Phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1%) were combined with different concentrations of extract (31.25 - 500 µg/mL) in water. For 20 minutes, the mixture was incubated at 50 °C. The mixture was then centrifuged for 10 minutes after a quantity (2.5 mL) of trichloroacetic acid (10%) was added. The absorbance at 700 nm was measured after the top layer of solution (2.5 mL) was combined with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%). Enhanced reaction mixture absorbance indicated enhanced reducing power.

3. RESULTS AND DISCUSSIONS

3.1. Qualitative Phytochemical Screening

The result of the Qualitative Phytochemical Screening is given in Table 02.

Table 02: Results of qualitative phytochemical screening.

Phytochemicals	Result of Leaf Extract	Result of Flower Extract
1. Carbohydrate	++	++
2. Reducing Sugar	+++	++
3. Tannin	+++	+++
4. Flavonoid (Lead Acetate test)	++	++
5. Saponin	-	-
6. Anthraquinone	+	+
7. Glycoside	+	+
8. Phenol	+++	+++
9. Terpenoid	+	-
10. Steroid		+
11. Phlobatannin	++	+++
12. Anthraquinone Glycoside	++	++
13. Cardiac Glycoside	+++	+++
14. Flavonoid (Dilute Ammonia test)	++	++
15. Volatile Oil	-	+
16. Alkaloid	+++	++

(+++ : Very Strong Positive; ++ : Strong Positive; + : Positive; - : Negative)

From the qualitative phytochemical screening, we can see that there was no presence of saponin in both extracts. There was also no presence of steroid or volatile oil in leaf extract but flower extract had given positive results for these compounds; whereas flower extract did not have any terpenoid but leaf extract showed positive result for this compound.

Tests for anthraquinone and glycoside showed positive results for both extracts; tests for carbohydrate, flavonoid (both tests), anthraquinone glycoside, and alkaloid showed strong positive results for both extracts; tests for tannin, phenol, and cardiac glycoside showed very strong positive results for both extracts.

From these results, we decided to conduct various quantitative analyses to determine total flavonoid content, total phenolic content, and total vitamin C content; perform DPPH radical scavenging assay, H₂O₂ scavenging assay, and reducing power assay.

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3.2. Quantitative Analysis

3.2.1. Determination of Total Flavonoid Content

The absorbance of quercetin at 415 nm and the standard curve modeled from the data is shown in Figure 01.

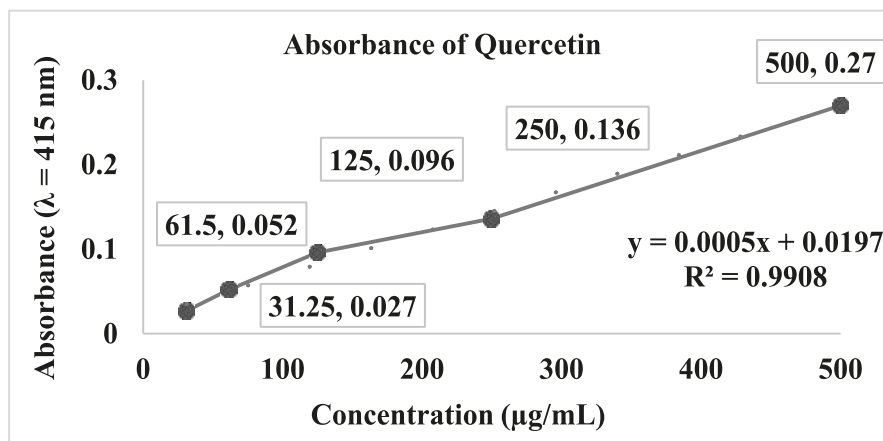


Figure 01: Standard curve of quercetin at 415 nm.

Total flavonoid contents in the ethanolic extracts measured from the standard curve are given in Table 03.

Table 03: Total Flavonoid Content in ethanolic extracts.

Extract	Total Flavonoid Content (mg/g of QE)
Leaf Extract	297.27 ± 41.962
Flower Extract	98.49 ± 54.655

An illustration of the total flavonoid contents in the ethanolic extracts is given in Figure 02.

