WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 19, 990-999. Research Article ISSN 2277–7105

PHYTOCHEMICAL CONSTITUENTS, PHYSICOCHEMICAL PROPERTIES, ANTI-HYPERGLYCEMIC, AND ANTIOXIDANT PROPERTIES OF AERIAL PARTS OF *EUPHORBIA HIRTA*

[1]Mohammad Abu Bin Nyeem, [*1]Md. Abdul Mannan

[1]*Associate Professor, Department of Unani Medicine, Hamdard University Bangladesh.

Article Received on
16 August 2025,

Revised on 05 Sept. 2025,
Accepted on 25 Sept. 2025

https://doi.org/10.5281/zenodo.17277615

***Corresponding Author**
Md. Abdul Mannan
Associate Professor,
Department of Unani Medicine, Hamdard University Bangladesh.

ABSTRACT

Euphorbia hirta is a medicinal plant with anti-diabetic and antioxidant properties. This study aimed to assess the phytochemical compositions, physicochemical properties, the antioxidant potential, and anti-hyperglycemic activity of *E. hirta* extracted using methanol, water, and chloroform with a Soxhlet apparatus. The values of physicochemical parameters were measured according to WHO guidelines while phytochemical screening identified the secondary metabolites. DPPH and FRAP assays were used to determine the antioxidant activity and anti-hyperglycemic activity of the OGTT system in Swiss albino mice. The physicochemical analyses of *E. hirta* indicate its quality and purity. Phytochemical studies revealed the presence of alkaloids, flavonoids, tannins, glycosides, phenols, carbohydrates, and proteins in the methanolic and water extracts, and the chloroform extract contained saponins, terpenoids, and steroids. The anti-hyperglycemic activity of methanolic extract showed a dose-dependent manner. At 400 mg/kg ($p< 0.01$), significantly reduced blood glucose is comparable to glibenclamide and also, at 200 and 100 mg/kg, it is statistically significant ($p<0.05$), indicating an insulin-unoriginal or insulin-sensitizing mechanism. Antioxidant activity of methanolic extract was observed. THE DPPH assay showed concentration-dependent radical scavenging activity exceeding ascorbic acid at higher concentrations ($p<0.01$). FRAP assay indicates a stronger ferric reducing capacity ($p<0.001$). The results that passed the statistical significance test indicate that *E. hirta* is an effective source of natural antioxidants that works with cure-reliance. In conclusion, the methanolic extract of *E. hirta* is a great anti-oxidant and anti-hyperglycemic source of natural remedial accouterments in the operation of oxidative stress-related and diabetic conditions.

KEYWORDS: *Euphorbia hirta*, physicochemical analysis, phytochemicals, anti-hyperglycemic, and antioxidant.

INTRODUCTION

Euphorbia hirta L. also called asthma weed, belonging to the Euphorbiaceae family that grows in India, Southeast Asia, and Africa. Traditionally, used as Ayurveda, Unani and folk medicine in the treatment of asthma, diarrhea, and skin-related problems and diabetes (Sharma, 2014; Kaur et al., 2011). The key role of oxidative stress is due to the disproportion between reactive oxygen species (ROS) and the antioxidant defense system that leads to the development of diabetes (Maritim et al., 2003). Antioxidant and bioactive plants *E. hirta* have also demonstrated the ability to scavenge oxidative damage in addition to the measures of controlling the level of glucose in the blood (Parekh & Chanda, 2007). Phytochemical analysis indicate that *E. hirta* is also rich in, among other compounds, flavonoids (e.g., quercetin, rutin), tannins, saponins, terpenoids, alkaloids and phenolic acids; all of which have provided some of the pharmacological effects, whereas oxidative stress modulation and glucose homeostasis stabilization predominate (Basma et al., 2011; Igbinosa et al., 2009). According to experimental evidence, its extracts have appreciable hypoglycemic effects on diabetic models, which could be related to its insulin-like effect and inhibition of carbohydrate digesting enzymes (Sharma et al., 2007). Moreover, the quality and safety of herbal formulas require physicochemical standardization, like the moisture content, the values of ash, and extractive values (WHO, 1998). These aspects were assessed in this study to justify the therapeutic potentiality of *E. hirta*.

MATERIALS AND METHODS

Plant Materials Collection and Authentication

Aerial parts of the *Euphorbia hirta* plant were obtained in May 2024 in Hamdard University Bangladesh campus, Gazaria, Munshiganj, Bangladesh. A qualified taxonomist examined the material of the plant to authenticate it and a voucher specimen (HUB/HB/LB No. 124) was lodged in the university herbarium as a future reference material.

