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BIOACTIVE PROFILE AND PHARMACOLOGICAL ACTIVITIES OF *ECLIPTA PROSTRATA* L.

NAHID SULTANA, ANIKA TABASSUM, TANIA HOSSAIN[1], MD MAHFUZUR RAHMAN[2], LIPA MONDAL[1] AND FARHANA ISLAM KHAN*

Department of Botany, Jagannath University, Dhaka-1100, Bangladesh

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Abstract

Eclipta prostrata L. has been used in traditional medicine due to its potential phytochemicals. In the current study, bioactive compounds such as tannins, terpenoids, flavonoids, and steroids were identified in the leaf extract. Moreover, the antibacterial assay showed moderate activity against bacteria. The HeLa cell line was treated with varying concentrations of methanolic leaf extract of *E. prostrata* which showed, approximately 95% of HeLa cells had died after 48 hours of incubation. The LD_{50} value for the methanolic extract was 75 mg/ml for HeLa cells. *Eclipta prostrata* extract exhibits significant cytotoxic effects on HeLa cells.

Introduction

Herbal medical products have been used since ancient times to treat a wide range of diseases and infections. Secondary metabolites, also known as phytochemicals, play a major role in the biological activity of plant extracts. Several well-known phytochemicals, including tannins, steroids, quinine, saponins, and flavonoids, have been found to possess various pharmacological properties, such as antibacterial, antiviral, antioxidant, anti-inflammatory, and cytotoxic properties (Puente-Garza *et al.* 2017, Hassan *et al.* 2018, Sarwar *et al.* 2025). Isolated antibacterial compounds from higher plants appear to be one of the most significant alternative strategies for combating antibiotic resistance and managing illness (Rahman *et al.* 2017). Researchers have also shown that some traditional medicinal plants have *in vitro* toxic and carcinogenic properties. It may cause serious health issues to take medication with non-prescribed medicinal plants (Tulay 2012). Besides, in the discovery of cytotoxic pharmaceuticals, natural products have played an essential role in avoiding serious problems associated with chemotherapy and radiotherapeutics (Cassileth and Deng 2004). *Eclipta prostrata* belongs to Asteraceae grows in tropical and subtropical regions of the world. It is commonly known as kalokeshi and bhringoraj (Priya *et al.* 2018). In Bangladesh, it is used for a variety of skin conditions, including burns and wounds, respiratory diseases, jaundice, diabetes, hair loss, exhaustion, and fever (Rahmatullah *et al.* 2010). Therefore, phytochemical studies based on the biological activities of medicinal plants can lead to the discovery of new chemical components of remarkable therapeutic interest (Wink 2015). This study's objective were to examine phytochemical screening, *in vitro* antibacterial activity and evaluate cytotoxicity on the Cervical Epithelial Carcinoma (HeLa) Cell Line.

Materials and Methods

Fresh leaves of *E. prostrata* were collected from Gazipur and Dhaka University. The herbarium specimen was authenticated by the Bangladesh National Herbarium and the Jagannath University Herbarium. A voucher specimen with the accession number DACB 87046 has been

*Author for correspondence: <tasnova152@gmail.com>. [1]Centre for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, Bangladesh. [2]Bangladesh Council of Scientific and Industrial Research (BCSIR), Chattogram Laboratories, Chattogram-4220, Bangladesh.

officially recorded and archived by the Bangladesh National Herbarium in Dhaka, Bangladesh. The leaves were quickly cleaned with tap water. The powdered materials were stored in properly sealed bottles at room temperature after being dried in the shed and finely ground.

The powdered leaves were soaked in 1 L (Ajax AR grade) methanol for 15 days in an airtight container at 23-25^{o}C. After filtering through filter paper, the extract was air-dried to eliminate the solvent. The final pellet was stored in an airtight screw-cap tube at 4°C. To ensure sterility, the pellets were filtered through a 0.45-µm Millipore filter paper after being dissolved in 2.5% dimethyl sulfoxide (DMSO) for the extraction procedure. These were diluted to the appropriate concentrations using distilled water for the antibacterial assays and cytotoxicity test.

