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Full Length Article

Exploration of Pharmacological and Toxicological Properties of Aerial Parts of *Blumea lacera,* a Common Weed in Bangladesh

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ABSTRACT

Background: *Blumea lacera* (Family Asteraceae) is a herb found in the uncultivated lands of many Asian countries. This plant is used by quack doctors for some medicinal purposes.

Objective: Works presented in this manuscript were conducted on the ethanol extract of aerial parts of *B. lacera*. Various pharmacological tests like antioxidant, analgesic, anti-inflammatory, antidiarrheal, diuretic, antibacterial, anthelmintic and toxicological tests like brine shrimp lethality and acute toxicity were evaluated.

Methods: Antioxidant test was conducted by determining secondary metabolites content and DPPH free radical scavenging assay. Acetic acid-induced writhing method, xylene-induced ear edema method, castor oil-induced diarrheal method, and urinary volume test were performed to evaluate the analgesic, anti-inflammatory, antidiarrheal and diuretic effects, respectively. The antibacterial test was performed by disk diffusion method, anthelmintic activity by recording the paralysis and death time of *P. cervi* and finally, cytotoxic activity was performed by brine shrimp lethality bioassay.

Results: TPC, TFC and TTC contents of *B. lacera* extract were found to be 9 mg GAE/g, 31 mg QE/g and 18 mg GAE/g. The SC_{50} value of the DPPH radical scavenging assay was found to be higher than 3000 $\mu g \cdot mL^{-1}$. The LD_{50} value of *B. lacera* extract in mice was higher than 5000 $mg \cdot kg^{-1}$. This extract significantly inhibited writhing reflexes by 24.5% and 43.6% and reduced ear edema up to 24.6% and 41% at 250 and 500 $mg \cdot kg^{-1}$ bw doses, respectively. It also increased the latency of the first defecation period up to 52.8 min and 106.6 min as well as decreased the stool count by 34.1% and 48.2% at 250 and 500 $mg \cdot kg^{-1}$ bw doses, respectively. It also showed an increase in urinary output in the diuretic test. Good antibacterial activity of *B. lacera* extract was confirmed by retardation in bacterial growth and significantly killed *P. cervi* in a dose-dependent manner. In brine shrimp lethality bioassay, the LC_{50} value was found to be 5.4 $\mu g \cdot mL^{-1}$.

Conclusion: *B. lacera* showed mild analgesic, anti-inflammatory and antidiarrheal properties. It could be used as a diuretic, antibacterial, anthelmintic and cytotoxic drug which justify the plant's traditional uses in folk medicine.

1. Introduction

Nature has always blessed us with its numerous medicinal plants. To get a cure for diseases, the dependence on medicinal plants is as old as human civilization. Most of the prescribed drugs are still derived from the plant kingdom. Due to the high expense of treatment, people of third-world countries often rely on medicinal plants to get cures for diseases. In developing countries, up to 80% of the population still use herbal medicines to meet their primary health care needs (Hoyler et al., 2018). *Blumea lacera* (Burm. F.) is a perennial, branched Rabi weed of India, Bangladesh, Srilanka, Nepal (Fig. 1A). This 1–1.5 m long herb is from the Asteraceae family and is locally known as Janglimuli, Kukurshunga, Kakaronda, Siyalmutra, Susksampatra, etc. (Mishra et al., 2015). In Bangladesh, this herb is almost found all over the world mainly in uncultivated lands. Leaves of this plant are obovate, 5–12 cm long, have a very pungent odor due to turpentine. The bright yellow flowers are arranged in axillary cymes. The yellow flowers are spiked in shape. Small fruits appear normally in December-March (Mishra et al., 2015; Pratap and Parthasarathy, 2012).

Khatri et al. reported that the essential oils obtained from its leaves are enriched with many important compounds like flavones, triterpenes, β-sitosterol, stigmasterol-3-O-β-D-glucopyranoside, cineol, campesterol, hentriacontane, lupeol, artemisinin, protocatechuic acid, etc. (Khatri et al., 2016) while Mokat et al. reported the presence of isooctane, α-copaene, β-caryophyllene, γ-cadinene, phytol, palmitic acid, stearic acid, pentadecanoic acid, methyl palmitate, etc. (Mokat et al. 2020) by conducting gas chromatography-mass spectroscopy. This plant has much importance in ethnomedicine. Traditionally, this plant is important for many therapeutic properties

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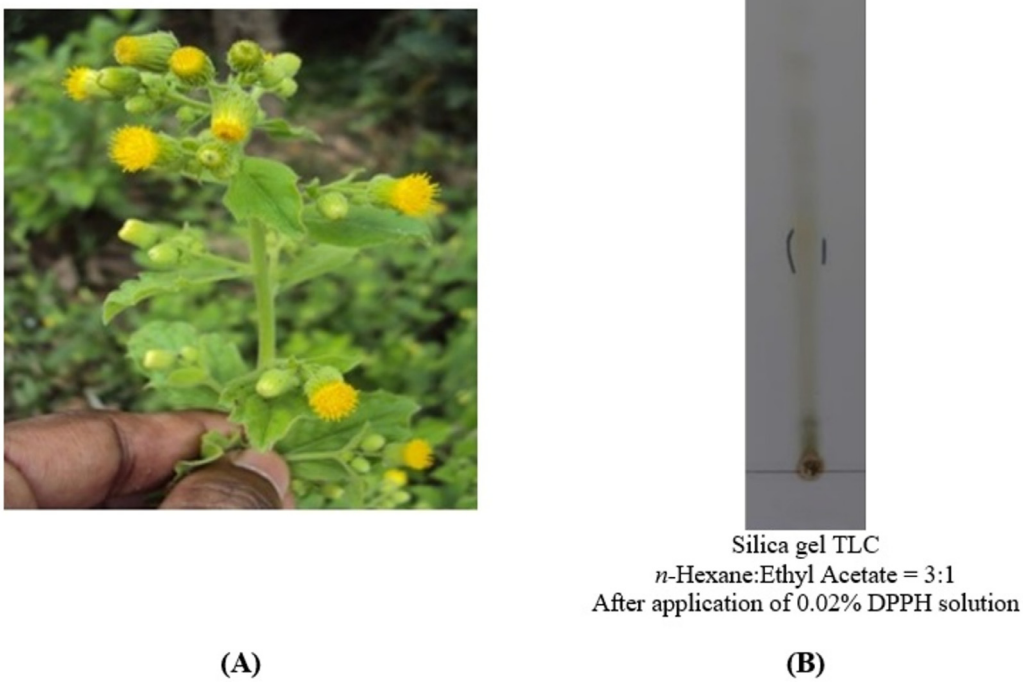

Fig. 1. **(A)** Photo of *Blumea lacera* plant, **(B)** Chromatogram of *B. lacera* extract on silica gel TLC.

like antipyretic, anti-inflammatory, anthelmintic, diuretic, antidiarrheal, antimicrobial, cytotoxic, astringent, liver tonic, tranquilizing, etc. (Hossen et al., 2021; Khatri et al., 2016). Compared to the abundance of many bioactive compounds and diverse medicinal importance, there are very few scientific reports were conducted on this plant. So, we aimed our focus to investigate different types of biological experiments on this plant to rationalize its uses.

