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Antioxidant, Anti-inflammatory, Antimicrobial and Thrombolytic Activities of *Eclipta alba* L. Growing in Bangladesh

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Abstract

Eclipta alba L. (Family: Asteraceae) is an evergreen herb of ethnomedicinal significance in the Indian subcontinent. The present study was carried out to determine the *in vitro* antioxidant, anti-inflammatory, antimicrobial and thrombolytic activities of *n*-hexane, chloroform and aqueous soluble fractions of ethanol crude extract of *E. alba* whole plant. Preliminary phytochemical screening was performed by qualitative tests which revealed the presence of alkaloids, steroids, glycosides, tannins, flavonoids, saponins and amides in *E. alba.* During the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay, the aqueous soluble fraction of *E. alba* at a dose of 100 µg/ml showed maximum 87.5% inhibition in scavenging the DPPH radical with IC_{50} value of 7.86 µg/ml. Anti-inflammatory activity was evaluated by egg albumin denaturation technique and RBC membrane stabilizing method. In both experiments, all the test samples of *E. alba* showed a concentration-dependent anti-inflammatory potential while the aqueous soluble fraction at the dose of 500 µg/ml demonstrated the maximum 90.12% inhibition of egg albumin denaturation and 78.42% inhibition of RBC membrane hemolysis against hypotonic solution. Antimicrobial sensitivity was evaluated by disc diffusion assay against several bacterial and fungal species. All the test samples at a concentration of 500 µg/disc exhibited noticeable antibacterial (zone of inhibition = 11-23 mm) and antifungal (zone of inhibition = 10-21 mm) activities when compared to the respective standard drug. The aqueous soluble fraction showed maximum activity against *Lactobacillus casai* (zone of inhibition = 23 mm). During antifungal test, the maximum activity was exhibited by the *n*-hexane fraction against *Pityrosporum ovale* (zone of inhibition = 21 mm). During *in vitro* thrombolytic assay, the aqueous soluble fraction revealed 56.84% lysis of blood clot, as compared to the standard streptokinase (84.60%). The results of our study suggest that *E. alba* can be considered for further investigation in order to discover the pharmacologically active natural products.

Key words: *Eclipta alba* L, antioxidant, anti-inflammatory, antimicrobial, thrombolytic, phytochemical.

Introduction

Medicinal plants have been serving as the major sources of therapeutic agents for maintenance of human health. These medicinal plants have been utilized by the early human beings, for the treatment of various diseases and ailments. Plant, the molecular architect, still offers a great potentiality for drug discovery, as they can synthesize a diverse range of bioactive compounds having interesting skeletons. Therefore, bioactivity guided phytochemical investigation of medicinal plants may yield newer chemical constituents of remarkable therapeutic interest (Wink 2015).

Eclipta alba L. is a traditionally important medicinal plant in many countries, especially in the tropical and subtropical regions. It is common in waste places, muddy lands and roadsides area. *E. alba* is a small branched annual herb that has a bitter,

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hot and dry taste. The plant is used in Ayurveda for the treatment of 'Kapha' and 'Vata' (Jahan *et al*., 2014; Kumar *et al*., 2005). In ayurvedic formulation, the plant is used as pain reliever, anthelmintic and anti-inflammatory agent. The fresh juice of the leaves is valuable as hair tonic (Kumar *et al*., 2005). It is also used in the treatment of hepatitis (Wagner *et al*., 1986), hyperlipidemia (Kumari *et al*., 2006) and snake venom poisoning (Melo *et al*., 1994). The herb contains anti-hepatotoxic compound wedelolactone which is a coumestan derivative (Jaglan *et al*., 2013). Recent reports showed that the triterpenoid saponins isolated from this plant exhibited antimicrobial, immunosuppressant and anti-venom potentials (Arunachalam *et al*., 2009). The plant is rich in phytochemicals such as wadeoloctone, eclalbasaponin, *β*-amyrin, stigmasterol and luteolin-7-glucoside (Asolkar *et al*., 1992). Rahman *et al*. (2005) reported oleanane glycosides from *Eclipta prostrata*. Eclalbasaponin II, an isolated compound of *E. prostrata* was reported to show potent antidiabetic activity in animal model (Rahman *et al*., 2011). In another study, Rahman *et al*. (2008) described the antimicrobial potency and cytotoxicity of the extractives of *E. prostrata* and its two purified compounds eclalbasaponin I and II.

Due to the above-mentioned biological effects of *E. alba*, the aim of our study was to evaluate antioxidant, anti-inflammatory, antimicrobial and thrombolytic activities of this plant species using established *in vitro* methods.