The leaf extract was also utilized to test its phyto-ingredients. To determine the primary classes of compounds, the plant extract was screened qualitatively for phytochemicals. Extracts that dissolved in 2.5% DMSO were tested for alkaloids and tannins, and plant extract that dissolved in methanol was subjected to standard procedures for the measurement of reducing sugar, glycosides, terpenoids, flavonoids, saponins, steroids, and volatile oils (Oodebiyi and Sofowora 1978, Trease and Evans 1989, Dohou *et al.* 2003, Talukdar and Chaudhary 2010, Alamzeb *et al.* 2013, Thusa and Mulmi 2017).

Bacterial isolates i.e., *Salmonella typhi, Citrobacter* sp., *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, B. subtilis* and *Staphylococcus aureus*, were obtained from the Food Analysis and Research Laboratory, Centre for Advanced Research in Sciences, University of Dhaka. The bacteria were cultured by using bacteriological peptone and Mueller-Hinton agar. The antibacterial sensitivity was assessed using the disc-diffusion method (Bauer *et al.* 1966). Each sterile 6 mm diameter paper disc was separately soaked in 30 µl of 200 mg/ml leaf extracts, and each disc was dried to provide 6 mg of dried extract per disc. After being swabbed with the test organisms at a concentration of 10^5 CFU/ml, these discs were then put on Mueller-Hinton agar plates. Control discs containing DMSO without any test compounds were included. The plates were then incubated at 37°C for 24 hrs. Following incubation, the growth inhibition zones around the discs were examined for each microbe, and their diameter were measured and recorded. Each experiment was run in duplicate and repeated three times. The diameter of the clear inhibitory zones created around the discs was measured, and the results were evaluated using areas devoid of bacterial growth.

Total phenolic content (TPC) was measured by the Folin-Ciocalteu assay (McDonald *et al.* 2001). In brief, 0.5 ml of methanolic extract (200 µg/ml) was prepared and mixed with 0.5 ml of Folin-Ciocalteu reagent and 3 ml of 20% Na_2CO_3. Absorbance was taken at 760 nm. The standard graph was prepared with gallic acid as a reference compound and expressed as mg/g of gallic acid equivalent (GAE).

Evaluating antioxidant capacity is essential for assessing the quality of medicinal, bioactive, and functional compounds in plants. The DPPH radical scavenging assay, described by Koleva *et al.* (2002), was used for evaluating the free radical neutralizing potential of plant extracts. The synthetic antioxidant BHT (tert-butyl-1-hydroxytoluene) is commonly used as a reference standard for comparison.

The HeLa cell line was grown in Dulbecco's Modified Eagle's Medium (DMEM) provided with 1% penicillin-streptomycin (1 : 1), 0.2% gentamycin, and 10% fetal bovine serum (FBS). Cultures were kept at 37°C in a humidified atmosphere containing 5% CO_2. Subculturing was performed once a monolayer formed in the flask. Cell detachment was achieved using trypsin, followed by the addition of a complete medium to halt the trypsin reaction. Cytotoxicity was measured using CellTiter 96 Non-Radioactive Cell Proliferation Assay Kit (Promega, USA). To conduct the test, 96-well plates were seeded with 2×10^4/100 µl of HeLa cells. The cells were then

incubated for 24 hrs at 37°C with 5% CO_2. Subsequently, the cells were exposed to different doses of *E. prostrata* methanol extract. A control group treated with 2.5% DMSO was also included. After 48 hrs of incubation, cytotoxicity was observed using an inverted light microscope (Trinocular microscope Optika, Italy). Subsequently, after adding 15µl of CellTiter 96 Non-Radioactive Cell Proliferation Assay Kit to each well, the wells were incubated for four hrs. Following incubation, an ELISA microplate reader (EPOCH, BioTek, USA) was used to assess absorbance at 570 nm. The results were expressed as a percentage of the optical density of cells treated compared to control cells. The test was performed in duplicate to determine the LD_{50} value for the extract using the following equation:

Viability (%) = (optical density of sample/optical density of control) × 100.

