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Possible Role of Phenolics and Flavonoids in Antioxidant, Cytotoxic and Thrombolytic Activities of *Eclipta prostata* L.

Md. Harun-Or-Rashid[1], Juthi Rani Das[1], Anika Akter[1], Jalal Uddin[1], Naznin Parvin[1], Farhina Rahman Laboni[1], Md. Moklesur Rahman Sarker[2,3] and Nurul Karim[4]

[1]Department of Pharmacy, World University of Bangladesh, Avenue 06, Sector # 17/H Uttara, Dhaka-1230, Bangladesh
[2]Department of Pharmacy, State University of Bangladesh, 77 Satmasjid Road, Dhanmondi Dhaka-1205, Bangladesh
[3]Pharmacology and Toxicology Research Division, Health Med Science Research Limited 3/1 Block F, Lalmatia, Dhaka 1207, Bangladesh
[4]Department of Biochemistry and Molecular Biology, Jahangirnagar University Savar, Dhaka, Bangladesh

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Abstract

Eclipta prostata (*E. prostata*) L, is a herbal medicinal plant, which is reported to contain phenolics and flavonoids, but very little is known about its bioactive potentials. In this report, we reexamined the phenols and flavonoid content and evaluated different bioactivities of organic soluble fractions from the shoots of *E. prostata* for the first time. Among the extractives, chloroform soluble fraction (CSF) possessed the highest content of phenols (156.03 ± 0.52 mg gallic acid equivalent /g of dry extract) and flavonoids (67.47 ± 0.56 mg quercetin equivalent /g of dry extract) and exhibited the most potent antioxidant properties in terms of total antioxidant capacity (198.71 ± 0.89 mg ascorbic acid equivalent/g of dry extract) and DPPH free radical scavenging ability (IC_{50}; 17.68 ± 0.32 µg/ml). Similarly, CSF showed the highest cytotoxicity with LC_{50} values of 16.03±0.23 µg/ml, and thrombolytic activity with 36.06 ± 0.39% clot lysis. A significant correlation was observed between flavonoid content and total antioxidant capacity (r^2 = 0.894, $p < 0.05$), while high correlation was seen between polyphenols and flavonoid content with free radical scavenging, total antioxidant capacity and cytotoxicity (r^2 = 0.612 - 0.928). These findings suggest that *E. prostata* is a precious source of polyphenols having potential bioactivities, which could be utilized for the management of lifestyle diseases.

Key words: *Eclipta prostata*, antioxidant, free radical scavenging, cytotoxicity, thrombolytic.

Introduction

Medicinal plants and their bioactive phytoconstituents are the important natural resources for human beings. They have been playing important role in health care service to local people from ancient times (Willett, 2002**)** and act as important raw materials to prepare traditional medicines (Ghani, 2005). Numerous phytoconstituents such as flavonoids, polyphenols, tannins, carotenoids, tocoferol, folic acid, cinnamic acid etc. act as natural antioxidants (Reza *et al.*, 2021). Among the phytoconstituents, phenolics and flavonoids have much appeal as they contain hydroxyl groups and are able to neutralize the free radicals or decompose peroxides to share an electron or a hydrogen atom. They were found to be effective in the prevention and repair of oxidative damage of cells (Zhang *et al.*, 2015; Costa *et al.*, 2017; Ramana *et al.*, 2018). Free radicals in physiological system arise oxidative stress (OS) that damage cellular constituents such as protein, DNA, RNA, nucleic acids etc. and assist to develop various lifestyle diseases like cognitive disorder, cardiac disease, arthritis, cancer, and aging process (Praca *et al.*, 2002; Maxwell, 1995; Das *et al.*, 2021). Endogenous antioxidative systems

Corresponding author: Md. Harun-Or-Rashid; E-mail: drmhrashid9092@gmail.com
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scavenge free radicals and inhibit the development of chronic and age-related degenerative disorders. However, insufficient endogenous antioxidants cause OS-oriented several diseases, which can be overcome by dietary intake of exogenous antioxidants. Hence, dietary antioxidants can lower the risk of these types of disorders by preventing or slowing down the OS (Franco *et al.*, 2019).