Materials and Methods

Collection and identification: The aerial parts of *E. alba* were collected from the hills of Forest Research Institute, Chattogram, Bangladesh in January, 2016. The plant was identified by a taxonomist in Bangladesh Forest Research Institute (BFRI), Chattogram, Bangladesh.

Preparation of plant samples by extraction: After harvesting, the plant parts were cleaned with water and shed-dried at 35-50°C. The dried plants were ground into coarse powder. About 600 g of the powder was taken in an amber colored glass container and soaked in 95% ethanol (3 L) at room temperature for several days. Then the mixture was filtered through Whatman filter paper number 1. The filtrate was evaporated with a rotary evaporator at reduced temperature and pressure. Later, modified Kupchan method (VanWagenen *et al*., 1993) was employed for solvent-solvent partitioning of the crude extract of *E. alba* (15.0 g) to obtain *n*-hexane (3.27 g), chloroform 2.05 g) and aqueous soluble fraction (2.13 g). All these plant samples were screened for phytochemical and pharmacological evaluation.

Preliminary screening for phytoconstituents: Preliminary phytochemical screening (Ahamed *et al*., 2021) was carried out to ensure the phytochemical constituents of the different solvent fractions of *E. alba* by using standard methods as mentioned in table 1.

DPPH assay method: DPPH assay was utilized to estimate the antioxidant potential of the plant samples according to the reported method (Brand-Williams *et al*., 1995) with minor modification (Islam *et al*., 2019).

Anti-inflammatory activity

Inhibition of egg albumin denaturation: Anti-inflammatory effect of the *E. alba* extractives was evaluated by inhibition of egg albumin denaturation assay (Ahamed *et al*., 2021). Briefly, 1.0 ml of 5% egg albumin solution and 2.8 ml phosphate buffer (pH 6.4 ± 0.2) were added to sterile test tubes. Later on, aspirin (0.1 mg) and tween-80 were added as positive- and negative controls, while the test sample of *E. alba* (500 µg/ml in tween-80) was considered as the test group, respectively. After heating at 57°C for 20 min, the reaction mixtures were allowed to cool down and filtered. Absorbance of reaction mixture was measured for each concentration (125, 250 and 500 µg/ml) at 660 nm using a UV-visible spectro-photometer. Anti-inflammatory activity was estimated by measuring the percentage of inhibition of protein using the following formula (Ahamed *et al*., 2021).

$$\% \text{ Inhibition of protein denaturation} = \frac{A_{control} - A_{test}}{A_{control}} \times 100\%$$

Here, A= Absorbance for respective group.

RBC membrane stabilization assay: RBC membrane stabilization assay method described by Chowdhury *et al.* (2021) and Ahamed *et al.* (2021) was utilized to assess the *in vitro* anti-inflammatory activity of *E. alba.* In this test, 1.0 ml of phosphate buffer (pH 7.4), 2.0 ml of hyposaline and 0.5 ml of human RBCs suspension were added to sterile tubes. Later on, aspirin (0.1 mg) was mixed for standard group, Distilled water (1.0 ml) was added to the control tube while 1.0 ml of plant sample (125 µg/ml, 250 µg/ml and 500 µg/ml) was mixed to the test groups. After incubation (37ºC for 30 min) and centrifugation (10 min at 3000 rpm), the absorbance of the supernatant was measured by using a UV-visible spectrophotometer at 560 nm. Membrane stabilization activity of each test sample was estimated as follows.

$$\% \text{ Inhibition of hemolysis} = \frac{A_{control} - A_{test}}{A_{control}} \times 100\%$$

Here, A= Absorbance for respective group.

Antimicrobial activity: Antimicrobial activity of *E. alba* extracts was evaluated by the agar diffusion assay (Akhter *et al.,* 2020) using a variety of test microorganisms (Table 2). Azithromycin and fluconazole were used as standards. The organisms were obtained as pure culture from the Faculty of Biology, University of Chittagong, Bangladesh. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

Thrombolytic activity assay: The plant extracts were evaluated for blood clotting ability following the guidelines explained by Chowdhury *et al*. (2021). Streptokinase (SK) was used as standard in this assay.

Results and Discussion

Medicinal plant is the reservoir of valuable bioactive constituents which could serve as leads for new drug design. Phytochemical screening is a qualitative analysis which is considered as a basic step in finding the bioactive principles present in the plant extracts (Jitendra *et al*., 2011). The main objective of phytochemical studies is to provide information about the class of secondary metabolites present in the plant extracts. Knowledge of these chemical compounds is very useful for development of pharmaceuticals (Jitendra *et al*., 2011). Our preliminary phytochemical study exposed the occurrence of important phytoconstituents such as alkaloids, glycosides, steroids, tannins, saponins, reducing sugars and flavonoids in the *n*-hexane, chloroform and ethanol extracts of *E. alba* (Table 1). The finding of this study closely resembles the published report on phytochemical composition of *E. alba* (Thenmozhi *et al*., 2019).

