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Khurshid Jahan
Lecturer, Dept. Of Pharmacy, World University Of Bangladesh, Dhaka-1205
E-mail: nimmy45@yahoo.com

Sukalyan Kumar Kundu
Professor, Jahangirnagar University, Bangladesh.

Md. Abdul Bake
Gono University, Savar, Dhaka, Bangladesh.

Correspondence:
Khurshid Jahan
Lecturer, Dept. Of Pharmacy, World University Of Bangladesh, Dhaka-1205.
Email: nimmy45@yahoo.com

Evaluation of antimicrobial and cytotoxic activities of the mehanolic and petroleum ether extract of *Blumea lacera* Burm.f in Bangladesh

Khurshid Jahan, Sukalyan Kumar Kundu, Md. Abdul Bake

ABSTRACT

The crude extract of *Blumea lacera* Burm.f (Family, Asteraceae) was evaluated for its possible antimicrobial and cytotoxic properties. After collection of the plant, it was (whole plant) powdered and was successfully extracted with petroleum ether and methanol. Phytochemical screening of the extracts showed the presence of alkaloids, tannins, steroids, gums, reducing sugar. The antimicrobial investigation showed that the petroleum ether and methanol extracts showed antimicrobial activity against two bacteria (one is gram positive and another is gram negative) and a fungus. From the literature review, it was also found that the plant has therapeutic effects on diabetic, sedation, stomach infection and cholera. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Keywords: Blumea lacera, methanolic extract, petroleum ether extract, antimicrobial activity, cytotoxic activity, gram positive, gram negative.

1. Introduction

Blumea Lacera Burm.f, synonyms: Conyza lacera Burm. f. (Bengali name- Kukurshinga, Shialmutra, English name- Asteraceae), is an annual herb with a strong odor and is distributed throughout the country as a weed in fallow lands throughout Bangladesh. It is also found in plains of India. *Blumea* consists of about 80 species [1]. Flowering time January to April [2]. *Blumea lacera* is described as a valuable medicinal plant in many popular systems of medicine including *Ayurveda*, homoeopathy, and unani. Stimulatory allelopathy of different parts of *B. lacera* on many agricultural crops has also been reported. Essential oil from *Blumea* has been shown analgesic, hypothermic, and tranquilizing activities [3]. There is a heavy demand of different parts (fresh and dry both) of this weed in national and international drug markets [4]. Leaf juice is astringent, stimulant, anthelmintic and diuretic. The plant is also act as a good stomachic, antispasmodic. The essential oil of the leaves possesses antimicrobial properties. Roots are astringent and febrifuge and mixed with pepper. They are given in cholera. The plant has mild antimicrobial properties [5] .This plant is used in folk medicine for the treatment of cough, bronchitis, dysentery, wound healin [6]. Plants, plant parts, plant products of all descriptions, particularly those with medicinal properties are invariably used as principal components or ingredients of various traditional medicines. More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh. Due to the introduction of large number of plants into traditional medicine based on only empirical evidence many of these so called medicinal plants are now found to be therapeutically useless. Their continued use in traditional medicine is now justified by arbitrarily calling them as necessary associates (excipients) of the active ingredients [7]. Various parts of *Blumea Lacera* Burm.f, yield an essential oil containing cineol, fenchone and Blumea camphor. Leaves also contain coniferyl alcohol derivatives, campesterol and flavones. Ethanolic extract of the aerial parts contain hentriacontane, hentriacontanol, a-amyrin, lupeol and its acetates and ß-sitosterol. Root and root bark contain triterpenes and sterols. Farmers can earn extra income after selling various parts of *Blumea* with the help of co-operatives [8]. Fresh leaves of *Blumea* are the most valuable part. Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases in human [9]. *Blumea Lacera* also possesses anticancer activities. Now a day, Ayurvedic and Unani medicines are also popular. They are usually dispensed as coarse and fine powder, broker pieces, as liquid preparation and in the form of cream and ointment.