Results and Discussion

Phytochemical screening of plant extracts has demonstrated the presence or absence of various bioactive constituents, including alkaloids, tannins, reducing sugars, glycosides, terpenoids, flavonoids, saponins, steroids, and volatile oils. The leaves of *Eclipta prostrata* have been reported to contain a substantial amount of these biologically active compounds, which play a crucial role in its pharmacological properties.

In the present investigation, leaves of *E. prostrata* contain tannins, terpenoids, flavonoids and steroids while alkaloids, glycosides, reducing sugar, saponin, and volatile oil were absent in the tested plant extract. Preliminary phytochemical analyses by Shukla *et al.* (2023) indicated that the therapeutic potential of *E. prostrata* in treating various diseases is attributed to the presence of carbohydrates, amino acids, alkaloids, tannins, phenolic compounds, terpenoids, steroids, flavonoids, cardiac glycosides, saponins, anthraquinones, and other glycosides. Likewise, Timalsina and Devkota (2021) identified several major bioactive constituents in *E. prostrata*, including coumestan derivatives, phenolic acid derivatives, flavonoids, triterpenoids, steroidal saponins, and substituted thiophenes, which are responsible for its diverse pharmacological activities.

The *in vitro* antibacterial potential of *E. prostrata* was screened against Gram-positive and Gram-negative bacteria using the disc diffusion method. Out of the eight bacterial species *B. subtilis, E. coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* showed better zone of inhibition (7, 9, 10, and 18 mm, respectively) (Table 1).

Table 1. Antibacterial activity of leaves extract of *Eclipta prostrata.*

Gram positive bacteria	Inhibition zone (mm)	Gram negative bacteria	Inhibition zone (mm)
Staphylococcus aureus	0	*Citrobacter* sp.	0
Bacillus cereus	0	*Escherichia coli*	0
Bacillus subtilis	7	*Salmonella typhi*	9
		Klebsiella pneumoniae	10
		Pseudomonas aeruginosa	18

Previous studies have consistently demonstrated the antibacterial potential of *E. prostrata* leaf extracts against a wide range of pathogenic microorganisms. For instance, Megala *et al.* (2021) reported that the ethanol extract of *E. prostrata* leaves exhibited notable antibacterial activity against several clinical isolates, including *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, with inhibition zones ranging from 18 to 20 mm. Similarly, Gurrapu and Mamidala (2017) revealed that alkaloids isolated from the leaves possess significant antimicrobial activity against *E. coli*, *P. aeruginosa*, *Shigella boydii*,

S. aureus, and *Streptococcus faecalis*, as confirmed through agar-well diffusion and broth microdilution assays. Furthermore, Goutam (2011) identified wedelolactone, a coumestan derivative isolated from *E. prostrata*, which demonstrated promising antibacterial efficacy at a concentration of 10 µg/ml against multiple bacterial strains, including *Staphylococcus epidermidis*, *Salmonella typhimurium*, *S. aureus*, *P. aeruginosa*, *Shigella flexneri*, and *E. coli*. Collectively, these findings highlight the broad-spectrum antibacterial activity of *E. prostrata*, which may be attributed to the presence of bioactive compounds such as alkaloids and coumestan derivatives, supporting its potential as a source of natural antimicrobial agents.

The total phenolic content (TPC) was expressed in mg/g of gallic acid equivalents (GAE), based on a standard calibration curve ($y = 0.024x + 0.0051$, $R^2 = 0.9996$). The TPC in the methanolic extract of *E. prostrata* was determined to be 59.41 mg/g GAE (Fig. 1). In this study, the methanolic extract also exhibited notable free radical scavenging activity, with an IC_{50} value of 34.9 µg/ml, compared to the standard IC_{50} value of 14.25 µg/ml (Fig. 2).