2. Materials and Methods

2.1. Plant collection and identification

The aerial parts of *B. lacera* were collected from the Teknaf, Cox's Bazar, Bangladesh in February 2016. Precautions were taken during plant collection to avoid adulterants. After collection, the dried plant samples were sent to Bangladesh National Herbarium, Dhaka and the expert provided an authentication No. DACB- 43,174 for future reference.

2.2. Extract preparation

After collection, the leaves were shade dried for 50 days. The dried leaves were then ground to have fine powder by a suitable grinder. Cold extraction with 96% ethanol of 250 g powder extract was carried out for 15 days. Finally, 8.95 g gummy crude extract was obtained (yield = 3.58% w/w) with the help of a rotary evaporator.

2.3. Chemicals and reagents

For the analytical and pharmacological test, laboratory-grade reagents were used such as Na_2CO_3 (Loba, India), $NaNO_2$ (Loba, India), $AlCl_3$ (Loba, India), NaOH (Loba, India), Folin-Ciocalteu (FC) reagent (Merck, Germany), quercetin (Merck, Germany), gallic acid (Sigma Aldrich, USA), $FeCl_3$ (Merck, Germany), ascorbic acid (Sigma-Aldrich, USA), xylene (Sigma Aldrich, USA), acetic acid (Merck, Germany). Diclofenac sodium, frusemide and loperamide were procured from Square Pharmaceuticals Ltd. And Ibuprofen was purchased from ACI Pharmaceuticals Limited, Bangladesh.

2.4. Animals

To conduct analgesic, anti-inflammatory, antidiarrheal, diuretic and acute toxicity tests, 5–6 weeks aged male Swiss albino mice (*Mus musculus*), possessing body weight of 25–30 g were purchased from Animal house of Pharmacology Laboratory, Jahangirnagar University. The mice were fed with normal rodent food and water and kept in 2–3 weeks in the Animal house of Pharmacy Discipline, Khulna University for their adaptation. To conduct the pharmacological tests, a total of 100 mice were used. The tests were conducted in a noiseless, calm environment in a separated room and for each test conducted on mice, every experimental group consisted of 5 mice. *P. cervi* were collected from intestines of freshly slaughtered cattle from local abattoirs to conduct the anthelmintic test and eggs of brine shrimp were collected from local fish hatchery for the cytotoxic test.

2.5. Phytochemical assessment

To conduct the phytochemical test, a 5% (w/v) solution of *B. lacera* extract was prepared. Then, qualitative phytochemical screening of the extract was conducted by the methods with some modifications to determine the presence of different phytochemical groups (Ayoola et al., 2008; Chimahali et al., 2019; Yadav and Agarwala, 2011; Amer et al., 2004).

2.5.1. Preparation of the reagents

- *Mayer's Reagent:* 1.36 g mercuric iodide in 60 mL of water was mixed with a solution containing 5 g of potassium iodide in 20 mL of water.
- *Dragendroff's Reagent:* 1.7 g basic bismuth nitrate and 20 g tartaric acid ware dissolved in 80 mL water. This solution was mixed with a solution containing 16 g potassium iodide and 40 mL water.

- *Fehling's Solution A:* 34.64 g copper sulfate was dissolved in a mixture of 0.5 mL of sulfuric acid and sufficient water to produce 500 mL.
- *Fehling's Solution B:* 176 g of sodium potassium tartrate and 77 g of sodium hydroxide were dissolved in sufficient water to produce 500 mL. Equal volumes of solutions A and B were mixed at the time of use.
- *Benedict's Reagent:* 1.73 g cupric sulfate, 1.73 g sodium citrate and 10 g anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 mL with water.
- *Molish Reagent:* 2.5 g of pure α-naphthol was dissolved in 25 mL of ethanol.

2.5.2. Phytochemical test

- *Carbohydrates/ reducing sugar test:*
  a) Benedict's test: 0.5 mL of aqueous extract of *B. lacera* was taken in a test tube. 5 mL of Benedict's solution was added to the test tube, boiled for 5 min and allowed to cool spontaneously. A red color precipitate of cuprous oxide indicates the presence of reducing sugar.
  b) Fehling's test: 2 mL of an aqueous extract of *B. lacera* was added to 1 mL of a mixture of equal volumes of Fehling's solutions A and B and boiled for a few minutes. A red or brick red color precipitate formation indicates the presence of reducing sugar.
- *Tannins test:* 5 mL solution of the *B. lacera* extract was taken in a test tube. Then 1 mL of 5% ferric chloride solution was added. Greenish black precipitate indicates the presence of tannins.
- *Phenols test:* 3 mL distilled water was added in 1 mL *B. lacera* extract. A few drops of 10% ferric chloride solution were added. Greenish black precipitate indicates the presence of phenols.
- *Flavonoids test:*
  a) 5 mL of dilute ammonia solution was added to a portion of the aqueous filtrate of *B. lacera* extract followed by the addition of concentrated H_2SO_4. A yellow coloration indicates the presence of flavonoids. The yellow color will disappear on standing.
  b) 5 mL of *B. lacera* extract was dissolved in dilute sodium hydroxide and then neutralized with dilute hydrochloric acid. The formation of yellow color and the disappearance of color indicates the presence of flavonoids.
- *Saponins test:* 1 mL solution of the *B. lacera* extract was diluted with distilled water to 20 mL and shaken in a graduated cylinder for 15 min. A layer of foam above the solution indicates the presence of saponins.
- *Steroids test:* 1 mL of *B. lacera* extract was taken in 5 mL chloroform and then 1 mL sulphuric acid was added. A red color indicates the presence of steroids.
- *Alkaloids test:*
  a) *Mayer's test:* 2 mL solution of the *B. lacera* extract and 5 mL of dilute hydrochloric acid (1%) were taken in a test tube. Then 1 mL of Mayer's reagent was added. A white or creamy white color precipitate indicates the presence of alkaloids.
  b) *Dragendroff's test:* 2 mL solution of the *B. lacera* extract and 5 mL of dilute hydrochloric acid (1%) were taken in a test tube. Then 1 mL of Dragendroff's reagent was added. Orange-brown precipitate indicates the presence of alkaloids.
- *Glycosides test:* A small amount of an alcoholic extract was taken in 1 mL of water. A few drops of aqueous NaOH were added. A yellow color indicates the presence of glycosides.
- *Terpenoids test:* 2 mL of chloroform was added to 0.5 g of the *B. lacera* extract, followed by the addition of 3 mL concentrated H_2SO_4 to form a layer. A reddish-brown coloration at the interface indicates the presence of terpenoids.
- *Gums test:* 5 mL solution of the *B. lacera* extract was taken and added with 1 mL of Molish's reagent and 1 mL of H_2SO_4 was added. Red violet ring produced at the junction of two liquids indicates the presence of gums.
- *Acidic compounds test:* Sodium bicarbonate solution was added to the alcoholic extract and observed for the production of effervescences. Production of effervescences indicates the presence of acidic compounds.