They are also packed in sachets, packets, aluminium foils, plastic containers and in glass bottles. When containers are used then clear labelled containing indication, contraindication, doses, uses, storage conditions are used. The individual parts of the plants such as the leaves, flowers, fruits and roots are used far trading cough as stimulant, as diuretic, as astringent, as sedative, as antispasmodic or in the treatment of whooping cough. The drugs are employed in different from such as fresh juice, decoction, infusion and powder.
So, proper uses of medicinal plants possess beneficial properties [10]. The cytotoxicity of the plant extract was determined when observation was done upon brine shrimp. In this method, the sample solution was used in different concentrations. The capability of the sample solution to kill brine shrimp indicated the cytotoxicity of the plant extracts. The powdered plant was extracted with methanol and petroleum ether and the phytochemical screening showed the presence of alkaloids, tannins, steroids, gum, reducing sugar. The antimicrobial activities of the discolorized extracts were investigated against some bacteria and one fungus. Petroleum ether extracts showed mild antimicrobial proper ties.

2. Materials and Methods

2.1 Collection and identification of plant materials:

For this present investigation the *Blumea Lacera* Burm.f was collected from Savar region, Bangladesh in July, 2013 and was identified from Bangladesh National Herbarium. The whole body of the plant was used for these purposes.

2.1.1. Preparation of the plant sample:

The collected and identified plant's body was cut into small pieces and was dried in the sun and finally was dried in oven at 50-60 ^{0}C for 48 hours. After complete drying, the small pieces were reduced to coarse powder with the help of mechanical grinder and the powder was stored in a suitable container for extraction.

2.1.2. Extraction of the plant material (method):

The drug to be extracted was packed in a 'thimble' made of filter paper which was then placed into the wider part of the extractor. Solvent was placed in the flask and was boiled, the vapors were allowed to pass through the side tube to the condenser where they were condensed and fall on to the packed drug, through which it extracted out the active constituents. As the volume of the solvent in the extractor increased, the level of the liquid in the siphon also increased till it reached the maximum point from where it was siphoned out into the flask. On further heating, the solvent was vaporized and the dissolved active constituents remained into the flask. By this way, the active constituents were collected in the flask. The part was then separated and marked.

2.1.3 Drug:

Drug employed in the study were: Cefalexin (1st generation Cephalosporin) as standard.

2.2 Phytochemical screening:

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the Dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with Libermann-Burchard reagent. Reducing sugars with Benedict's reagent [11, 12, 13].

2.3 Antimicrobial screening of plant extracts

2.3.1 Preparation of Culture media (nutrient agar media)

It is a general culture media which may be used as an enrichment medium by incorporating 10% blood or other biological fluid. Nutrient agar media was used for the subculture of test organisms in which proper growth of the organisms were ensured.

Table 1: To prepare nutrient agar media following formula are used:

Ingredients	Gm/L
Agar	15
Lab-lemco powder	1
Peptic digest of animal tissue	5
Sodium chloride	5
Yeast extract	2

(Hi Media Laboratories Ltd. India)

To prepare required volume of this medium, amount of each of the constituents were calculated from the composition chart given for 1L. Distilled water was added to it and the content was heated in a water bath to make a clear solution. The pH was adjusted to7.4±0.2 using 10% sodium hydroxide solution. Agar was added to the solution in calculated amount and distilled water was added sufficiently to make final volume. The prepared medium was transferred to each number of the required number of bottles which were previously washed. The medium was sterilized by autoclaving for 15 minutes at 121 ^{0}C under a pressure of 15l b/inch^2.

2.3.2 Preparation of antibiotic assay medium (Sabouraud Dextrose Agar, SDA)

65.0 gm of Sabouraud Dextrose Agar was suspended in 100 ml of distilled water and was boiled for about 15 minutes till complete dissolution. The pH of the solution was adjusted to 6.6±0.2 by adding 10% sodium hydroxide (NaOH). The medium was then sterilized by autoclaving for 15 minutes at 121 ^{0}C under a pressure of 15l b/inch^2.

Table 2: Test organisms used for antimicrobial test

Serial No	Microorganisms	Code No	Type of bacteria
01	**Fungus** *Candida albicans*	F-01	NA
02	**Bacteria** *Staphylococcus aureus*	B-02	Gram positive
	Escherichia coli	B-03	Gram negative

2.3.3 Sample preparation
Test samples were prepared by dissolving the plant extract into sterile distilled water where concentration was adjusted to 300 µg/ml. About 0.15 gm of extracts was dissolved in 500 µl sterile distilled water and the solution was dissolved by sonicator to get the desire concentration.