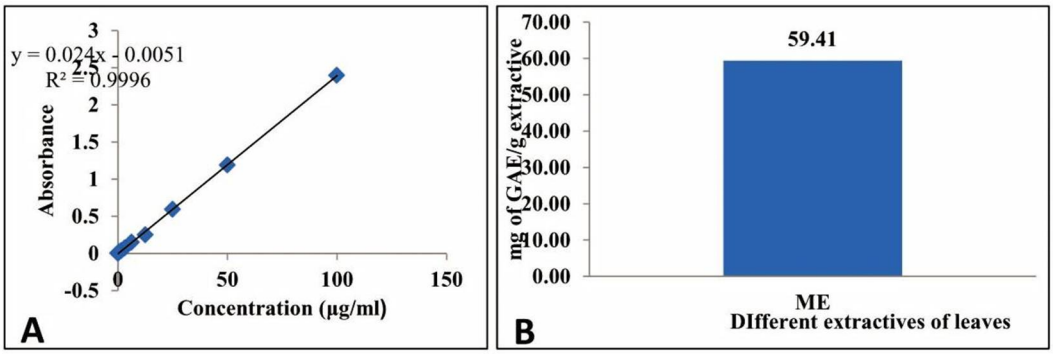

Fig. 1. Standard curve of gallic acid for total phenolic determination: (A) and total phenolic content (B) of leaves extract of *Eclipta prostrata*.

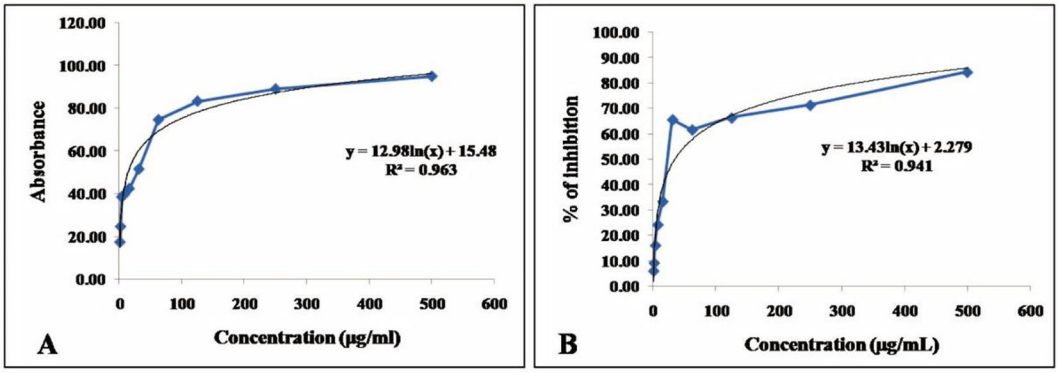

Fig. 2. IC_{50} value of tert-butyl-1-hydroxytoluene (BHT): (A) and IC_{50} value (B) of leaves extract of *Eclipta prostrata*.

This investigation examined the cytotoxic effects of *E. prostrata* methanolic extracts. The CellTiter 96 Non-Radioactive Cell Proliferation Assay and microscopic examination of the morphological changes in the cell cultures 48 hrs after the extracts were applied to determine the outcomes of cytotoxicity on HeLa cells. The microscopic observations revealed that the number of dead cells grew in proportion to the increasing concentrations of the extract treatment in HeLa cell

lines. Cells also showed cellular atrophy, cell shrinkage due to loss of water and cellular content, condensation of the cytoplasm and nucleus, loss of cell-cell contacts, and tissue disintegration denoting cell death (Fig. 3). In the CellTiter 96 Non-Radioactive Cell Proliferation assay, *E. Prostrate* extract exhibited an LD_{50} value of 75 mg/ml on the HeLa cell line. It was found that *E. Prostrate* methanolic extract with the concentration of 100 mg/ml was very effective and only 39.31% of HeLa cells were viable (Fig. 4).