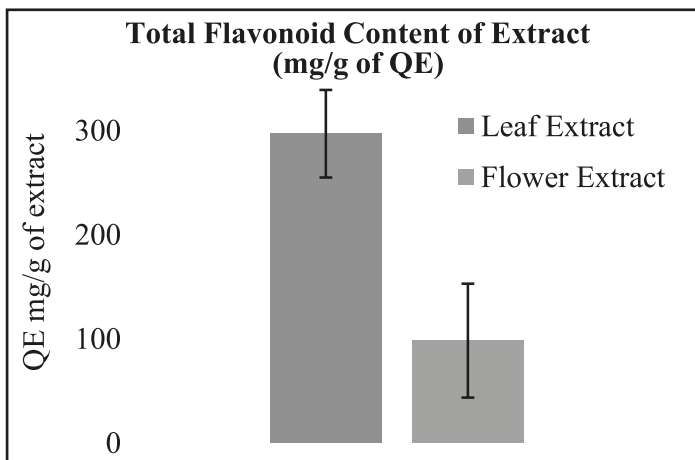


Figure 02: Total flavonoid content in extracts.

From the results we can see that, leaf extract has a total flavonoid content of 297.27 ± 41.962 mg/g of QE, while flower extract has a much lower total flavonoid content of 98.49 ± 54.655 mg/g of QE.

3.2.2. Determination of Total Phenolic Content

The absorbance of gallic acid at 765 nm and the standard curve modeled from the data is shown in Figure 03.

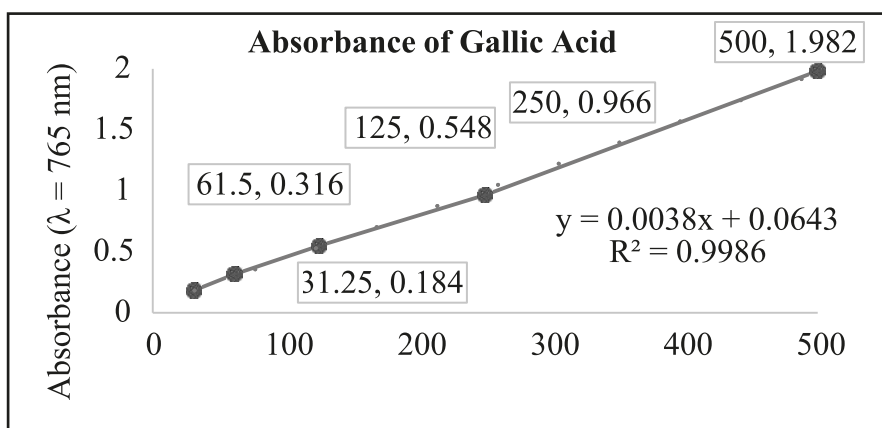


Figure 03: Standard curve of gallic acid at 765 nm.

Total phenolic contents in the ethanolic extracts measured from the standard curve are given in Table 06.

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Table 04: Total Phenolic Content in ethanolic extracts.

Extract	Total Phenolic Content (mg/g of GAE)
Leaf Extract	191.816 ± 154.76
Flower Extract	88.539 ± 81.84

An illustration of total phenolic contents in the ethanolic extracts is given in Figure 04.

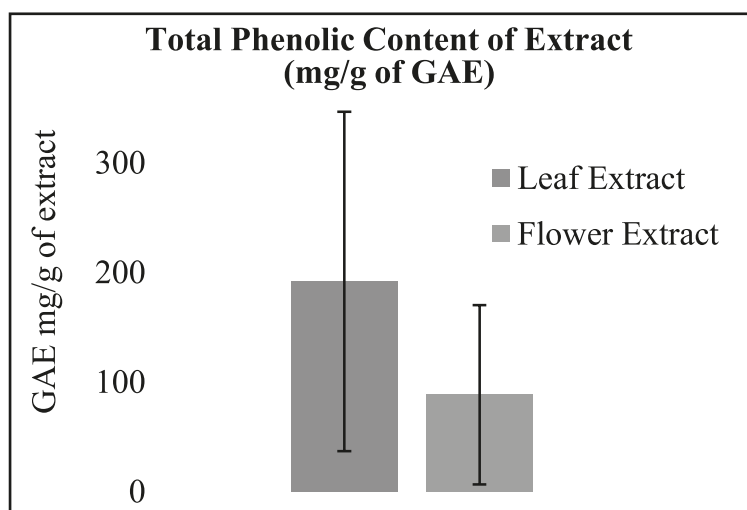


Figure 04: Total phenolic content in extracts.

From the results, we can see that, leaf extract had a total phenolic content of 191.816 ± 154.76 mg/g of GAE, and flower extract had a total phenolic content of 88.539 ± 81.84 mg/g of GAE.

The mean value of the leaf extracts' total phenolic content was more than twice the flower extracts' value of that. However, they both had very high variability of the total phenolic contents.

3.2.3. Determination of Total Vitamin C Content

The absorbance of ascorbic acid at 513 nm and the standard curve modeled from the data is shown in Figure 05.