2.3.4 Preparation of test plate
Plates of the Nutrient agar medium were taken. By using sterile loop, organisms were spreaded over the plates. A sterilized Pasteur pipette was taken and then three holds were made on each of the plate. The plates were marked properly by writing name of the organisms in one side and marking the position of sample and standard disc on the other side. About 100 µl of prepared sample solution was poured into one hole of each of the plate, the other two holes contained the standard disc (CFX) and test sample of different extracts (extracts of petroleum ether and extract of methanol). The plates were then carefully stored in incubator for the development of the zone. Observation was done after 24 hours.

2.4 Cytotoxic investigation
2.4.1 Preparation of sample solution
20mg methanol extract was weighed out and dissolved in 1 ml of DMSO (di-methyl-sulfoxide) by sonicator and finally taken in a test tube. Similarly petroleum ether extract was dissolved in DMSO.

2.4.2 Preparation of salt water
About 38 gm of sodium chloride (NaCl) was weighed out and taken in a volumetric flask. Then it was dissolve in 1L distilled water. The flask was shaken gently for proper dissolved.

2.4.3 Hatching of Brine shrimp: Brine shrimp eggs collected from pet shops and was hatched to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time.

2.4.4 Method:
About 16 test tubes were taken and marked as 1A, 1B, 5A, 5B, 10A, 10B, 20A, 20B, 50A, 50B, 100A, 100B, 200A, 200B, 500A, 500B where remarking was according to the concentration. From the sample solution about 0.5ml extract solution was taken in a test tube. With it 9.5 ml salt water was added and the test tube was allowed to sonicator. The concentration of the mother solution should always be 1 mg/ml. By using micropipette, about 10 brine shrimps were taken carefully in each of the 16 test tubes. Then mother solution was taken by the concentration 5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml, 2500 µg/ml respectively.
Observation was done after 24 hours. The number of brine shrimp died was calculated out after 24 hours.

3. Results
3.1 Chemical group test
Results of different chemical tests on the methanolic extract and petroleum ether extract of *Blumea lacera* Burm.f (whole plants) showed the presence of carbohydrate (reducing sugar), alkaloids, tannins, steroids, gums (Table 1).

Table 3: Results of different chemical tests on the methanolic extract and petroleum ether extract of *Blumea lacera* Burm.f (whole plants).

Plant Extracts	Alkaloids	Tannins	Saponins	Glycosides	Steroids	Gums	Reducing Sugar
ME	+	+	-	-	+	+	+
PEE	+	+	-	-	+	+	+

ME: Methanol extract; PEE: Petroleum ether extract; +: Positive result; -: Negative result

3.2 Microbiological Investigation:
The antimicrobial activities of the methanol extracts and petroleum ether extracts of *Blumea Lacera* Burm.f were compared with that of a standard antibiotic cefalexin. The antimicrobial activities of the extracts were tested against two bacteria and one fungus which were responsible for various infectious diseases.

Table 4- shows that the inhibition zones of staphylococcus aureus in methanol extract, petroleum ether extract, and CFX was 12 mm, 15 mm and 18 mm in diameter respectively. The inhibition zone of *E. coli* was 10 mm, 16 mm, and 20 mm after methanol extract, petroleum ether extract and CFX respectively. No inhibition zone was appears for fugus (candida albecans) in methanol and petroleum ether extract.

Table 4: Result of antimicrobial activities of the extracts of *Blumea Lacera* Burm.f

Microorganisms	Inhibition Zones (Diameter in mm)		
Bacteria	Ethanol extract	Petroleum ether extract	CFX
Staphylococcus aureus	12	15	18
E. coli	10	16	20
Fungus			
Candida albecans	No	No	15

Concentration of Plant extracts were 300 µl/hole. CFX means Cefalexin in Bangladesh (1st generation cephalosporin).

3.3 Cytotoxic investigation of the plant extracts
Following the procedure of Meyer, the extracts showed lethality indicating the biological activity of the compounds present in the extracts [14]. Test samples showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the sample.