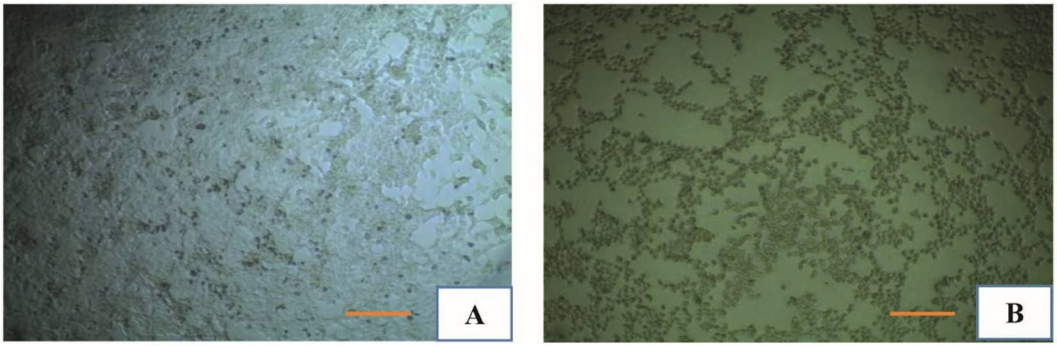

Fig. 3. Cytotoxic activity of cell free extract of selected bacteria on the untreated control of HeLa cell line (A) and *E. prostrata* plant extract (B) showing reduced cells size and many floating dead cancerous cells. 1 Bar = 20 µm.

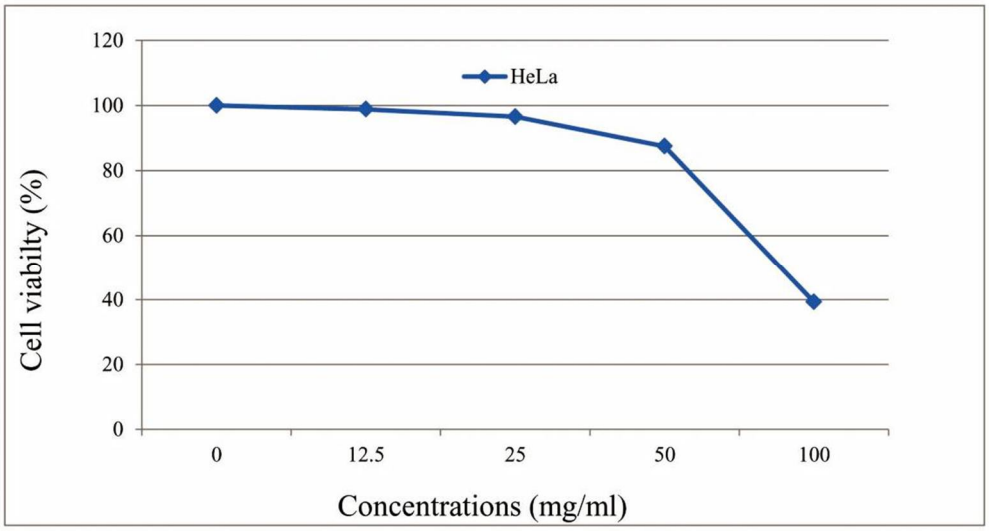

Fig. 4. CellTiter 96 Non-Radioactive Cell Proliferation Assay and cytotoxicity test of methanol extracts of *E. prostrata* on HeLa cell line.

In a previous study, Yang *et al.* (2023) reported the impact of different concentrations of *E. prostrata* on AGS, HT-29, and A549 cancer cells using MTT assay. The concentration of 100 µg/mL EtOAc extract induced approximately 70% cancer cell death while no significant difference in cell viability was observed in normal HEK-293 cells.

The available evidence indicates that *E. prostrata* possesses notable anticancer potential through multiple mechanisms. *In vivo* studies using Ehrlich ascites carcinoma (EAC)-bearing mice demonstrated that oral administration of the methanolic extract (250 and 500 mg/kg) significantly

prolonged survival, reduced tumor volume, and decreased viable tumor cell counts compared to untreated controls. Additionally, the extract contributed to the restoration of hematological parameters, including red blood cell count and hemoglobin levels, while improving immune response by increasing lymphocyte percentage and reducing neutrophil levels (Sing *et al.* 2017).