2.6. In vitro antioxidant assays

2.6.1. Qualitative antioxidant assay

To find the existence, of the antioxidant compounds, a qualitative antioxidant test of *B. lacera* extract was conducted by the method of using the thin layer chromatography (TLC) technique (Sadhu et al., 2003). A minute amount of plant solution (in methanol) was spotted on silica gel precoated TLC plates and a chromatogram was allowed to be developed by placing them in different solvent systems. After drying, those were observed under Ultraviolet (UV) light at 254 nm and 364 nm wavelength, and 0.02% 2,2-diphenyl-1-picryl hydrazyl (DPPH) solution was sprayed on the plates for the confirmation of antioxidant compounds.

2.6.2. Quantitative antioxidant assay

a) *Determination of secondary metabolites content*

Determination of Total Phenolic Content (TPC)

TPC of *B. lacera* extract was measured by using Folin Ciocalteu (FC) reagent where gallic acid was used as standard (Jahan et al., 2021). TPC was calculated from the calibration curve and expressed as mg of gallic acid equivalent (GAE) per gram of dry extract.

Determination of Total Flavonoid Content (TFC)

TFC of *B. lacera* extract was measured by the method described by Jahan et al. (2021) by using aluminum chloride colorimetric assay where quercetin was used as standard. TFC was calculated from the calibration curve and expressed as mg of quercetin equivalent (QE) per gram of dry extract.

Determination of Total Tannin Content (TTC)

TTC of *B. lacera* extract was measured by the method described by Jahan et al. (2021) by using Folin Ciocalteu (FC) reagent where gallic acid was used as standard. TTC was calculated from the calibration curve and expressed as mg of gallic acid equivalent per gram of dry extract.

b) *DPPH free radical scavenging assay*

This was done by the method described by (Biswas et al., 2018) by the modified microplate method. Different concentrations 1–4096 $\mu g \cdot mL^{-1}$ of *B. lacera* extract and ascorbic acid were prepared and 0.008% DPPH solution (in methanol) was added with each concentration. Taking the absorbance at 517 nm wavelength, free radical scavenging activity was calculated from the calibration curve of log concentration vs percent inhibition and was expressed in SC_{50} (concentration required to scavenge 50% DPPH free radicals).

2.7. Assessment of acute toxicity

The acute toxicity property of *B. lacera* extract on Swiss albino mice was evaluated by the method described by (Khan and Akhtar, 2012) with some modifications. Crude extract of *B. lacera* was prepared at 1000, 3000 and 5000 $mg \cdot kg^{-1}$ bodyweight (bw) doses in 1% tween-80 in water and orally given to the mice of different groups. The mice were observed for 24 h and the death of any mice was recorded for the next 7 days to determine the LD_{50} (50% lethal dose) value. During that period particular attention was given to observing different conditions such as convulsion, tremor, lethargy, sedation, etc.

2.8. Assessment of peripheral analgesic activity

Peripheral analgesic activity of *B. lacera* extract was evaluated by the acetic acid-induced writhing method in mice described by (Debnath et al., 2021). Mice of negative control group were treated with 1% tween-80 in water solution. Plant extract at 250 $mg \cdot kg^{-1}$ and

500 mg·kg^{-1} and diclofenac sodium at 25 mg·kg^{-1} bw doses were prepared in 1% tween-80 in water solution and those were orally administered to different mice groups. 0.7% acetic acid was intraperitoneally injected 30 min later to induce writhing. The inhibition of writhing compared to the control group was calculated by the following formula:

$$\text{Inhibition of writhing } (\%) = \left[\left(W_c - W_t\right)/W_c\right] \times 100$$

Where, W_c = average number of writhing in control group
W_t = average number of writhing in test group

2.9. Assessment of anti-inflammatory activity

Xylene-induced ear edema method in mice was performed by the method of (Karim et al., 2019) with some modifications. Mice of negative control group were treated with 1% tween-80 in water solution. Plant extract at 250 mg·kg^{-1} and 500 mg·kg^{-1} and ibuprofen at 100 mg·kg^{-1} bw doses were prepared in 1% tween-80 in water solution and those were orally administered to different mice groups. After 1 h, 20 µL xylene was rubbed on both the anterior and posterior surfaces of the right ear lobe of each mouse at 0.01x doses (x = body weight of mice in g). After 1 h of xylene application, a circular section of 3 mm diameter of each ear was taken with a cork borer and weighed. Finally, the inhibition of edema was calculated by the following formula:

$$\text{Inhibition of edema or inflammation } (\%) = \left[\left(I_c - I_t\right)/I_c\right] \times 100$$

Where I_c = average weight of ear section in control group
I_t = average weight of ear section in test group

2.10. Assessment of antidiarrheal activity

Castor oil induced-diarrheal method in mice was conducted to mice by the method described by (Jahan et al., 2021) for assessing the antidiarrheal activity of *B. lacera* extract. Mice of negative control group were treated with 1% tween-80 in water solution. Plant extract at 250 mg·kg^{-1} and 500 mg·kg^{-1} and loperamide at 3 mg·kg^{-1} bw doses were prepared in 1% tween-80 in water solution and those were orally administered to different mice groups. After 30 min, 0.5 mL castor oil was orally fed to each mouse to induce diarrhea and those were placed in separate cages having blotting paper. Then, the mice were observed for the next 4 h for measuring the latent defecation time and no. of excreted stools. Percent inhibition of defecation was calculated by the following formula:

$$\text{Defecation reduction } (\%) = \left[\left(S_c - S_t\right)/S_c\right] \times 100$$