3.3.1 Observation data
For methanol extract

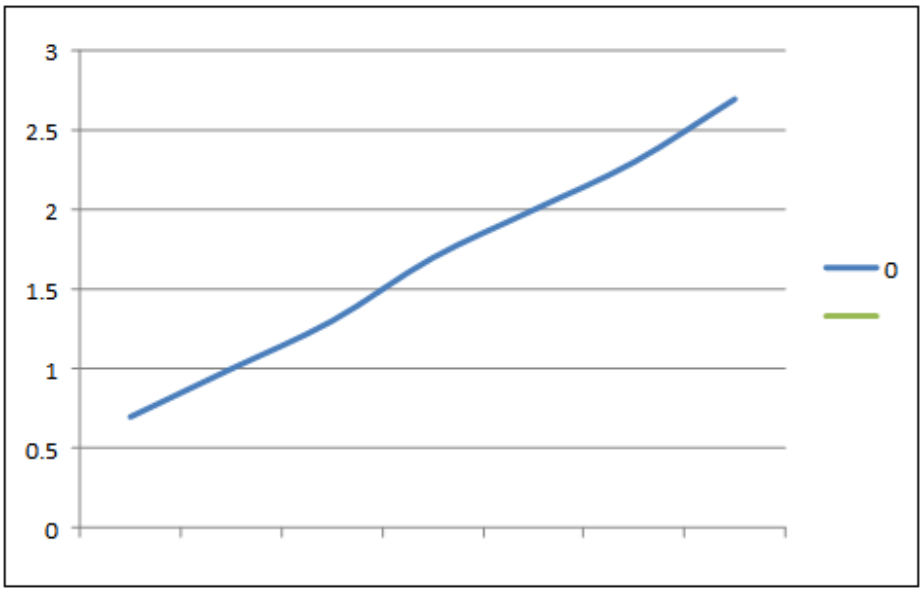

Fig 1: Graphical representation of log C Vs % mortality of brine shrimp

For petroleum ether extract

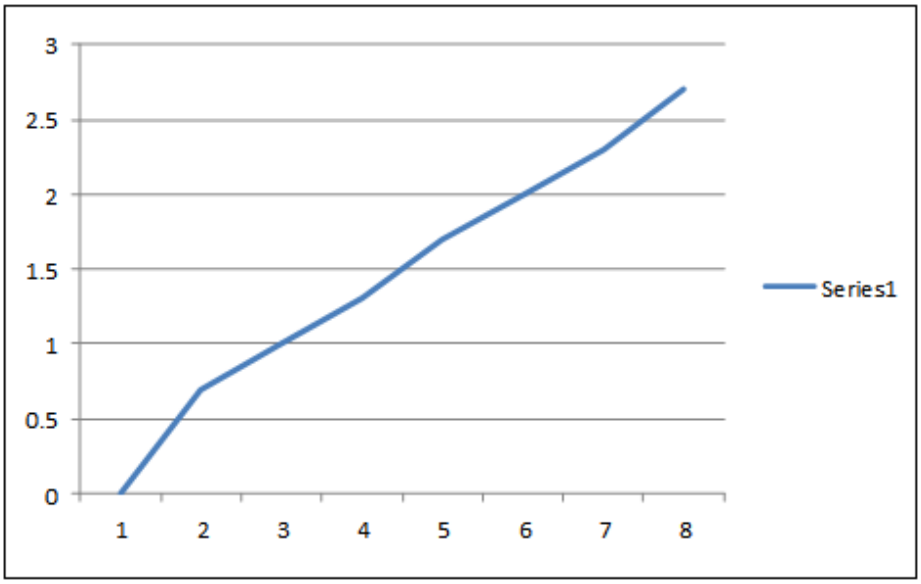

Fig 2: Graphical representation of log C vs % mortality of brine shrimp

4. Discussion

The powdered plant was extracted with methanol and petroleum ether and the phytochemical screening showed the presence of alkaloids, tannins, steroids, gum, reducing sugar. Earlier studies showed that, anti-dysenteric and antidiarrhoeal properties of medicinal plants were due to alkaloids, tannins, sterols, flavonoids [15, 16]. The antimicrobial activities of the discolorized extracts were investigated against two bacteria and one fungus. Petroleum ether extracts showed mild antimicrobial properties. The cytotoxicity of the plant extracts in vitro was investigated by using the plant extract in different concentrations. Observation was done upon brine shrimps. The lethality of brine shrimp indicated the cytotoxicity of the plant extracts. The plant extracts showed cytotoxic properties.

5. Conclusion

The research work indicates that the plant *Blumea Lacera* Burm.f showing many properties where it possess antimicrobial properties, mild cytotoxic properties. However, further studies are needed to understand the origin of their activity.

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