Thrombosis is a pathophysiological process, which causes clot formation within the blood vessel and retards the blood flow through the circulatory system. The consequence of thrombosis is embolism, ischemia, heart attack, stroke and so forth (Monie1 *et al.*, 2017) and responsible for mortality and morbidity. Various drugs, including tissue plasminogen activator, urokinase and streptokinase are currently being used (Mackman, 2008) and have a risk of hemorrhage and anaphylactic shock. Therefore, there is an extreme need for the development of alternative drugs for its treatment. It was reported that plants with a high content of polyphenols exhibit good thrombolytic potentials (Ali *et al.*, 2013; Uddin *et al.*, 2018).

Eclipta prostata (*E. prostata*), commonly known as false daisy, local name bhangra or bhringaraj, belongs to Asteraceae family. It is a perennial herbaceous plant with small branches that grows in tropical and subtropical regions (Rao EV, 2000). *E. prostata* is bitter, hot, sharp, and dry in taste and appears as white to yellow flower (Puri, 2003). It is traditionally used as medicine in Ayurvedic (Sittichai and Picheansoothon, 2014) although there is lack of clinical evidences for its effective uses. The plant has been used as a liver tonic and in spleen enlargement. It is a famous hair tonic maintaining dark hair and is used externally for inflammatory head problems such as headaches, sinusitis and ear infections (Caldecott, 2006). It is also used for skin diseases and in catarrhal jaundice (Kapoor, 2001). Flowers of *E. prostata* are well known to use in analgesic, antispasmodic, fungicidal, digestive and bactericidal preparations.

E. prostata contains phytosterol, β-amyrin, triterpenes such as ecalbatin, echinocystic acid and flavones coumestans, polypeptides, polyacetylenes, thiophene derivatives, steroids, sterols, triterpenes, and flavonoids (Chopra *et al.*, 1966). These phytoconstituents are responsible for many pharmacological activities (Handa *et al* 1986; Chung *et al.*, 2017). Due to the presence of numerous natural antioxidants and folk medicinal value but no complete data for total phenols, flavonoids and antioxidant capacity, this study aimed to evaluate for the bioactive constituents and their free radical scavenging effect. The study was further extended to evaluate cytotoxicity and thrombolytic effect to show the correlation with phenolic and flavonoid contents of chloroform soluble extractives of shoots of *E. prostata*.

Materials and Methods

Collection, pulverization and extraction of plant: The shoots of *E. prostata* was collected from the rural areas of Dhaka district, Bangladesh when it became mature during July - August, 2019. It was taxonomically identified by the expert member of Bangladesh National Herbarium (BNH), Mirpur, Dhaka, Bangladesh and a voucher specimen was preserved under the Accession no: DACB-46509. The plants were cleaned, cut into pieces followed by air dry and in an oven at 45°C for 24 hrs. The dried plants were crushed and preserved in a closed container for future use. Dried powder materials (about 250 gm powder) were soaked in an amber color extraction bottle with sufficient volume of 90% methanol. The bottle was plugged and allowed to stand for 7 days for extraction. After seven days, solvents were filtered through clean cloth and subsequently with cotton and finally through Whatman filter paper No.1. The filtrate was concentrated with a vacuum pump rotary evaporator at 50□ and allowed to evaporate to dryness (MSF: 24 gm, 9.6%). A portion (10 gm) of crude methanol extract (CME) was fractionated by the Kupchan method (van Wagenen *et al.*, 1993). The resulting fractions i.e., chloroform (CSF), petroleum ether (PSF), ethyl acetate (ESF) and aqueous (AQSF) soluble materials were allowed to dry. The dried

materials were subjected to determine the percentage of yield PSF (1.75 g, 1.68%), CSF (1.95 g, 1.872%), ESF (3.55 g, 3.408%) and ASF (1.85 g, 1.776%). The yield was calculated on the basis of starting materials. The dried fractions were preserved in a refrigerator until further use.