Plant extraction Preparation Methods

Euphorbia hirta was dried for 15 days without the direct contact of sunrays. When the moisture content below 5% by loss on drying at 100˚C, the *E. hirta* was powdered and weighed (300gm), after which solvents were using a Soxhlet apparatus with methanol, distilled water, and chloroform by standard procedures (Harborne, 1998; Kokate, 2008). The

extraction was continued until the color of the solvent in the siphon tube became clear. Chloroform was taken to extract the non-polar compounds; methanol was taken to the compounds of the intermediate polarity, and water on highly polar constituents. The percentages of extraction were: methanol 10.3% w/w, aqueous 8.41% w/w, and chloroform 4.54%w/w.

Drug

Glibenclamide (5 mg tablet; Square Pharmaceuticals Limited, Bangladesh) was used in this study.

Animals

Swiss albino mice of either sex (20-25 gm body weight) bred in the animal house of the department of pharmacology, Hamdard University Bangladesh, were collected from the animal resources branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-60%, r.t 23±2^{o}C and 12-hour light: dark cycle). The animals were fed with a standard diet and water *ad libitum.* The institutional animal ethical committee approved the study protocol (Approval No. HUB/IAEC/2024/05).

Acute toxicity test

Acute oral toxicity study was performed as per OECD 423 guidelines (OECD, 2001). Test animals (n=5) of either sex selected by random sampling technique were for the study. The animals were kept fasting for overnight providing only water, after which the methanolic extracts were administrated orally at the dose label of 5 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administrated was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 50, 300, and 2000 mg/kg body weight.

Physicochemical analysis

Physicochemical parameters of the powdered leaves of *Euphorbia hirta* were determined according to WHO guidelines (2011) and validated protocols (Haruna et al., 2023; AOAC, 2006; De & Sharma, 2023; Khandelwal, 2008; Kokate et al., 2010). Results are presented in Table 1. All values are expressed as % w/w, unless otherwise stated.

Table 1.

Parameter	Value
Moisture content (Loss on Drying)	9.38% w/w
Total Ash Value	7.42% w/w
Acid-Insoluble Ash	1.39% w/w
Water-Soluble Ash	2.63% w/w
Alcohol-Soluble Extractive Value	12.9% w/w
pH Determination	6.28
Swelling Index	3.55 mL/g
Bulk Density	0.50 g/mL

Preliminary phytochemical screening

Phytochemical screening such as alkaloids, flavonoids, tannins and phenols, saponins, glycosides, terpenoids, steroids, carbohydrates, and proteins were employed, as per established protocols (Harborne, 1998; Trease & Evans, 2002; Tiwari et al., 2011; Kokate, 2014; Yadav & Agarwala, 2011; Evans, 2009; Sofowora, 1993). The results are summed up in Table 2.

Table 2.

Phytochemical Constituent	Observation	Methanol	Aqueous	Chloroform
Alkaloids (Mayer's, tests)	Cream-colored precipitate	+	+	–
Alkaloids (Wagner's, tests)	Reddish-brown precipitate	+	+	–
Alkaloids (Dragendorff's tests)	Reddish-brown precipitate	+	+	–
Alkaloids (Hager's tests)	Yellow precipitate	+	+	–
Flavonoids (Shinoda test)	Pink to red coloration	+	+	-
Tannins (Ferric chloride test)	Blue-black or green precipitate	+	+	–
Saponins (Froth test)	Persistent froth >10 minutes	–	+	+
Glycosides (Keller–Killiani test)	Reddish-brown ring	+	+	–
Phenols (Ferric chloride test)	Deep blue or green color	+	+	+
Terpenoids (Salkowski test)	Reddish-brown layer	+	–	+
Steroids (Liebermann–Burchard test)	Green or blue color	+	–	+
Carbohydrates (Molisch's test)	Violet ring	+	+	–
Proteins (Biuret test)	Violet color	+	+	–

Anti-hyperglycemic activity

Experimental design

The methanolic extract was tested for anti-hyperglycemic activity by employing the Oral Glucose Tolerance Test (OGTT) in Swiss albino mice, as done in Ahmed et al. (2011). A total of 30 Swiss albino mice were deprived of food overnight by being placed in groups of six (n = 5) at random:

Group I: Vehicle control (dimethyl sulfoxide, DMSO)

Group II: Standard drug (Glibenclamide 10 mg/kg body weight)

Group III-VI: 50, 100, 200, and 400 mg/kg body weight of *Euphorbia hirta* methanolic extract, respectively.