Table 1. Phytochemical screening of *n*-hexane, chloroform and aqueous soluble fractions of ethanol extract of *E. alba* whole plant.

Phytochemical	Test sample		
	n-hexane fraction	Chloroform fraction	Aqueous fraction
Reducing sugar	+	-	-
Steroids	+	+	+
Glycosides	+	+	+
Tannins	+	+	+
Alkaloids	+	+	+
Flavonoids	-	+	+
Saponins	-	+	+
Gums	-	-	-
Amides	+	-	+

'+' = present, '-'= absent.

Quantitative antioxidant activity of the fractionated extracts of *E. alba* was investigated using the DPPH scavenging assay with ascorbic acid as reference. DPPH assay is based on accepting of hydrogen atom by DPPH compound from antioxidant molecule (Anjum *et al*., 2022). As shown in figure 1A, the DPPH radical scavenging activity of different solvent fractions revealed a significant and concentration-dependent antioxidant potential which

is similar to the standard ascorbic acid. The fractionated extracts of *E. alba* at maximum concentration of 100 µg/ml showed appreciable DPPH free radical scavenging activities with the inhibition of 87.5% for aqueous fraction, 85.75% for chloroform fraction and 73.98% for *n*-hexane fraction against ascorbic acid (90.61% inhibition). The half minimal inhibitory concentration (IC_{50}) was 78.6, 129.6 and 151 µg/ml for aqueous, *n*-hexane and chloroform soluble fraction, respectively whereas for ascorbic acid, it was 46.3 µg/ml (Figure 1B). These findings agree with previous report (Patel *et al.*, 2016) where the hydroalcoholic extract of *E. alba* efficiently showed potent antioxidant activity by scavenging DPPH radical in a dose-dependent manner. In another work, 80% alcoholic extract of *E. alba* also revealed the DPPH scavenging activity with IC_{50} value of 136.57 ± 6.83 µg/ml (Yadav *et al.*, 2017; Mittal *et al.*, 2018). The antioxidant property of a plant extract is generally associated with the presence of polyphenolic compounds like flavonoids, tannins and phenolic acids that can scavenge DPPH radicals by their hydrogen donating ability (Rashid *et al.*, 2022; Muley *et al.*, 2009). The findings of our present study suggest that all the extracts from *E. alba* showed antioxidant potential by free radical quenching capacity through the mechanism of electron transfer or hydrogen donation.

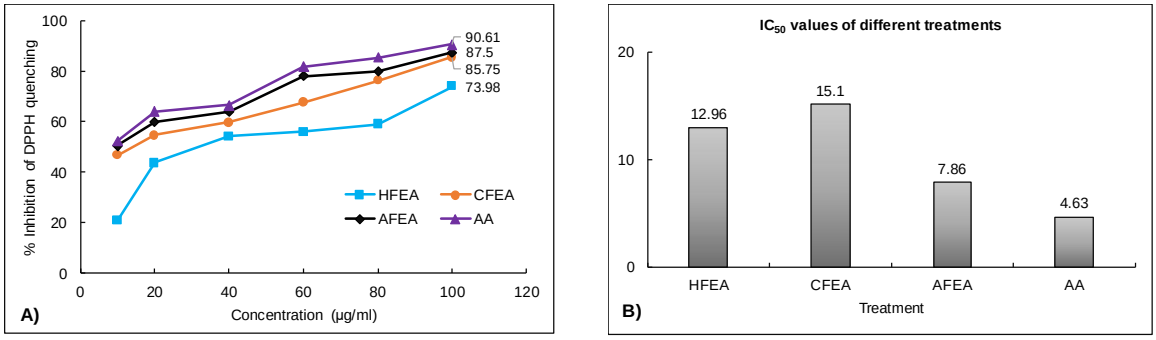

Figure 1. Antioxidant activities of *n*-hexane (HFEA), chloroform (CFEA) and aqueous soluble fractions (AFEA) of *E. alba* in comparison with standard ascorbic acid (AA).