Chemicals and reagents: Shrimp eggs were obtained from the Institute of Nutrition and Food Science, University of Dhaka, Bangladesh. Dimethyl sulphoxide (DMSO), DPPH, gallic acid, quercetin and Folin-Ciocalteau (FC) reagent were purchased from BDH Chemicals Ltd., Poole, England. The standard drugs streptokinase, ascorbic acid and vincristine sulfate (VC) used in this study were obtained as kind gifts from Beacon Pharmaceuticals Ltd., Bangladesh. Other chemicals and reagents used in this study were of the highest grade available.

Phytochemical analysis: The fresh CME of *E. prostata* was tested for various phytoconstituents, including saponins, tannins, reducing sugars, flavonoids and alkaloids by the reported procedures (Asaduzzaman *et al.*, 2014; Rashid *et al*., 2017).

Determination of phenols content: The phenolic content of different fractions of *E. prostata* were assayed calorimetrically according to the method as described by Harbertson and Spayd (2006) with a slight modification (Asad *et al*., 2022). Standard calibration curve was generated for gallic acid in the concentration ranges from 2.0 µg/ml to 20.0 µg/ml. The total phenolic contents (TPC) was expressed as mg of gallic acid equivalents (GAE)/g of dry samples.

Determination of flavonoid content: The flavonoid content of the fractions of *E. prostata* was estimated by a colorimetric method reported elsewhere (Zhishen *et al.*, 1999; Akter *et al*., 2022). Total flavonoid contents (TFC) was measured from the extrapolated standard curve developed for quercetin. The TFC was expressed as mg of quercetin equivalents (QE)/g of dry samples.

Determination of antioxidant capacity: Antioxidant capacity of *E. prostata* extractives was assayed calorimetrically as a procedure described previously (Prieto *et al.*, 1999; Akter *et al*., 2022) with simple modification. A 5.0 ml of reaction mixture was prepared containing 0.6 M sulphuric acid, 30 mM sodium phosphate and 1.0% ammonium molybodate with 0.2 ml test sample into a test tube. The test tube was incubated for 10 mins at 95□ for completion of reaction. The reaction mixture was cold at room temperature and centrifuged to remove the precipitate. The absorbance of supernatant was recorded at 695 nm in a UV-spectrophotometer (Shimadzu, Japan). The reaction mixture without sample/standard was used as blank solution. Total antioxidant capacity (TAC) was determined from standard curve and expressed as mg of ascorbic acid equivalents (AAE)/g of dry samples.

Free radical scavenging capacity: Free radical scavenging property was evaluated against DPPH according to the reported method (Brand-Williams *et al*., 1995) with simple modification (Rashid *et al.*, 2022).

Determination of cytotoxicity: The cytotoxicity of the fractions of *E. prostata* was measured by a well-known method, brine shrimp lethality bioassay reported by Meyer *et al.*, (1992) with simple modifications (Laboni *et al.*, 2016).

Determination of thrombolytic potential: The clot lysis activity of the fractions of *E. prostata* was measured as the method described by Prasad *et al.*, (2006) with simple modification (Rashid *et al.*, 2017).

Statistical analysis: Each experiment was done in triplicate of each sample. The experimental results were calculated by using Microsoft Excel and values were presented as mean ± SD. The *t-test* was used to determine the significant differences (p-value < 0.05) between the means. Correlation studies were performed using Pearson's correlation test.

Results and Discussion

The CME of shoots of *E. prostata* and its four fractions were subjected to determine the presence of constituents and their measurement by phytochemical analysis such as identification of constituents, phenolic and flavonoid content. The phyto-constituents showed antioxidant properties which

were evaluated in terms of total antioxidant capacity and free radical scavenging ability against DPPH. The antioxidant compounds persist various therapeutic aids evaluated by thrombolytic and cytotoxic activities.

Qualitative phytochemical analysis: Qualitative analyses of the CME of *E. prostata* showed the presence of phenols, flavonoids, alkaloids, glycosides and steroids (Table 1).