An hour after treatment, an oral load of glucose (4 g/kg) was given to all groups. The glucometer was used to check the blood glucose after two hours (Venkatesh et al., 2004). A $p< 0.05$ was considered statistically significant.

Antioxidant Assays

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The DPPH method was employed to determine free radical scavenging activity (Vani et al., 1997). The DPPH control reading was done by mixing 3 mL of methanol and 150 µL DPPH. A test solution of *E. hirta* extract at concentrations of 100, 150, and 200 mg/mL was made and diluted in methanol to a volume of 3 mL. The absorbance value was recorded at 517nm after 15 minutes. The standard used was ascorbic acid at a concentration of 50 mg/mL.

Radical Scavenging Activity = [(Absorption of Control-Absorption of Sample)/ Absorption of Control 100]

FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP activity was determined as indicated by Benzie & Strain (1996). A fresh working reagent was prepared with acetate buffer, TPTZ, and $FeCl_3$ at a ratio of 10:1:1. In the reaction, the mixture was composed of 900 L FRAP reagent, 90, and 30 L of distilled water, and a sample or standard, respectively. At 37°C, absorbance was done at 595 nm after 10 minutes.

RESULTS

Table 3: Anti-hyperglycemic activity of methanolic extract of *Euphorbia hirta* in Swiss Albino Mice.

Group	Treatment	Dose (mg/kg body weight)	Route of Administration	Mean Blood glucose (mmol/L) ± S.E	% of Reduction vs. control
Group I	Control (dimethyl sulfoxide, DMSO)	4 g/kg	Oral	6.70 ± 0.31	-
Group II	Standard drug (Glibenclamide)	10	Oral	3.42 ± 0.22[a]	48.96%
Group III	*E. hirta* extract	50	Oral	5.50 ± 0.14 [b]	17.91%
Group IV	*E. hirta* extract	100	Oral	4.76 ± 0.11[c]	28.96%
Group V	*E. hirta* extract	200	Oral	4.36 ± 0.13[c]	34.92%
Group VI	*E. hirta* extract	400	Oral	3.32 ± 0.16[a]	50.45%

Values are statistically significant at P < 0.05. [a]p<0.01, [b]not significant, [c]p<0.05 vs. control. Student's t-test; Values are mean ± S.E (N=5).

Antioxidant activity of methanolic extract of *Euphorbia hirta*

A. DPPH Assay

Table 4 indicated that the absorbance and Percentage Radical Scavenging Activity (%RSA) of *Euphorbia hirta* methanolic extracts at different concentrations in comparison to standard Ascorbic Acid in the DPPH Assay.

Table 4: DPPH Radical Scavenging Activity.

Treatment	Concentration (mg/mL)	Mean Absorbance (517nm) ± SE	% Radical Scavenging Activity (%RSA)
Control	-	0.980± 0.007	-
Standard (Ascorbic acid)	50	0.440 ± 0.014[a]	55.10%
E. hirta extract	100	0.550± 0.021 [b]	43.88%
E. hirta extract	150	0.420± 0.010 [a]	57.14%
E. hirta extract	200	0.290 ± 0.017[c]	70.41%

Values are statistically significant at P < 0.05. [a]p<0.05, [b]not significant, [c]p<0.01 vs. control. Student's t-test; Values are mean ± S.E (N=5).

B. FRAP Assay

Ferric-reducing antioxidant power (FRAP) of the methanolic extract of *Euphorbia hirta* was measured in concentrations of 50, 100, and 150 mg/mL and ascorbic acid was used as a reference standard. Results are presented in Table 5.

Table 5: FRAP Assay of Extracts of different concentration of *Euphorbia hirta* and Ascorbic acid.

Treatment	Concentration (mg/mL)	Mean Absorbance (595 nm) ± SE	Significance (*p- Value)*
Standard (Ascorbic acid)	50	0.324± 0.005	-
Standard (Ascorbic acid)	100	0.640 ± 0.007	-
Standard (Ascorbic acid)	150	0.916 ± 0.011	-
E. hirta extract	50	0.418± 0.010 [a]	*P < 0.001*
E. hirta extract	100	0.812± 0.016 [a]	*P < 0.001*
E. hirta extract	150	1.24 ± 0.089 [a]	*P < 0.001*

Values are statistically significant at P < 0.05. [a]*p*<0.001 vs. standard. Student's t-test; Values are mean ± S.E (*N*=5).