Where S_c = average number of stool in control group
S_t = average number of stool in test group

2.11. Assessment of diuretic activity

The diuretic effect of *B. lacera* extract was done by the method described by (Saha et al., 2021). Mice of negative control group were treated with 1% tween-80 in water solution. Plant extract at 250 mg·kg^{-1} and 500 mg·kg^{-1} and frusemide at 5 mg·kg^{-1} bw doses were prepared in 1% tween-80 in water solution. Those prepared solution were orally given to each mouse in such a way that each mouse was fed with 2 mL solution. The mice were placed in metabolic cages and after a certain period, the volume of urine was measured. The diuretic activity of *B. lacera* extract was calculated by the following equations:

$$\text{Urinary excretion} = \left[\text{Total urinary output } \left(V_o\right)/ \text{Total liquid administered } \left(V_a\right)\right] \times 100$$

Diuretic action was measured by dividing the urinary excretion of the test group and the control group. Finally, diuretic activity was measured by dividing the diuretic action of the test group and the control group.

2.12. Assessment of antibacterial activity

To determine the antibacterial activity of *B. lacera* extract, the disk diffusion assay method was performed according to the method of (Bauer, 1966). Six species of gram-negative bacteria (*Vibrio cholerae, Shigella dysenteriae, Salmonella typhi, Escherichia coli, Pseudomonas aurigenosa, Proteus* spp) and two species of gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogens*) were taken to conduct the study. Nutrient agar media and nutrient broth media were prepared and the bacteria were cultured 2 times. The second culture was incubated in nutrient broth media then transferred to separate petridishes containing nutrient agar media. Filter paper discs of 250 µg/disk and 500 µg/disk and kanamycin 30 µg/disk were placed in four regions of each petridish. After incubating those petridishes overnight at 37 °C, the zone of inhibition of bacterial growth was measured by a calibrated scale.

2.13. Assessment of anthelmintic activity

Anthelmintic activity of *B. lacera* extract was determined by the method described by (Saha et al., 2021). Live *Paramphistomum cervi* were collected from the intestine of freshly slaughtered cattle at the local slaughterhouse. *B. lacera* extract was prepared at 6.25–50 mg·mL^{-1} and albendazole at 15 mg·mL^{-1} were placed in separate petridishes while one petri dish was filled with normal saline solution and that was considered as a negative control group. Six *P. cervi* nematodes were placed in those petridishes. Then after waiting for a while, both the paralysis (movement after shaking) and death time (no movement even after vigorous shaking) of the nematodes were recorded.

2.14. Assessment of cytotoxic activity

Brine shrimp lethality bioassay technique was performed according to the method described by (Debnath et al., 2020) to investigate the cytotoxicity of *B. lacera* extract. Eggs of *Artemia salina* were hatched to obtain live nauplii. Different concentrations (0.1562–640 µg·mL^{-1}) of *B. lacera* extract and vincristine sulfate were prepared and 10 live nauplii of *A. salina* were put in each test tube and were kept for 24 h. Then percent mortality was calculated from the following equation to determine LC_{50} (concentration needed to kill 50% nauplii):

$$\%\ \text{Mortality} = \left[\left(L_c - L_s\right)/L_c\right] \times 100$$

Where L_c = average number of alive shrimp of control solution
L_s = average number of alive shrimp of sample solution

2.15. Statistical analysis

Values presented in this manuscript are expressed as average ± standard deviation (SD). A one-way Analysis of Variance test was used to conduct a statistical comparison of values among the groups and it was followed by Tukey as a post hoc test by using SPSS (version 25) (*IBM*, USA). In these results, the statistical significance level was considered as $P < 0.05$. The graphs were prepared using Graph pad prism software (version 6) (*GraphPad Software*, LLC, USA) (Saha et al., 2021).

3. Results

3.1. Phytochemical assessment

In the phytochemical test, *B. lacera* extract was found to contain carbohydrates, tannins, phenols, flavonoids, saponins, alkaloids, glycosides, terpenoids, gums, etc. (Table 1).

Table 1
Results of phytochemical assessment.

Test	Reagent	Standard	Observation for *B. lacera* extract	Inference
Reducing sugars test	Fehling's Solution	Dextrose	Brick red colored precipitate was formed	Presence of reducing sugar
	Benedict's reagent	Dextrose	Brick red colored precipitate was formed	Presence of reducing sugar
Tannins test	Ferric Chloride solution	Rose petal	Greenish black precipitate was formed	Presence of tannin
Phenols test	Ferric Chloride solution	Rose petal	Dark greenish precipitate was formed	Presence of phenols
Flavonoids test	Concentrated H_2SO_4	Quercetin	A yellow coloration was observed and the yellow coloration disappeared on standing	Presence of flavonoids
	Dilute HCl Dilute NaOH	Quercetin	A yellow coloration was observed and disappearance of color	Presence of flavonoids
Saponins test	Distilled water	Sugarcane juice	No layer of foam was produced	Absence of saponin
Steroids test	Sulphuric acid	Norgestrel tablet	No red color was observed.	Absence of steroids
Alkaloids test	Mayer's reagent	Nicotine	A yellowish buff-colored precipitate was obtained	Presence of alkaloids
	Dragendroff's reagent	Nicotine	An orange-brown precipitate was observed	Presence of alkaloids
Glycosides test	NaOH solution	Aloe vera	Yellow color was found	Presence of glycosides
Terpenoids test	H_2SO_4	Menthol	A reddish brown coloration at the interface was formed	Presence of terpenoids
Gums test	Molish's reagent	Liquid glue	Reddish ring	Presence of gum
Tests for acidic compounds	Sodium bicarbonate solution	Lemon juice	Effervescence was not produced	Absence of acidic compounds

Table 2
Total content of secondary metabolites and approximate SC_{50} values of DPPH free radical scavenging assay of *B. lacera* extract.

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg GAE/g)	DRSA (SC_{50} μg·mL^{-1})
B. lacera extract	9	31	18	> 3000
Ascorbic acid				15

TPC (Total Phenolic Content), TFC (Total Flavonoid Content), Total Tannin Content (TTC), DRSA (DPPH Radical Scavenging Activity).

3.2. In vitro antioxidant tests

3.2.1. Qualitative antioxidant test

Upon development, *B. lacera* extract produced a few yellowish spots on TLC plates after spraying of DPPH solution which indicated the presence of minimal antioxidant component (Fig. 1B).