Table 1. Phytochemical screening of the CME of *E. prostata.*

Constituents	Results
Phenols	+
Flavonoids	+
Alkaloids	+
Saponins	-
Tanins	+
Steroids	+
Glycosides	+

'+' = presence; '-' = absence.

Total phenolic content (TPC): TPC of the fractions of *E. prostata* was estimated by the Folin-Ciocalteu method (Figure 1A). The results were expressed as GAE and are shown in Table 2. The content of phenolics of PSF, CSF, ESF, AQSF and CME were found to be 34.64 ± 0.21, 156.03 ± 0.52, 76.47 ± 0.57, 55.23 ± 0.45 and 59.77 ± 0.41 mg of GAE/g of dry sample, respectively. The results indicated the presence of moderate amount of phenolics in all the fractions. The CSF exhibited the highest phenolic content followed by ESF, CME, AQSF and PSF. The phenolic content of CME was reported earlier by Xiong *et al.*, 2021; Le *et al.*, 2021, which is consistent with our results. A number of phenolic compounds such as 4-hydroxy benzoic acid, chlorogenic acid, vanillic acid, protocatechuic acid, syringic acid, caffeic acid and its ethyl ester, ferulic acid ethyl ester etc. have been reported from the shoots of *E. prostata* (Zhang *et al.*, 2001; Xiong *et al.*, 2021; Le *et al.*, 2021). Our results support that *E. prostata* is an important source of phenolics that might have potential health benefits.

Total flavonoid content (TFC): TFC of the fractions of *E. prostata* was measured by aluminium chloride colorimetric assay (Figure 1B). The results were expressed as QE and are shown in Table 2. The TFC of CME, PSF, CSF, ESF and AQSF were 50.28 ± 0.48, 27.51 ± 0.52, 67.47 ± 0.56, 54.93 ± 0.39 and 50.28 ± 0.39 mg of QE/g of dry sample, respectively, suggesting that the flavonoids are distributed in all fractions. Similar to phenolics, the highest content of flavonoid was found in the CSF followed by ESF, CME, AQSF and PSF. In previous study, a sufficient amount of flavonoid in the methanol fraction has been reported by Lee *et al.*, 2009; Zhao *et al.*, 2015; Li *et al.*, 2018, which is consistent with our results. The common flavonoids such as luteolin, luteolin-*7-O-β*-D-glucoside, acacetin, kaempferol, kaempferol-7-*O*-α-D-ramnoside, quercetin and quercetin-3-*O-b*-D-glucoside etc have been isolated from the *E. prostata* by *et al.*, (Zhao *et al.*, 2015; Li *et al.*, 2018; Xiong *et al.*, 2021; Le *et al.*, 2021). In this report we evaluated the highest content of flavonoid from CSF among the shoot extractives of *E. prostata.*

Table 2. Total phenolic and flavonoid contents of the different fractions of *E. prostata*.

Samples	TPC (mg GAE/g of extract)	TFC (mg QE/g of extract)
CME	59.77 ± 0.41	50.28 ± 0.48
PSF	34.64 ± 0.21	27.51 ± 0.52
CSF	156.03 ± 0.52	67.47 ± 0.56
ESF	76.47 ± 0.57	54.93 ± 0.39
AQSF	55.23 ± 0.45	50.28 ± 0.39

TPC, Total phenolic content; TFC, total flavonoid content; CME, Crude methanol extract; PSF, Petroleum ether soluble fraction; CSF, Chloroform soluble fraction; ESF, Ethyl acetate soluble fraction; AQSF, Aqueous soluble fraction.

Total antioxidant capacity (TAC): TAC of the different fractions of *E. prostata* was determined on the ability of reduction of Mo (VI) to Mo (V). TAC of CME, PSF, CSF, ESF and AQSF of *E. prostata* were found to be 112.32 ± 0.58, 89.37 ± 0.48, 198.71 ± 0.89, 182.17 ± 0.53 and 127.94 ± 0.53 mg of

AAE/g of dry samples, respectively (Table 3). Among the fractions, CSF showed the highest capacity (Figure 1C). Pearson's correlation analysis revealed a significant correlation between TFC and TAC (r^2=0.894, p<0.05), whereas a high correlation was seen between TPC and TAC (r^2=0.649) (Table 4). The plant polyphenols act as singlet oxygen scavenger, reducing agent and proton donor (Karamon *et al.*, 2010; Xiong *et al.*, 2021; Zhao *et al.*, 2015). Thus, it was rational to determine total antioxidant capacity in the extractives of *E. prostata* and found to have potential antioxidant capacity in different fractionates.