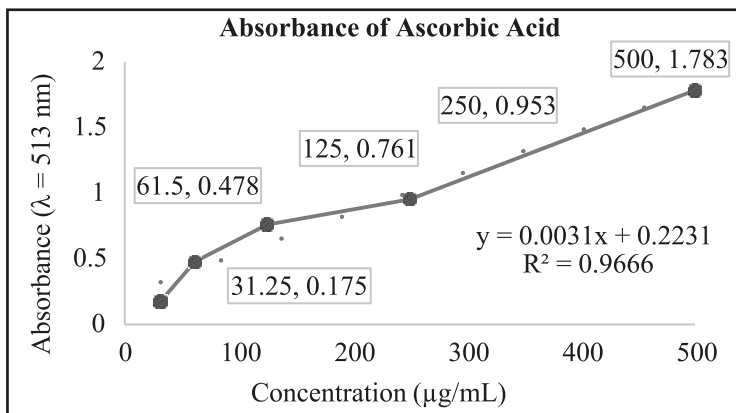


Figure 05: Standard curve of ascorbic acid at 513 nm.

Total vitamin C contents in the ethanolic extracts measured from the standard curve are given in Table 05.

Table 05: Total vitamin C content in ethanolic extracts.

Extract	Total Vitamin C Content (mg/g of AAE)
Leaf Extract	274.49 ± 141. 6
Flower Extract	263.74 ± 155 16

An illustration of the total vitamin C content in the ethanolic extracts is given in Figure 06.

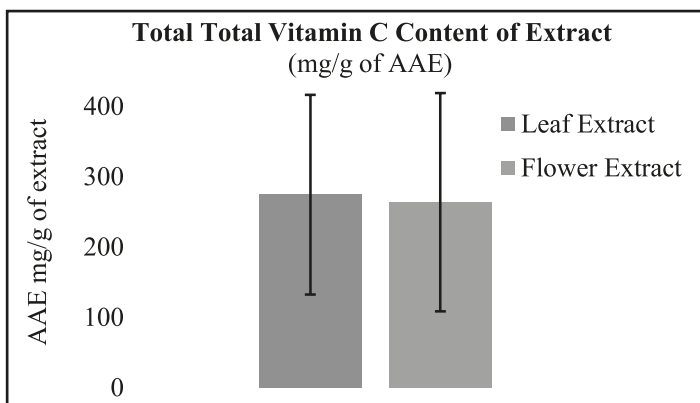


Figure 06: Total vitamin C contents in extracts.

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From the results we can see that, leaf extract had a total vitamin C content of 274.49 ± 141.86 mg/g of AAE, and flower extract also had quite the same total vitamin C content of 263.74 ± 155.16 mg/g of AAE.

3.2.4. Anti-oxidant Assays

3.2.4.1. DPPH Radical Scavenging Assay

The absorbance of standard and extracts at 517 nm is given in Table 06.

Table 06: Absorbance of standard (ascorbic acid) and extracts at 517 nm

Concentration ($\mu\text{g/mL}$)	Absorbance of Standard	Absorbance of Extracts ($\lambda = 517 \text{ nm}$)	
		Leaf Extract	Flower Extract
500	0.041	0.108	0.06
250	0.136	0.132	0.09
125	0.249	0.161	0.157
62.5	0.355	0.366	0.325
31.25	0.487	0.477	0.446

(Absorbance of control = 0.796)

A comparison of % inhibition by standard and extracts is shown in Figure 07.

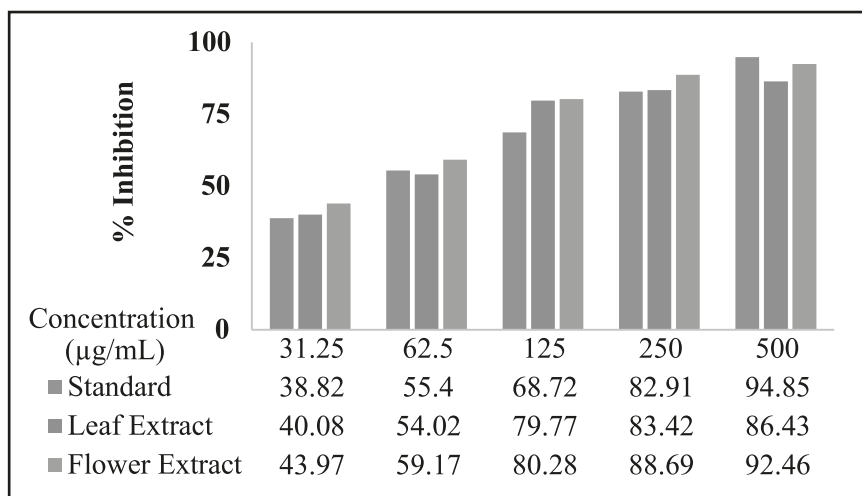


Figure 07: Comparison of DPPH radical scavenging assay

The regression line equations modeled from the absorbance of standard and extracts are given in Table 07.