3.2.2. Quantitative antioxidant tests

In the quantitative determination of secondary metabolites content of *B. lacera* extract, the calculated total content of phenolic, flavonoid and tannin were found to be 9 mg GAE/g, 31 mg QE/g and 18 mg GAE/g, respectively. In DPPH free radical scavenging assay, the calculated SC_{50} for *B. lacera* extract and ascorbic acid were > 3000 μg·mL^{-1} and 15 μg·mL^{-1}, respectively (Table 2).

3.3. Assessment of acute toxicity

Only one mouse died in both 3000 and 5000 mg·kg^{-1} dose groups of *B. lacera* extract during the observing period. So, LD_{50} could not be determined for *B. lacera* extract. However, in other animals, no abnormal behavior or toxicity was observed.

3.4. Assessment of peripheral analgesic activity

In the acetic acid-induced writhing test, the *B. lacera* extract significantly reduced the writhing impulse 24.5% and 43.6% at the doses of 250 and 500 mg·kg^{-1} bw, respectively. The positive control (diclofenac sodium) showed 82.7% writhing inhibition at 25 mg·kg^{-1} dose (Table 3)

3.5. Assessment of anti-inflammatory activity

In the xylene-induced ear edema method, *B. lacera* extract reduced the swelling of edema or weight up to 24.6% and 41% at doses of 250 and 500 mg·kg^{-1} bw, respectively. The positive control (ibuprofen) reduced 65.6% of ear edema at 100 mg·kg^{-1} bw dose (Table 4 and Fig. 2).

3.6. Assessment of antidiarrheal activity

B. lacera extract increased the time of the first defecation period up to 52.8 and 106.6 min at the doses of 250 and 500 mg·kg^{-1} bw, respectively whereas loperamide (3 mg·kg^{-1} bw dose) and the control group showed latent defecation period at 182.8 and 35.2 min, respectively. The extract at two doses and loperamide showed significant inhibition of defecation by 34.1%, 48.2% and 83.5%, respectively (Table 5).

3.7. Assessment of diuretic activity

B. lacera extract increased the urinary output in the observing period at both 250 and 500 mg·kg^{-1} bw doses. The urinary excretion and diuretic activity are plotted in Fig. 3A and Fig. 3B, respectively.

3.8. Assessment of antibacterial activity

B. lacera extract showed a good antibacterial effect for the tested gram-negative and gram-positive bacteria in both 250 μg/disk and 500 μg/disk doses. A wide zone of inhibition was found that indicated the retardation of bacterial growth (Table 6 and Fig. 4) and the responses were found in a dose-dependent manner.

3.9. Assessment of anthelmintic activity

B. lacera extract caused both paralysis and death to the *P. cervi.* Those times were recorded and found in a dose-dependent manner. At the doses of 6.25, 12.5, 25 and 50 mg·mL^{-1}of *B. lacera* extract, the paralysis time of *P. cervi* were 38.6, 32.2, 21.8 and 17 min, respectively whereas the paralysis time for albendazole was 8.76 min. In the same doses, the death time of *P. cervi* was 59, 52, 43.8 and 38.7 min, respectively whereas that for albendazole was 19.3 min (Table 7).

3.10. Assessment of cytotoxic activity

In brine shrimp lethality bioassay, *B. lacera* extract increased the mortality of *A. salina* with the increase in concentration. The calculated

Table 3
Effects of *B. lacera* extract on acetic acid-induced writhing in mice.

Treatment group	Dose (mg·kg^{-1})	Mean writhing	% inhibition of writhing
Negative control	——	22 ± 2.55 $^{\theta, \blacktriangle, \Delta}$	——
Standard (diclofenac Na)	25	3.8 ± 0.84$^{*\blacktriangle, \Delta}$	82.7
B. lacera extract	250	16.4 ± 1.14$^{* \theta, \Delta}$	24.5
B. lacera extract	500	12.4 ± 1.87$^{* \theta, \blacktriangle}$	43.6

Data are means of five replicates ± SD (standard deviation); * $P < 0.05$ vs. Control (Dunnett's *t*-test).
$^{\theta}$ $P < 0.05$ vs. diclofenac sodium 25 mg·kg^{-1}.
$^{\blacktriangle}$ $P < 0.05$ vs *B. lacera* 250 mg·kg^{-1}.
$^{\Delta}$ $P < 0.05$ vs. *B. lacera* 500 mg·kg^{-1} (pair-wise comparison by Post Hoc Tukey test).

Table 4
Effects of *B. lacera* extract on xylene-induced ear edema in mice.

Treatment group	Dose (mg·kg^{-1})	Mean weight or circular ear section (g)	% inhibition
Negative control	——	1.525 ± 0.096 $^{\theta, \blacktriangle, \Delta}$	——
Standard (ibuprofen)	100	0.525 ± 0.096$^{*\blacktriangle, \Delta}$	65.6
B. lacera extract	250	1.15 ± 0.123$^{* \theta, \Delta}$	24.6
B. lacera extract	500	0.9 ± 0.141$^{* \theta, \blacktriangle}$	41

Data are means of five replicates ± SD (standard deviation); * $P < 0.05$ vs. Control (Dunnett's *t*-test).
$^{\theta}$ $P < 0.05$ vs. ibuprofen 100 mg·kg^{-1}.
$^{\blacktriangle}$ $P < 0.05$ vs *B. lacera* 250 mg·kg^{-1}.
$^{\Delta}$ $P < 0.05$ vs. *B. lacera* 500 mg·kg^{-1} (pair-wise comparison by Post Hoc Tukey test).