Table 3. TAC and DPPH scavenging activity (IC_{50}) of the different fractions of *E. prostata*.

Fraction/ Standard	TAC (mg AAE/g of fraction)	IC_{50} value (µg/ml)
CME	112.32±0.58	138.55±0.52
PSF	89.37±0.38	ND
CSF	198.71±0.89	17.68±0.32
ESF	182.17±0.53	45.69±0.61
AQSF	127.94±0.53	38.02±0.61
Ascorbic acid (AA)	-	10.10±0.42

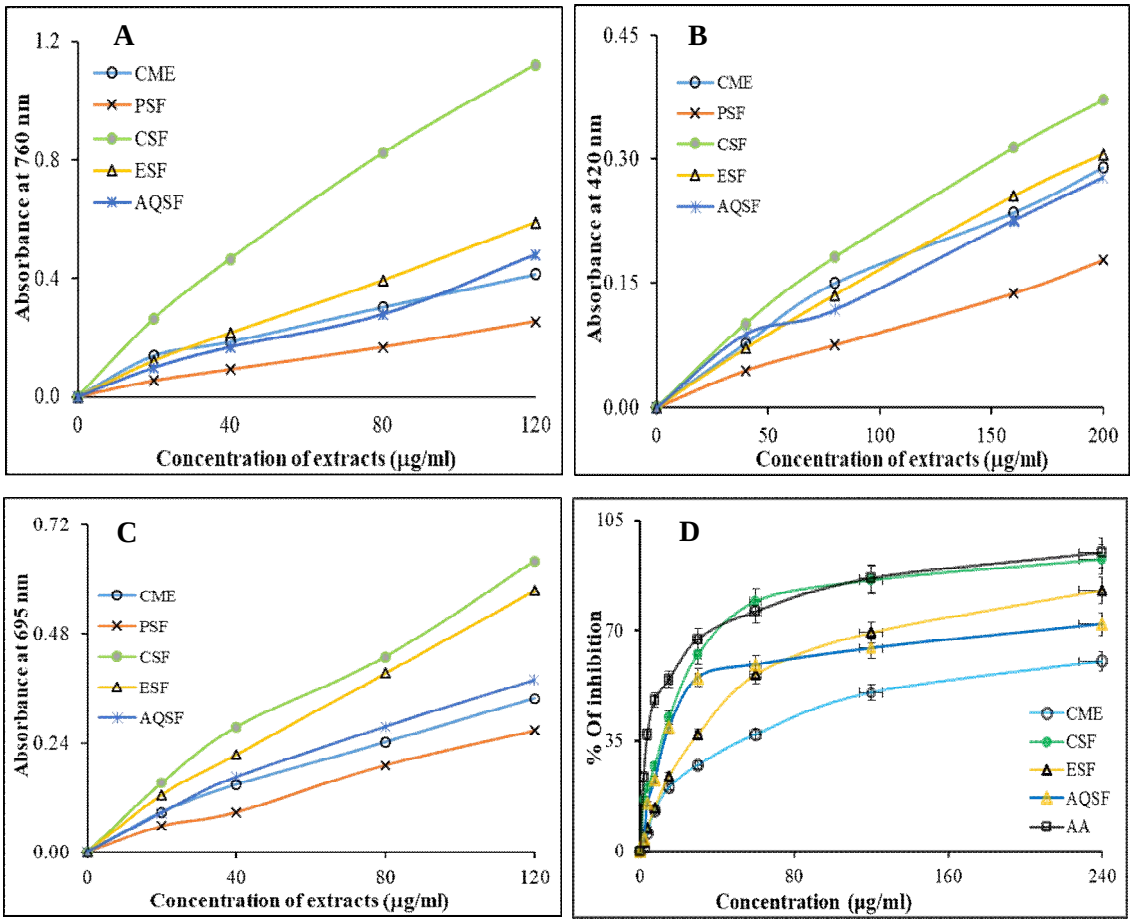

Figure 1. Evaluation of *in-vitro* antioxidant activities of the different fractions of *E. prostata*. (A) Evaluation of total phenolic content; (B) Evaluation of total flavonoid content; (C) Evaluation of total antioxidant capacity; (D) DPPH free radical scavenging activity. AA was used as a standard antioxidant. Data was expressed as mean ± SD (*n* = 3).

DPPH radical scavenging ability: Free radical scavenging potential of the fractions of *E. prostata* was evaluated by spectrophotometric method using the synthetic DPPH free radicals (Figure 1D). The IC_{50} values of the fractions were found within the range of 17.68 ± 0.32 µg/ml to 138.55 ± 0.52 µg/ml

(Table 3). Among the fractions, CSF showed the highest scavenging activity with IC_{50} value of 17.68 ± 0.32 µg/ml followed by AQSF, ESF and CME. PSF showed low scavenging activity and hence data were not shown in Table 3. AA was used as a standard with an IC_{50} value of 10.10 ± 0.42 µg/ml. The DPPH scavenging activity of the crude ethanol extract was examined earlier by Uddin *et al.,* (2010). This studies evaluated methanol extractive of *E. prostata* also showed DPPH free radical scavenging activity. From correlation analysis, we found a high correlation of TPC and TFC with DPPH scavenging activity (r^2 = 0.728 & 0.612) (Table 4). The presence of abundant polyphenols and flavonoids in the shoots of *E. prostata* (Lee *et al.*, 2009; Zhao *et al.*, 2015; Li *et al.*, 2018; Xiong *et al.*, 2021; Le *et al.*, 2021) acts as proton donor and easily scavenged DPPH free radical. This was justified by the highest presence of phenolic and flavonoid contents in CSF which showed strong correlation with DPPH scavenging activity in this study (Figure 3).