Inflammation is considered as a primary stage of healing process by removing the harmful stimuli such as pathogens, damaged cell, or irritants from the living system (Adebayo *et al.*, 2015). From previous evidence of other species in asteraceae family, different solvent fractions of *E. alba* were screened for anti-inflammatory activity using two authentic *in vitro* models- inhibition of egg albumin denaturation assay and RBC membrane stabilizing method. Albumin denaturation is associated with inflammatory response including arthritis. By inhibition of albumin denaturation, inflammatory activity can be inhibited (Adebayo *et al.*, 2015). Therefore, plant extract that can suppress the protein denaturation and stabilize cell membrane against lysis could serve as a probable source of anti-inflammatory drug candidates.

In the present study, all the test materials of *E. alba* have prominent effect to inhibit the albumin denaturation which is comparable to standard aspirin. The aqueous soluble fraction at the dose of 500 µg/ml showed the maximum inhibitory capacity (90.12%) followed by the chloroform (82.58%) and the *n*-hexane fraction (61.91%), while the standard drug aspirin exhibited 96.09% inhibition of albumin denaturation (Figure 2A). As part of our investigation, anti-inflammatory activity of *E. alba* was further analyzed by RBC membrane stabilization method. Human RBC membranes are comparable to the liposomal membrane, so the prevention of RBC membrane breakdown has been used as a measure for estimating the anti-inflammatory property of plant extract.

Concentration-dependent anti-inflammatory activity was observed in both *in vitro* models (Figure 2A and Figure 2B). Similar to egg albumin denaturation method, the various solvent fractions of *E. alba* also inhibited RBC membrane hemolysis induced by hypotonic solution. As shown in figure 2B, the aqueous soluble fraction showed 78.42% inhibition of hypotonic solution-induced hemolysis, which is comparable to the standard aspirin (85% inhibition of hemolysis) (Figure 2B). All the fractions protected RBC membrane against hypotonic solution-induced lysis and its stabilization implies that the plant, *E. alba* may stabilize lysosomal membranes. Stabilization of liposomal membrane is important in inhibiting the inflammatory response at the site of injury. The results of our study are similar to the published report (Kumar *et al*., 2005) where the anti-inflammatory effect of the chloroform extract of *E. alba* was explained by using various *in vivo* models (Ahamed *et al*., 2021). In another study, the methanol extract and its organic fractions of *E. prostrata* showed dose-dependent anti-inflammatory activity on carrageenan-induced paw edema in rats. Similarly, the methanolic extract of *E. prostrata* leaves at a concentration of 100-and 200 mg/kg showed the

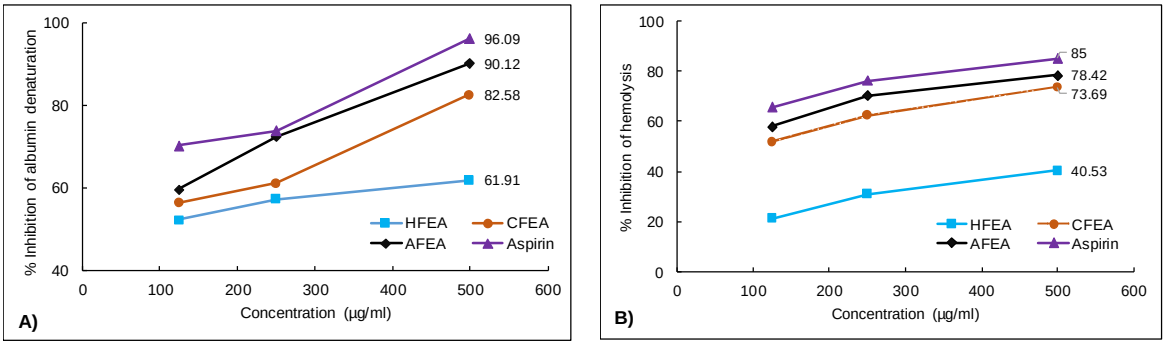

Figure 2. Anti-inflammatory activity of *n*-hexane (HFEA), chloroform (CFEA) and aqueous fractions (AFEA) of *E. alba* by inhibition of albumin denaturation (A) and by RBC membrane stabilization method (B).

Table 2. Antimicrobial activity of the *n*-hexane (HFEA), chloroform (CFEA) and aqueous (AFEA) soluble fractions of *E. alba*.

Test microorganisms	Zone of inhibition (mm)			
	HFEA	CFEA	AFEA	Standard
Gram positive bacteria				Azithromycin
Bacillus azotoformans	--	18	19	15
Bacillus cereus	11	18	21	20
Lactobacillus casai	13	22	23	21
Staphylococcus aureus	13	22	23	19
Gram negative bacteria				
Escherichia coli	--	16	18	16
Vibrio cholera	--	22	22	19
Fungus				Fluconazole
Aspergillus niger	12	13	10	20
Pityrosporum ovale	21	15	13	26
Trichophyton sp.	19	18	16	24

significant dose-dependent anti-inflammatory activity in carrageenan and egg albumin-induced hind paw edema in rats (Hossain *et al*., 2011). Previous studies have reported that phytochemicals such as

flavonoids, terpenoids and saponins showed anti-inflammatory activity by inhibiting the synthesis and release of prostaglandins and polypeptide kinins (Arunachalam *et al*., 2009). Therefore, the presence of flavonoids and saponins in the test extracts of *E. alba* may be responsible for its anti-inflammatory activity via the mechanism as discussed above.