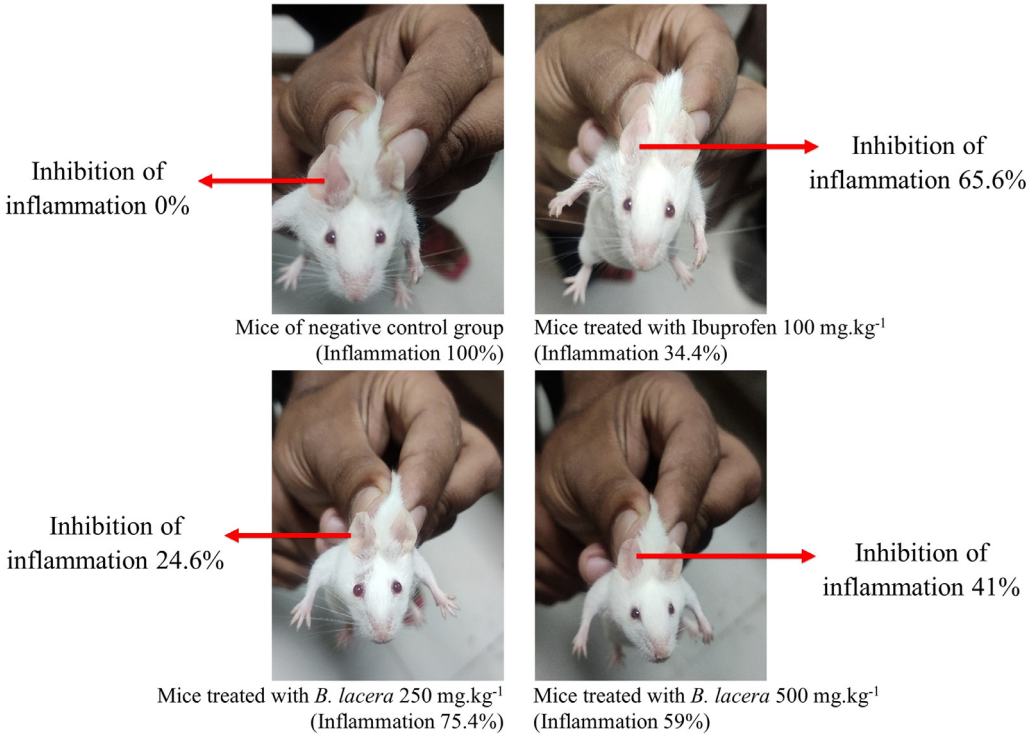

Fig. 2. Xylene-induced ear edema in different mice groups after treating with ibuprofen (100 mg·kg^{-1}) and *B. lacera* extract (250 mg·kg^{-1} and 500 mg·kg^{-1}).

Table 5
Effects of *B. lacera* extract on castor oil-induced diarrhea in mice.

Treatment group	Dose (mg·kg^{-1})	Average latent period of defecation	Average no. of stool	% inhibition of defecation
Negative control	——	35.2 ± 7.08 $^{\theta, \blacktriangle, \Delta}$	17 ± 2.35 $^{\theta, \blacktriangle, \Delta}$	——
Standard (loperamide)	3	182.8 ± 16.08 $^{*\blacktriangle, \Delta}$	2.8 ± 1.48$^{*\blacktriangle}$	83.5
B. lacera extract	250	52.8 ± 6.34$^{* \theta, \Delta}$	11.2 ± 1.64$^{* \theta, \Delta}$	34.1
B. lacera extract	500	106.6 ± 14.15 $^{* \theta, \blacktriangle}$	8.8 ± 0.84$^{* \blacktriangle}$	48.2

Data are means of five replicates ± SD (standard deviation); * $P < 0.05$ vs. Control (Dunnett's *t*-test).
$^{\theta}$ $P < 0.05$ vs. loperamide 3 mg·kg^{-1}.
$^{\blacktriangle}$ $P < 0.05$ vs *B. lacera* 250 mg·kg^{-1}.
$^{\Delta}$ $P < 0.05$ vs. *B. lacera* 500 mg·kg^{-1} (pair-wise comparison by Post Hoc Tukey test).

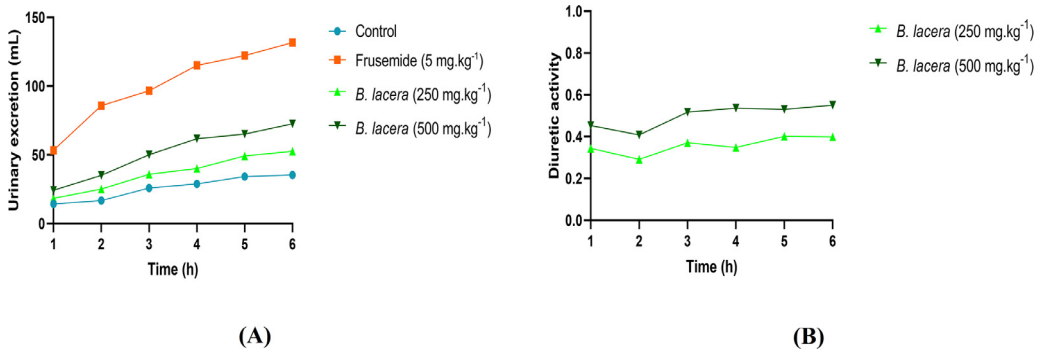

Fig. 3. **(A)** Urinary excretion of *B. lacera* extract and frusemide, **(B)** Diuretic activity of *B. lacera* extract.

Table 6
In vitro antibacterial activity of *B. lacera* extract.

Bacterial strains	Type of bacterial strains	Diameter of zone of inhibition in mm			
		Standard (Kanamycin) 30 µg/disk	Extract 250 µg/disk	Extract 500 µg/disk	Negative control
Staphylococcus aureus	Gram(+)	23	11	16	0
Streptococcus pyogens	Gram(+)	37	21	27	0
V. cholerae	Gram(-)	31	20	26	0
Shigella dysenteriae	Gram(-)	28	14	21	0
S. typhi	Gram(-)	28	12	18	0
E. coli	Gram(-)	21	10	16	0
Pseudomonas auriginosa	Gram(-)	26	11	19	0
Proteus spp	Gram(-)	20	12	16	0

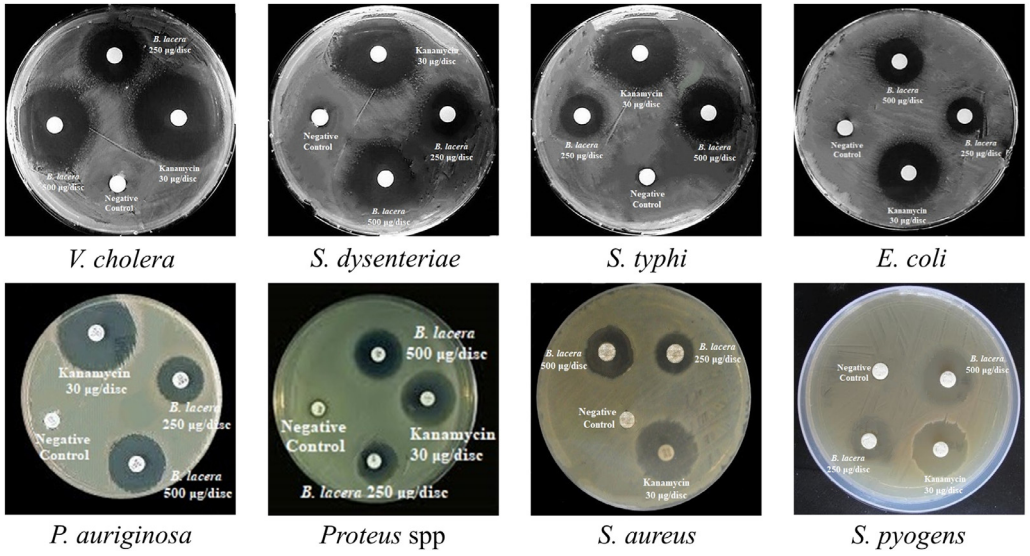

Fig. 4. Diameter (mm) of zone of inhibition for different bacterial culture in antibacterial test conducted with kanamycin (30 µg/disk) and *B. lacera* extract (250 µg/disk and 500 µg/disk).