The chloroform fraction of the methanolic extract showed marked inhibition of breast tumor growth, potentially through selective regulation of Hsp60, and also alleviated tumor-induced liver and kidney toxicity. Moreover, bioactive compounds isolated from the aerial parts of the plant exhibited cytotoxic effects against human ovarian cancer cell lines (Kim *et al.* 2015). Consistent with these observations, methanolic leaf extracts demonstrated dose-dependent cytotoxicity against oral cancer (KB) cell lines in MTT assays (Jayaraman *et al*. 2022).

Overall, these findings suggest that *E. prostrata* exerts significant anticancer effects by inhibiting tumor growth, enhancing host survival, restoring hematological balance, and inducing cytotoxicity in various cancer cell lines, highlighting its potential as a promising source of anticancer agents.

In the case of HeLa cells, LD_{50} of 75 mg/ml indicated that 50% of the test population exposed to the leaf extract of *E. prostrata* at a concentration of 75 mg/ml would be expected to die from cervical cancer (HeLa) cells after 48 hrs. In this research, the leaf extract of *E. prostrata* showed more significant cytotoxicity against HeLa cell lines. Similarly, findings reported by Kanagaraj *et al.* (2023) demonstrated that the methanolic extract of *E. prostrata* exhibited notable cytotoxic activity against HT-29 human colorectal cancer cells, with an IC_{50} value of 62.44 µg/ml. In agreement with this, Timalsina and Devkota (2021) also observed significant antiproliferative effects of the ethanolic extract of *E. prostrata* using the MTT assay. Their results indicated IC_{50} values of 22.1 ± 2.9 µg/ml for HepG2, 25.3 ± 3.6 µg/ml for A498, and 50.2 ± 8.7 µg/ml for C6 cell lines. Moreover, morphological alterations such as cell detachment, rounding, and increased floating cells were evident at higher extract concentrations across all tested cell lines, suggesting dose-dependent cytotoxic effects.

The present investigation showed the presence of potential phytochemicals in *E. prostrata* that can lead to therapeutic interest. The methanol extract expressed cytotoxic effects against the treated cell lines. This plant has the potential antiproliferative activity against cancer cells that indicates the plant can be used as a source of anticancerous drugs.

References

Alamzeb M, Khan MR, Ali S, Shah SQ and Rashid MU 2013. Antimicrobial properties of extracts and compounds isolated from *Berberis jaeschkeana*. Bangladesh J. Pharmacol. **8**(2): 107-109.

Bauer AW, Kirby WMM, Sherris JC and Truck M 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Path. **45**(4): 493-496.

Cassileth BR and Deng G 2004. Complementary and alternative therapies for cancer. Oncologist **9**(1): 80-89.

Dohou R, Yamni K, Tahrouch S, Hassani LI, Badoc A and Gmira N 2003. Screening phytochimique d'une endémique ibero marocaine *Thymelaea lythroides*. Bull. Soc. Pharm. Bord. **142**: 61-78.

Gautam TP 2011. Indigenous uses of some medicinal plants in Panchthar District, Nepal. Nepal. J. Biosci. **1**: 125-130.

Gurrapu S and Mamidala E2017. *In vitro* antibacterial activity of alkaloids isolated from leaves of *Eclipta alba* against human pathogenic bacteria. Pharmacogn. J. **9**(4): 573-577.

Hassan NKNC, Taher M and Susanti D 2018. Phytochemical constituents and pharmacological properties of *Garcinia xanthochymus*- a review. Biomed. Pharmacother. **106**: 1378-1389.

Jayaraman L, Sivaji S and Anandakumar S 2022. Phytochemical screening, cytotoxic activity and molecular docking studies of *Eclipta alba* leaves extract against oral cancer. RASAYAN J. Chem. **15**(1): 676-685.

Kanagaraj C, Mohanapriya B, Aravindh S, Sathyapriya A and Suresh SN 2023. *In vitro* cytotoxic effect of methanolic extract from *Eclipta prostrata* L. against human colorectal adenocarcinoma cell line. Med. Res. Arch. **11**(2): 1-6.