Table 07: Regression line equation, Regression coefficient (R^2), and IC_{50} of standard (ascorbic acid) and extracts at 517 nm against control.

Regression line equation	Standard	Leaf Extract	Flower Extract
$y = \% \text{ Inhibition} \times \text{Conc. } (\mu\text{g/m})$	$y = 20.136 \ln(x) - 29.083$	$y = 17.617 \ln(x) - 16.316$	$y = 18.251 \ln(x) - 15.208$
R^2	0.997	0.8831	0.9348
IC_{50} ($\mu\text{g/mL}$)	50.78	43.13	35.62

From the results, we can see that the standard and extracts had an increase in % inhibition as the concentration increased. Both extracts had lower IC_{50} values (43.13 and 35.62 $\mu\text{g/mL}$ for leaf and flower extract respectively) than that of the standard (50.78 $\mu\text{g/mL}$) that were calculated from their regression line equation (higher regression coefficients). So, both extracts showed more DPPH scavenging activity than the standard.

3.2.4.2. Hydrogen Peroxide (H_2O_2) Radical Scavenging Assay

The absorbance of standard and extracts at 230 nm is given in Table 08.

Table 08: Absorbance of standard (ascorbic acid) and extracts at 230 nm

Concentration ($\mu\text{g/mL}$)	Absorbance of Standard	Absorbance of Extracts ($\lambda = 230 \text{ nm}$)	
		Leaves Extract	Flower Extract
500	0.382	0.656	0.499
250	0.551	0.837	0.695
125	0.874	1.352	1.117
62.5	1.561	2.102	1.794
31.25	2.182	2.454	2.241

(Absorbance of control = 2.57)

A comparison of % inhibition by standard and extracts is shown in Figure 08.

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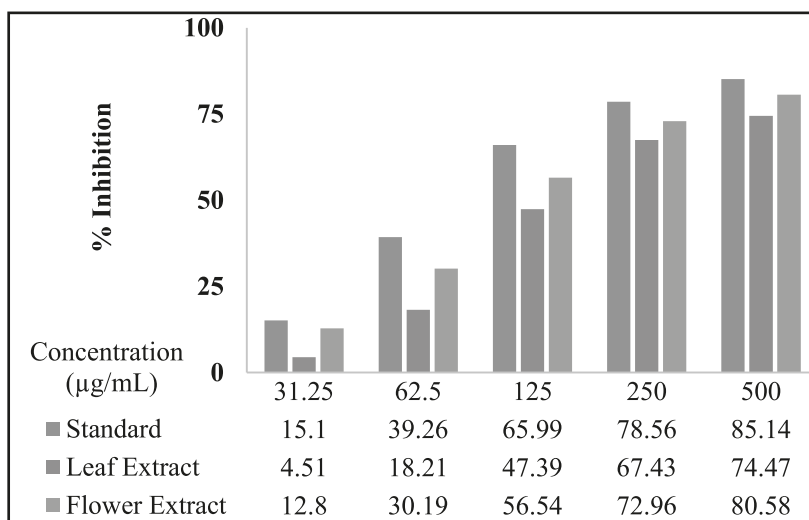


Figure 08: Comparison of Hydrogen Peroxide radical scavenging assay.

The regression line equations modeled from the absorbance of standard and extracts are given in Table 09.

Table 09: Regression line equation, Regression coefficient (R^2), and IC_{50} of standard (ascorbic acid) and extracts at 230 nm against control.

Regression line equation	Standard	Leaf Extract	Flower Extract
$y = \% \text{ Inhibition}, x = \text{Conc. } (\mu\text{g/mL})$	$y = 25.879 \ln(x) - 68.141$	$y = 27.288 \ln(x) - 89.349$	$y = 25.727 \ln(x) - 73.604$
R^2	0.9442	0.9667	0.9698
IC_{50} ($\mu\text{g/mL}$)	96.08	165.11	122.05

From the results, we can see that the standard and extracts had an increase in % inhibition as the concentration increased. Both extracts had higher IC_{50} values (165.11 and 122.05 $\mu\text{g/mL}$ for leaf and flower extract respectively) than that of the standard (96.08 $\mu\text{g/mL}$) that were calculated from their regression line equation (higher regression coefficients). So, both extracts showed less DPPH scavenging activity than the standard.