LC_{50} values for *B. lacera* extract and vincristine sulfate were 5.6 and 0.4 µg·mL^{-1}, respectively (Fig. 5A and 5B).

4. Discussion

Every plant is a storehouse of numerous phytochemicals. These phytochemicals are stored as primary or secondary metabolites by the plant itself. Mostly secondary metabolites are enriched in bioactive compounds responsible for eliciting different types of beneficial health effects for both plant and animal kingdoms. Dependence on plant-derived medicine is increasing day by day. Our phytochemical investigation conducted on aerial parts of *B. lacera* extract revealed the presence of many important phytochemical groups. These phytochemicals might be responsible for eliciting various biological responses.

Antioxidants are the most common types of phytochemicals present in most plants. These are helpful to neutralize different types of harmful free radicals that are regularly produced in the body. Free radicals cause different types of serious diseases like cancer, stroke, hyperglycemia, atherosclerosis, immunodeficiency, hepatitis, etc. (Saha et al., 2021). Antioxidants protect our bodies from these types of serious diseases. After determining the existence of antioxidant compounds in the qualitative antioxidant assay, we went to determine the total content of some antioxidant secondary metabolites such as phenols, flavonoids and tannins. We found very poor content of these secondary metabolites. In the DPPH free radical scavenging assay, the SC_{50} value of *B. lacera* extract was found too high. So, from the above results, we may conclude that *B. lacera* is not a good source of antioxidant components. However, those observations might be due to the presence of some antioxi-

Table 7
Anthelmintic activity of *B. lacera* extract.

Treatment group	Dose (mg·mL^{-1})	Mean paralysis time (min)	Mean death time (min)
Negative control	——	——	——
Standard (albendazole)	15	8.7 ± 0.44 $^{▲,Δ,*,Ψ}$	19.3 ± 0.85 $^{▲,Δ,*,Ψ}$
B. lacera extract	6.25	38.6 ± 1.35 ^{θ,Δ,*,Ψ}	59 ± 0.99 ^{θ,Δ,*,Ψ}
B. lacera extract	12.5	32.2 ± 0.91 $^{θ,▲,*,Ψ}$	52 ± 1.59 $^{θ,▲,*,Ψ}$
B. lacera extract	25	21.7 ± 0.65 $^{θ,▲,Δ,Ψ}$	43.8 ± 0.67 $^{θ,▲,Δ,Ψ}$
B. lacera extract	50	17.1 ± 0.91 $^{θ,▲,Δ,*}$	38.7 ± 0.41 $^{θ,▲,Δ,*}$

Data are means of six replicates ± SD (standard deviation).
^{θ} $P < 0.05$ vs. albendazole 15 mg·mL^{-1}.
$^{▲}$ $P < 0.05$ vs *B. lacera* 6.25 mg·mL^{-1}.
^{Δ} $P < 0.05$ vs. *B. lacera* 12.5 mg·mL^{-1}.
* $P < 0.05$ vs. *B. lacera* 25 mg·mL^{-1}.
^{Ψ} $P < 0.05$ vs. *B. lacera* 50 mg·mL^{-1} (pair-wise comparison by Post Hoc Tukey test).

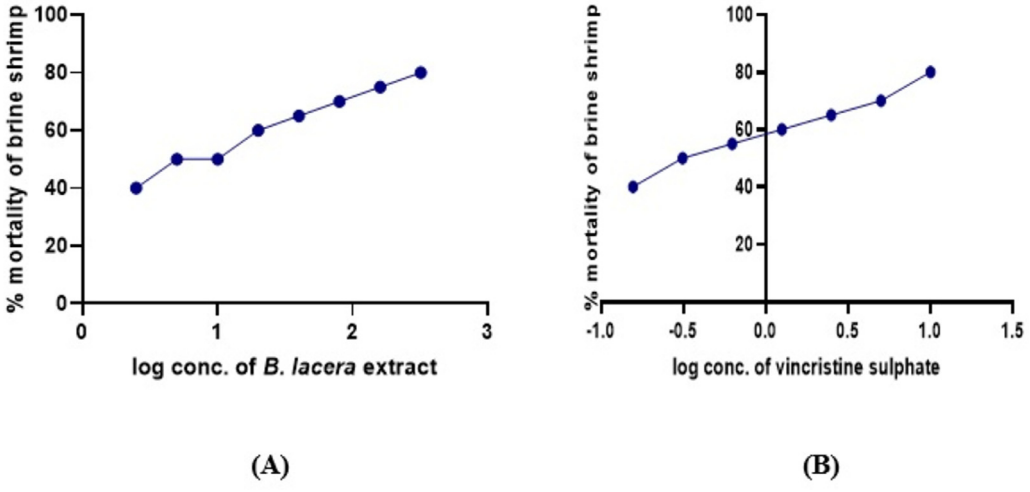

Fig. 5. **(A)** Brine shrimp lethality bioassay of *B. lacera* extract **(B)** Brine shrimp lethality bioassay of vincristine sulfate.

dant molecules like hentriacontane (Khajuria et al., 2017), artemisinin (Kim et al., 2015), protocatechuic acid (Kakkar and Bais, 2014), squalene (Güneş, 2013), etc.

From the acute toxicity test, we found that the LD_{50} values of *B. lacera* extract might be higher than 5000 mg·kg^{-1}. So we set to conduct the pharmacological screening on mice at 250 mg·kg^{-1} and 500 mg·kg^{-1} doses.