Kim HY, Kim HM, Ryu B, Lee JS, Choi JH and Jang DS 2015. Constituents of the aerial parts of *Ecliptaprostrata* and their cytotoxicity on human ovarian cancer cells *in vitro*. Arch. Pharm. Res. **38**(11): 1963-1969.

Koleva II, Van Breek TA, Linssen JPH, De Groot A and Evstatieva LN 2002. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. **13**:8-17.

McDonald S, Prenzler PD, Autolovich M and Robards K 2001. Phenolic content and antioxidant activity of olive extracts. Food Chem. **73**: 73-84.

Megala M, Kiruthiga N, Sekar DS and Punithavathi G 2021. Antioxidant and antimicrobial activity of *Eclipta prostrata* leaf extract J. Acad. Ind. Res. **10**(1): 5-7.

Odebiyi OO and Sofowora EA 1978. Phytochemical screening of Nigerian medicinal plants II. Lloydia. **41**(3): 234-246.

Priya K, John P, Usha PTA, Kariyil BJ, Uma R and Hogale MS 2018. Phytochemical analysis of *Eclipta prostrata* L. leaves. Int. J. Curr. Microbiol. App. Sci. **7**(8): 1069-1075.

Puente-Garza CA, García-Lara S and Gutiérrez-Uribe JA 2017. Enhancement of saponins and flavonols by micropropagation of *Agave salmiana*. Ind. Crops Prod. **105**: 225-230.

Rahman MM, Shahriar MR, Meghla NS, Ishika T, Roy PC and Kamruzzaman M 2017. Antimicrobial activity of some medicinal plant extracts against Gram positive and Gram negative bacteria in Bangladesh. Asian J. Med. Biol. Res. **3**(4): 405-411.

Rahmatullah M, Hasan ME, Islam MA, Islam MT, Jahan FI, Seraj S, Chowdhury AR, Jamal F, Islam MS, Jahan R and Chowdhury MH 2010. A survey on medicinal plants used by the folk medicinal practitioners in three villages of Panchagarh and Thakurgaon district, Bangladesh. Am. Eurasian J. Sustain. Agric .**4**(3): 291-301.

Sarwar AKM Golam, Hasan M, Farhan-Ul-Islam, Hossain TI and Ashrafuzzaman M 2025. Medicinal plant genetic resources of Bangladesh exhibiting anti-dengue activity: A review. Int. J. Minor Fruits Med. Aroma. Plants **11**: 29-40.

Shukla V, Chaurasia S and Singh R 2023. Phytochemical screening of *Ecliptaprostrata* (L.) Int. J. Nov. Res. Develop. **8**(9): 413-418.

Singh A, Singh A and Dwivedi V 2017. Screening of hydro-alcoholic extract of *Eclipta alba* for its anticancerous efficacy. Int. J. Sci. Res. **6**: 488-491.

Talukdar A and Chaudhary B 2010. Phytochemical screening of ethanolic extracts of *Rubia cordiofolia*. Pharma. Biol. Sci. **1**(4): 530-536.

Thusa R and Mulmi S 2017. Analysis of phytoconstituents and biological activities of different parts of *Mahonia nepalensis* and *Berberis aristata*. Nepal J. Biotechnol. **5**(1): 5-13.

Timalsina D and Devkota HP 2021. *Eclipta prostrata* (L.) L. (Asteraceae): ethnomedicinal uses, chemical constituents, and biological activities. Biomol. **11**(11): 1738.

Trease GE and Evans WC 1989 Pharmacognosy: phenols and phenolic glycosides. **12**: 343-383.

Tulay AC 2012. A compendium of essays on alternative therapy. *In:* Tech. Europe. pp. 234-249.

Wink M 2015. Modes of action of herbal medicines and plant secondary metabolites. Med. **2**(3): 251- 286.

Yang J, Kim JS, Kwon YS, Seong ES and Kim MJ 2023. Antioxidant and antiproliferative activities of *Eclipta prostrata* (L.) L. extract and isolated compounds. Mol. **28**(21): 7354.

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