Cytotoxicity: The *in-vivo* cytotoxicity of the fractions of *E. prostata* was measured against shrimp nauplii by brine shrimp lethality bioassay, which is a simple way to evaluate the anticancer activity of a cytotoxic agent. The cytotoxicity of the fractions was expressed in terms of LC_{50} values (Figure 2A & 2B). The LC_{50} values of CME, CSF, ESF and AQSF were found to be 23.07 ± 0.45 µg/ml, 16.03 ± 0.23 µg/ml, 33.27 ± 0.34 µg/ml and 58.75 ± 0.84 µg/ml, respectively. These results indicated the highest cytotoxicity in the CSF followed by ESF and CME. Vincristine sulphate was used as a standard cytotoxic agent, which showed LC_{50} value of 2.5 ± 0.43 µg/ml.

The methanol extractive of *E. prostrata* was effective against Ehrlich ascites carcinoma (EAC) cells in mice (Sing *et al.*, 2017). The inhibition of breast cancer cells by CSF was reported by Aryea *et al.*, (2015). The constituents from aerial parts of *E. prostata* reported to be cytotoxic against human ovarian cancer cell lines, SKOV3 (Kim *et al.*, 2015). Methanol and ethanol extracts have been reported to exhibit significant anti-proliferative effects against various cell lines **(**Chaudhary *et al.*, 2011). In this study, we observed a high correlation between phenolic content and cytotoxicity (r^2 = 0.928) (Table 4). A similar relationship between flavonoid content and anti-tumor activity in human ovarian cancer cell and brine shrimp lethality was reported earlier (Uddin *et al.*, 2010; Kim *et al.*, 2015). Our results provide the cytotoxic potential of the chloroform fraction which requires investigation against these cell lines.