Pain and inflammation are normal body responses to different external obnoxious stimuli. These stimuli tend to release several internal mediators such as prostaglandin, prostacyclin, thromboxane, bradykinin that initiate pain and inflammation. Although there are many analgesics and anti-inflammatory agents available in the market, those cause serious and chronic adverse effects like gastrointestinal upset, peptic ulcer, renal perfusion, bleeding disorders, etc. (Harirforoosh et al., 2013). As a result, searching for safe and effective novel analgesic and anti-inflammatory compounds is always demanding. In this regard, medicinal plants with traditional uses in pain and swelling management can be a crucial source to overcome this condition. Alkaloids, tannins, flavonoids, terpenoids are already reported for revealing these activities (Alemu et al., 2018). In the analgesic test, *B. lacera* extract significantly reduced the writhing or pain impulse and in the anti-inflammatory test, it also reduced the ear edema at both 250 and 500 mg·kg^{-1} bw doses. Thus, we can assume that *B. lacera* has mild analgesic as well as anti-inflammatory properties, and these results also support its traditional uses in folk medicine. There are many reported compounds such as β-sitosterol (Saeidnia et al., 2014), cineol (Juergens, 2014), lupeol (Siddique and Saleem, 2011), hentraicontane (Khajuria et al., 2017), artemisinin (Kim et al., 2015), protocatechuic acid (Kakkar and Bais, 2014), β-caryophyllene (Dahham et al., 2015), etc. found in this plant and their presence might be responsible for the analgesic and anti-inflammatory properties.

Diarrhea is a very common gastrointestinal disease that causes mortality in many children even adults. Children under 5 years old have been suffering from diarrhea accounts approximately 63% of the worldwide diarrheal burden (Ugboko et al., 2020). Castor oil's main component, ricinoleic acid irritates the gastric mucosal layer, and as a result the motility increases. In the castor oil-induced diarrheal test in mice, *B. lacera* extract reduced the frequency of diarrheal episodes. It not only elongates the latent defecation period but also reduced the number of excreted stools over the observed time. The reason for this antidiarrheal activity of the plant extract might be due to the presence of artemisinin (Shahrajabian et al., 2020) protocatechuic acid (Li et al., 2019), β-stigmasterol-β-D-glucopyranoside (Muhammad et al., 2021), etc. compounds as their antidiarrheal properties are already reported.

Diuretics are the compounds that increase the urinary output. They help the body to get rid of excess fluids, salts and toxic substances. They reduce the volume of water and ultimately blood of any individuals without any significant side effects (Jannat et al., 2018). From that point, diuretics are used as the first-line therapy to treat hypertension which has been increasing for the last centuries. Moreover, diuretics are also helpful to manage heart failure, hypertension, liver cirrhosis, chronic renal failure, water poisoning, etc. In the diuretic test, the *B. lacera* extract increased the urinary volume over the period in a dose-dependent manner. Our results showed good diuretic nature of the ex-

tract and this property may be due to the presence of some reported diuretic compounds like hentriacontane (Jalalpure and Gadge, 2011), artemisinin (Blanch and Mónica, 1998), lupeol (Vidya et al., 2002), etc.

From the last 2–3 decades, there is a sudden increase in antimicrobial resistance that has become a great threat to human health. Many bacterial species have altered their defensive process to fight against different types of antimicrobials. As a result, many antibiotics gradually lose their capacity to destroy bacteria. So, there arises a great demand for newer antimicrobials nowadays. Scientists from all over the world are trying with their best efforts to invent better antimicrobials with no microbial resistance. Plant and algae-derived antimicrobials have become a great focus to researchers to discover potent antimicrobials. From our antibacterial test, we observed that *B. lacera* extract showed potent retardation in bacterial growth. Those results revealed good antibacterial properties of *B. lacera* and this may be due to the presence of some antibacterial compounds like lupeol (Siddique and Saleem, 2011), hentriacontane (Khajuria et al., 2017), artemisinin (Kim et al., 2015), protocatechuic acid (Kakkar and Bais, 2014), β-caryophyllene (Dahham et al., 2015), phytol (Islam et al., 2018), stearic acid (da Silva et al., 2002), etc. present in this plant.

Helminths are very common nematodes living in the gastrointestinal tract of mammals. These parasites steal nutrients, vitamins, minerals, blood from the host bodies and as a result create different types of diseases. Around 3.5 million people are being affected by these protozoal diseases. Due to the increasing spread of anthelmintic drug resistance over the world, there is a growing interest in newer sources of anthelmintics, and as a result, plant-derived anthelmintics have become a great focus to scientists (Grzybek et al., 2016). From our anthelmintic test, we observed that *B. lacera* extract caused the paralysis and death of the *P. cervi*. Our result justified the traditional use of this plant as an anthelmintic and some anthelmintic compounds present in this herb such as lupeol (Siddique and Saleem, 2011), phytol (Islam et al., 2018), α-copaene (Turkez et al., 2014), etc. might be responsible for this effect.

After conducting the antibacterial and anthelmintic tests, we aimed to conduct the brine shrimp lethality bioassay to determine the cytotoxic nature of *B. lacera* extract. This technique is based on the killing capability of the cultured *A. salina* eggs by any compound. *B, lacera* extract showed a good lethal effect on *A. salina.* This result indicates that the herb may have good cytotoxic properties. Compounds like lupeol (Siddique and Saleem, 2011) hentriacontane (Khajuria et al., 2017), protocatechuic acid (Kakkar and Bais, 2014), squalene (Güneş, 2013), α-copaene (Turkez et al., 2014) were reported for anticancer or antitumor properties and their presence in this extract might be responsible for the above cytotoxic effect.

5. Conclusion

B. lacera belonging to the family Asteraceae is a neglected weed grown in many Asian countries. However, this weed has also some uses in traditional medicine, and considering those uses, we aimed to conduct some scientific experiments on this plant. Our results indicated this herb might be helpful for different types of ailments like pain, inflammation, diarrhea, bacterial and protozoal infection. However, it is somewhat toxic in nature as found from toxicological tests. So, we recommend using the purified bioactive compounds from it rather than using the crude extract for pharmacological applications. The toxicological nature of this herb might be helpful to discover new anticancer or antibiotic molecules in the future.

Ethical Approval

All experimental protocols in this current study involving animals were performed following the standard ethical guidelines of Animal Ethics Committee (AEC), Khulna University, Khulna-9208, Bangladesh [Ref: KUAEC-2021/03/02].

Data Availability

All experimental data are preserved by the authors. Those will be available upon request.

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Declaration of Competing Interest

The authors declare no financial or non-financial competing interests related to this publication.

CRediT authorship contribution statement

PK: Project design, conduction of all laboratory tests, literature search, statistical analysis, graphical presentation, writing and editing the original paper.

SLD: Assistance in conduction of laboratory tests.

SKS: Project design, idea generation, resources, paper writing and correction, overall supervision.

All authors reviewed the manuscript and give their consent for publication.

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Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccmp.2022.100038.

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