3.2.4.3. Total Reducing Power Assay

The absorbance of ascorbic acid at 700 nm and the standard curve modeled from the data is shown in Figure 09.

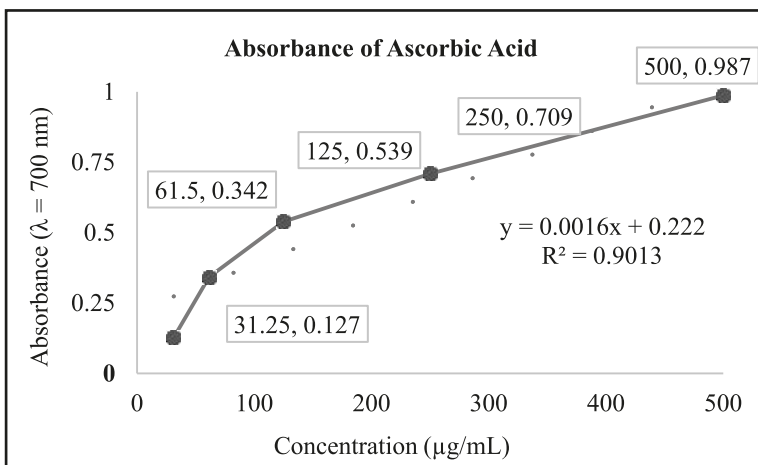


Figure 09: Standard curve of ascorbic acid at 700 nm.

The total reducing power contents in the ethanolic extracts are given in Table 10.

Table 10: Total reducing power contents in ethanolic extracts.

Extract	Total Reducing Power Content (mg/g of AAE)
Leaf Extract	151.37 ± 94.702
Flower Extract	633.84 ± 90.934

An illustration of the total reducing power content in the ethanolic extracts is given in Figure 10.

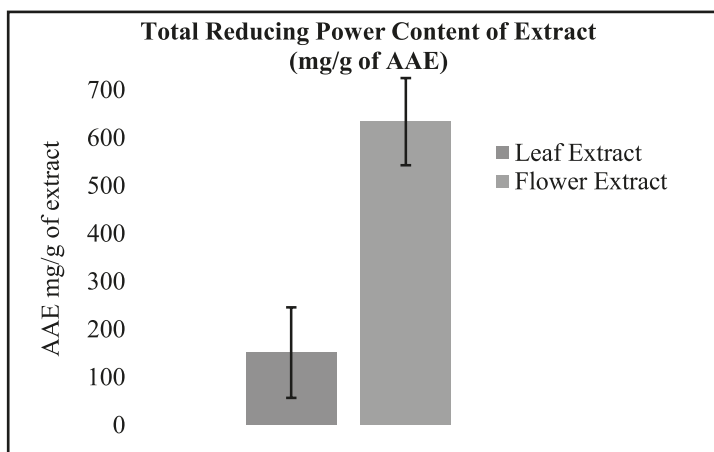


Figure 10: Total reducing power content in extracts.

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From the results we can see that, leaf extract has a total vitamin C content of 151.37 ± 94.702 mg/g of AAE, while flower extract has a much higher total vitamin C content of 633.84 ± 90.934 mg/g of AAE.

4. CONCLUSIONS

This study consisted of qualitative phytochemical screening, quantitative determination of some of the phytochemicals, and anti-oxidant assays followed by preparing ethanolic leaf and flower extract of *E. hirta L.* Presence of 14 types of phytochemicals (e.g., carbohydrate, reducing sugar, tannin, flavonoid, anthraquinone, glycoside, phenol, terpenoid, steroid, phlobatannin, anthraquinone glycoside, cardiac glycoside, volatile oil, and alkaloid) helped us design the in vitro anti-oxidant effects test. The leaf extract had 200% more total flavonoid content and 100% more total phenolic content than the flower extract, while they both had similar total vitamin C contents. Both extracts exerted better DPPH scavenging assay and poor H₂O₂ scavenging assay than the standard ascorbic acid. Lastly, the flower extract had 300% more total reducing power content than the leaf extract. More studies on this plant should be considered by researchers in phytochemistry and pharmacology in discovering newer and potential bioactive compounds, etc.

5. RECOMMENDATIONS

Further study can be done with specific lead compounds of the crude extracts.

6. ACKNOWLEDGEMENT

This work has been carried out at the Department of Pharmacy, University of Information Technology & Sciences (UITS), Dhaka, Bangladesh. The authors would like to express their wholehearted gratitude to all faculty members and students of pharmacy, UITS for their enormous support during the whole study.

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