Thrombolytic effect: Thrombolytic activity of different fractions of *E. prostata* was assayed by determining the ability to clot lysis. Streptokinase (SK) was used as a positive control that showed 63.09 ± 0.51% (w/w) lysis as compared to the 0.9% w/v NaCl solution (7.92 ± 0.23%). In this study, the different fractions of *E. prostata* exhibited varying degrees of thrombolytic activities ranging from 14.07 ± 0.15% to 37.38 ± 0.35% (Table 5). CME showed the highest clot lysis activities (37.38 ± 0.35%) followed by CSF, ESF, AQSF and PSF. Vascular blockage due to thrombus formation in the blood vessels leads to death. Commonly used thrombolytic agents such as streptokinase, urokinase, plasminogen activator, etc having risk of hemorrhage and anaphylactic reactions. So, there is still a need for newer thrombolytics from natural sources and research is ongoing to develop new ones for this purpose (Sikder *et al.*, 2012; Anjum *et al.*, 2022). This study is the first attempt in *E. prostata* to develop newer thrombolytic and its crude methanol extract and CSF shown to possess significant (37.38 ± 0.35% and 36.06 ± 0.39, respectively) clot lysis activity.

Table 4. Correlation of total phenolic and flavonoid content with antioxidant and cytotoxic activities.

Activities	Correlation value (r^2)	
	TPC	TFC
TAC	0.649	0.894
DRSA	0.728	0.612
Cytotoxicity	0.928	0.473

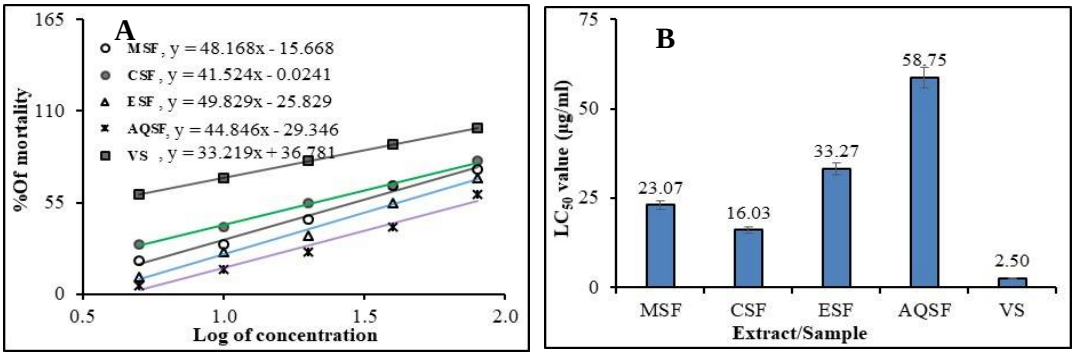

Figure 2. Cytotoxicity of the different extractives of *E. prostata* against brine shrimp nauplii. (A), A Graph for log of concentration versus % of mortality of nauplii; (B), LC_{50} values of the different fractions. Vincristine sulphate (VS) was used as a standard drug for comparison. Data expressed as mean ± SD (n=3).

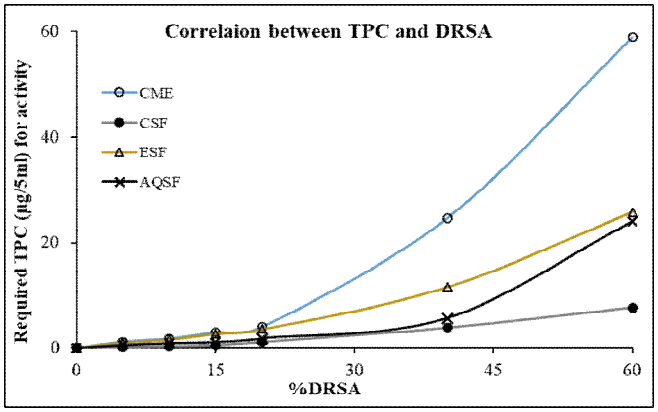

Figure 3. Correlation of TPC of the extractives of shoots of *E. prostata* with its antioxidant properties % DRSA. Data expressed as mean ± SD (n=3, p< 0.*001).*