Active principles isolated from medicinal plants appear to be one of the important alternative approaches to encompass antibacterial activity and hence antibiotic resistance and the management of infectious diseases. Compared to synthetic antibiotics, plant-based therapeutic agents have less or no side effects (Hossan *et al*., 2018). From this view point, fractionated extracts (*n*-hexane, chloroform and aqueous fraction) of *E. alba* were screened for antimicrobial potential against six bacterial strains and three fungal strains (Table 2). All the test samples of *E. alba* at a concentration of 500 µg/disc exhibited pronounced antibacterial activity with zone of inhibition ranging from 11 to 23 mm, while the fungal strains were relatively less susceptible to the test materials with zone of inhibition ranging from 10 to 21 mm (Table 2). Compared to the hexane soluble fraction, both chloroform and ethanol fractions exhibited strong growth inhibitory activity against most of the test microbes. The chloroform soluble fraction showed prominent activity against *Lactobacillus casai* (22 mm), *Staphylococcus aureus* (22 mm) *and Vibrio cholera* (22 mm). The results of antifungal test indicated that both *Pityrosporum ovale* and *Trichophyton sp.* were mostly sensitive to all the test samples. Our results are in agreement with published reports where the authors confirmed the antibacterial potential of aerial parts extracts of *E. alba* by agar well diffusion methods (Bakhit *et al.*, 2011). Recently, Sollepura *et al.* (2019) reported the antifungal activity of the methanol fractions of *E. alba* against pathogens present in cereal grain. The observed antimicrobial activity in our experiment may be due to secondary metabolites such as tannins, alkaloids, phenolic compounds and flavonoids present in the test extracts of *E. alba.*

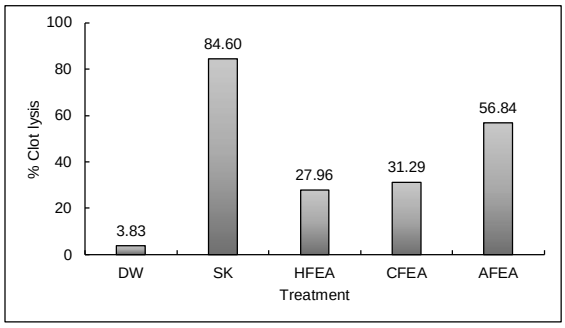

Figure 3. Thrombolytic activity of *n*-hexane (HFEA), chloroform (CFEA) and aqueous soluble fractions (AFEA) of *E. alba.*

During the screening for thrombolytic property, addition of 100 µl of streptokinase (SK), as positive control to blood clot showed 84.60% clot lysis, while 100 µl of deionized water (DW) as negative control exhibited only negligible (3.83%) clot lysis. Treatment of clots with 100 µl of aqueous, chloroform and *n*-hexane soluble fractions of *E. alba* showed 56.84, 31.29% and 27.96% clot lysis, respectively (Figure 3). The above results indicate that all the test samples from *E. alba* showed mild to low thrombolytic activity. Medicinal plants can serve as the treatment of atherothrombosis by various mechanisms of the action. Blood clotting property of plant extracts primarily appears in their manifold effects such as antioxidant, anti-inflammatory, hypotensive, lipid-lowering, anti-thrombotic, etc. Moreover, the plants-derived secondary metabolites such as flavonoids, tannins, phenolics etc. are characterized as potential thrombolytic agents (Kirichenko *et al*., 2020).

Conclusion

Our study emphasized the *in vitro* antioxidant, anti-inflammatory, antimicrobial and thrombolytic activities of different solvent fractions of *E. alba*. Among all, the aqueous fraction showed promising antioxidant activity via scavenging the DPPH radical. Egg albumin denaturation and RBC membrane stabilization tests revealed anti-inflammatory activity of the plant samples comparable to standard aspirin.

The antimicrobial test displayed that all the solvent fractions have significant growth inhibitory activity against majority of the tested microorganisms. Thus, the study rationalizes the traditional uses of *E. alba* as folk medicine. However, further study is needed to find out the mechanisms underlying these bioactivities.

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