DISCUSSION

In the current study, acute toxicity indicated that *E. hirta* methanolic extract can be used safely and should no mortality up to the dose of 2000 mg/kg body weight. So the extract is safe for long-term administration.

The phytochemical screening recognized the presence of secondary metabolites such as alkaloids, flavonoids, tannins, phenols, glycosides, terpenoids and steroids, which have been identified to play a significant role in enhancing glycemic control as well as antioxidant defense systems (Harborne, 1998; Kokate, 2014; Sofowora, 1993).

The methanolic extract proved to have a substantial effect on the anti-hyperglycemia in Swiss albino mice (Table 3) that had been subjected to oral glucose. The dose-dependent normalization of blood glucose levels was also seen for all doses, with the observed lowest blood glucose levels being 400 mg/kg ($p<0.01$), similar to standard drug glibenclamide. This is an indication that *E. hirta* can exert an insulin-mimetic or insulin-sensitive mechanism which could possibly be mediated by its constituent flavonoid and phenolic compounds, which have been reported to increase the activity of the pancreatic β-cells or promote better glucose uptake at the periphery (Ahmed et al., 2011; Venkatesh et al., 2004).

The Antioxidant capacity that was assessed through DPPH and FRAP analysis also showed a dose-dependent increase in activities involving free radical scavenging and ferric reducing powers. It is worth noting that the extract at 200 mg/mL had 70.41% DPPH radical-scavenging activity, which was better than the 55.10% RSA of 50 mg/mL ascorbic acid ($p<0.05$) in table 4. The absorbance of the extract at 150 mg/L was much higher (significance

value p<0.001) than that of the standard. This was a reflection that the extract has a greater antioxidant potential at higher dose levels. These results are similar to previous reports indicating that the polyphenolic-rich extracts of *E. hirta* have strong antioxidant powers (Basma et al., 2011; Sharma et al., 2014).

Combined, the anti-hyperglycemic activity and anti-oxidative activity, evident in this study, could be auxotrophic and synergistic. In diabetes mellitus, oxidative stress is a major factor in causing beta cell dysfunction and insulin resistance. Thus, the effect of the antioxidant activity of *E. hirta* can be indirectly related to its anti-diabetic effect i.e. preventing oxidative stress.

CONCLUSIONS

The anti-hyperglycemic and antioxidant potentials of the methanolic extract of *Euphorbia hirta* are facilitated by the richness in phytochemicals and bioactivity of the extract in a dose-dependent manner in vitro and in-vivo model. The observations imply that *E. hirta* can be an important source of natural adjunct and therapy of hyperglycemia and oxidative stress-related diseases such as type 2 diabetes mellitus. It is suggested that prospective research on further confirmation of its safety and pharmacological effects through bioassay-guided fractionation and elucidation of molecular mechanisms are necessary.

ACKNOWLEDGEMENT

Research work has been performed in Pharmacy Graduate Laboratory, Department of Pharmacy, Hamdard University Bangladesh, Munshiganj-1510.

REFERENCES

1. Ahmed, F., Hudeda, S., & Urooj, A. (2011). Anti-hyperglycemic activity of *Ficus racemosa* bark extracts in type 2 diabetic individuals. *Journal of Diabetes,* 3(4): 318–319. https://doi.org/10.1111/j.1753-0407.2011.00152.x
2. Ahmed, F., Rahman, S., Ahmed, N., Hossain, M., Biswas, A., Sarkar, S., Banna, H., Khatun, A., Chowdhury, M. H., & Rahmatullah, M. (2011). Evaluation of *Neolamarckia cadamba* (Roxb.) Bosser leaf extract on glucose tolerance in glucose-induced hyperglycemic mice. *African Journal of Traditional, Complementary and Alternative Medicines,* 8(1): 79–81. https://doi.org/10.4314/ajtcam.v8i1.60549
3. Association of Official Analytical Chemists. (2006). *Official methods of analysis* (18th ed., W. Horwitz, Ed.). AOAC International.