Table 5. Thrombolytic effects of the different fractions of *E. prostata.*

Samples	Weight of empty vial (W_1 gm)	Weight of vial with clot (W_2 gm)	Weight of clot W_3= W_2-W_1 gm	Weight of vial after clot lysis W_4 gm	Weight of lysis clot W_5 = W_2-W_4 gm	% Of clot lysis = 100 × W_5/W_3
CME	5.295	6.044	0.749	5.764	0.28	37.38 ± 0.35
PSF	5.624	6.406	0.782	6.296	0.11	14.07 ± 0.15
CSF	5.426	6.147	0.721	5.887	0.26	36.06 ± 0.39
ESF	4.907	5.826	0.919	5.596	0.23	25.03 ± 0.11
AQSF	5.024	5.935	0.911	5.766	0.169	18.55 ± 0.23
Blank	5.329	5.958	0.631	5.908	0.05	7.92 ± 0.23
SK	5.128	5.873	0.745	5.403	0.47	63.09 ± 0.48

CME, Crude methanol extract; PSF, Petroleum ether soluble fraction; CSF, Chloroform soluble fraction; ESF, Ethyl acetate soluble fraction; SK, Streptokinase. Data expressed as mean ± SD (n = 3, p < 0.01) for all tested dosages.

This study focused on the phenolics and flavonoids of *E. prostata* to gain insights into their bioactive potential. Quantitative analysis of the extractives showed the high content of phenolics and flavonoids in this plant. A causative relationship

between phenolics and flavonoids and antioxidant activity has been reported in many plants (Karamon *et al.*, 2010; Zhao *et al.*, 2015; Esmaeili *et al.*, 2015; Xiong *et al.*, 2021). We reported here for the first time a significant correlation between total phenol content and antioxidant activity, indicating the strong antioxidant activity of the phenolics. Free radicals have emerged as a crucial factor in the pathogenesis of numerous diseases, including cancer and neurodegenerative disorders. They can attack the endogenous molecules like protein, DNA, RNA, and nucleic acid in human cells and damage them resulting in initiation and development of several diseases (Praca *et al.*, 2002; Maxwell, 1995). Natural antioxidants such as phenolics and flavonoids have been found to be effective in prevention and treatment of these diseases (Steinmetz *et al.*, 1996; Willett, 2002; Ramana *et al.*, 2018). In this study, all the extractives were found to have cytotoxic activity and the activity was correlated with the phenolic and flavonoid content (Table 4). Apart from these activities, a positive correlation was evident between phenolics and flavonoids with thrombolytic activity (Table 5). Phenolics are abundant in fruits and vegetables and have potential health benefits. The activity of the phenolic compounds has been attributed to the reactivity of phenol moiety having redox properties which is able to donate an electron or a hydrogen atom. Thus, phenolics have the ability to scavenge the free radicals, neutralize singlet and triplet oxygen, or decomposing peroxides (Dembinska-kice *et al.*, 2008). Flavonoids are also ubiquitous in plants and have good antioxidant potential. They contain the conjugated ring structure and hydroxyl group that can share an electron or a hydrogen atom to scavenge free radical and reactive oxygen species (Birt *et al.*, 2000). In *E. prostata*, the association of phenolics and flavonoids with bioactivities warrants further studies on the extractives to identify and characterize the active compounds and the mechanisms underlying the bioactivity.

Conclusion

In conclusion, this study showed that *E. prostata* is a potential source of phenolics and flavonoids having antioxidant, cytotoxicity and thrombolytic activities, which could be useful for further development of high value of phytomedicinal preparations for the management of life-style disease.

Conflict of interest

The authors declare that there are no conflicts of interest.

Authors' contributions

JRD, AA and NP were involved in collection of plant parts and laboratory experiments. JU and FRL were involved in work supervision, extensive literature search, data acquisition and interpretation of results. MMRS and NK did critical statistical analysis, experiments and report revision. Concept development, experiment design, overall monitoring, report writing and final approval of the manuscript was done by MHR.

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