4. Basma, A. A., Zakaria, Z., Latha, L. Y., & Sasidharan, S. (2011). Antioxidant activity and phytochemical screening of the methanol extracts of *Euphorbia hirta* L. *Asian Pacific Journal of Tropical Medicine,* 4(5): 386–390. https://doi.org/10.1016/S1995-7645(11)60109-0
5. Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, 239(1): 70–76. https://doi.org/10.1006/abio.1996.0292
6. De, M., & Sharma, L. (2023). A comparative physico-chemical, phytochemical and spectroscopic analysis of two medicinal plants belonging to Euphorbiaceae family: *Acalypha indica* L. and *Euphorbia hirta* L. growing in Paschim Medinipur District, West Bengal, India. *International Journal of Experimental Research and Review*, 32: Article 18. https://doi.org/10.52756/ijerr.2023.v32.018
7. Evans, W. C. (2009). *Trease and Evans Pharmacognosy* (16th ed.). Elsevier Health Sciences.
8. Hamad, K. J., Al-Shaheen, S. J., Kaskoos, R. A., Ahamad, J., Jameel, M., & Mir, S. R. (2013). Essential oil composition and antioxidant activity of *Lavandula angustifolia* from Iraq. *International Research Journal of Pharmacy,* 4(4): 117–120. Doi:10.7897/2230-8407.04421
9. Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.
10. Haruna, S. Y., Isah, A. M., Garba, A. A., Alkali, M., Magaji, B., & Zubairu, M. S. (2023). Phytochemical constituents and physicochemical properties of medicinal plant *Euphorbia hirta* leaves. *International Journal of Modern Chemistry,* 15(1): 30–41. https://www.modernscientificpress.com
11. Igbinosa, O. O., Igbinosa, E. O., & Aiyegoro, O. A. (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and Pharmacology,* 3(2): 58–62.
12. Kaur, G., Alam, M. S., Jabbar, Z., Javed, K., & Athar, M. (2006). Evaluation of antioxidant activity of *Cassia siamea* flowers. *Journal of Ethnopharmacology,* 108(3): 340–348. https://doi.org/10.1016/j.jep.2006.05.021
13. Khandelwal, K. R. (2008). *Practical Pharmacognosy: Techniques and Experiments* (17th ed.). Nirali Prakashan.
14. Kokate, C. K. (2008). *Practical Pharmacognosy* (4th ed.). Vallabh Prakashan.
15. Kokate, C. K. (2014). *Practical Pharmacognosy* (4th ed.). Vallabh Prakashan.

16. Kokate, C. K., Purohit, A. P., & Gokhale, S. B. (2010). *Pharmacognosy* (45th ed.). Nirali Prakashan.
17. Maritim, A. C., Sanders, R. A., & Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: A review. *Journal of Biochemical and Molecular Toxicology*, 17(1): 24–38. https://doi.org/10.1002/jbt.10058
18. OECD. (2001). OECD guideline for testing of chemicals No. 423: Acute oral toxicity – Acute toxic class method. https://doi.org/10.1787/9789264071001-en
19. Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, 10(2): 175–181. https://doi.org/10.4314/ajbr.v10i2.50624
20. Sharma, N. K., Dey, S., & Prasad, R. (2007). In vitro antioxidant potential evaluation of *Euphorbia hirta* L. *Pharmacologyonline*, 1: 91–98.
21. Sharma, N., Samarakoon, K. W., Gyawali, R., Park, Y. H., Lee, S. J., Oh, S. J., Lee, T. H., & Jeong, D. K. (2014). Evaluation of the antioxidant, anti-inflammatory, and anticancer activities of *Euphorbia hirta* ethanolic extract. *Molecules*, 19(9): 14567–14581. https://do i.org/10.3390/molecules190914567
22. Sofowora, A. (1993). *Medicinal Plants and Traditional Medicine in Africa* (2nd ed.). Spectrum Books.
23. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, 1(1): 98–106.
24. Trease, G. E., & Evans, W. C. (2002). *Pharmacognosy* (15th ed.). Saunders Ltd.
25. Vani, T., Rajani, M., Sarkar, S., & Shishoo, C. J. (1997). Antioxidant properties of the Ayurvedic formulation *Triphala* and its constituents. *International Journal of Pharmacognosy*, 35(5): 313–317. https://doi.org/10.1080/09251619708951274
26. Venkatesh, S., Reddy, G. D., Reddy, Y. S., Sathyavathy, D., & Reddy, B. M. (2004). Effect of *Helicteres isora* root extracts on glucose tolerance in glucose-induced hyperglycemic rats. *Fitoterapia*, 75(3–4): 364–367. https://doi.org/10.1016/j.fitote.2003.12.025
27. World Health Organization. (1998). *Quality control methods for medicinal plant materials*. https://apps.who.int/iris/handle/10665/41986
28. World Health Organization. (2011). *Quality control methods for herbal materials* (Updated ed.). https://apps.who.int/iris/handle/10665/44479
29. Yadav, R., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, 3(12): 10–14. https://www.journalphytology.com