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Phytochemical screening, antimicrobial and anticancerous activities of two different plant Extracts

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Abstract
The phytochemical screening of ethanolic leaf extract of *L. glutinosa* and *C. quadrangularis* confirmed the presence of alkaloids, steroids, flavonoids, terpenoids, tannins, anthraquinones and alkaloids, flavonoids, terpenoids respectively. Disk diffusion method was performed to evaluate the antimicrobial activity of these plant extracts. *L. glutinosa* showed its highest activity against a gram negative strain *S. paratyphi* which exhibited a ring of 12 mm in diameter at 400 µg/disc. In the case of *C. quadrangularis*, highest activity was found against *S. paratyphi* which showed a ring of 12.33 mm in diameter at 400 µg/disc. On the contrary, standard Kanamycin showed its highest zone of inhibition against *E. coli* which is 28.66 mm in diameter at 30 µg/disc. In the case of anticancerous activity, standard vincristine sulphate showed 12.59 µg/ml activity whereas ethanolic leaf extract of *L. glutinosa* & *C. quadrangularis* demonstrated minimal activity which is 530.05 µg/ml and 639.68 µg/ml respectively.

Keywords: *Litsea glutinosa, Cissus quadrangularis,* Phytochemical screening, Disk diffusion method, Kanamycin, Vincristine sulphate

Introduction
Plant-based medicine comprise significant amounts of bioactive compounds, which is allowing for desirable health benefits. According to the report of World Health Organization, 80% of the world populations depend primarily on indigenous medicine and that the majority of traditional therapies involve the use of plant extracts or of their active constituents [1]. For thousands of years nature has been a great source of medicinal agents and modern drugs isolated from natural sources many of which were investigated based on their traditional use as medicine. It has been noted that the lead of many important pharmaceuticals currently in use have been plants used by indigenous people. Herbal medicine or phytomedicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. The potential for medicinal plants can be assessed by finding new chemical entities of wide structural diversity. These new chemical substances can also serve a template for producing more effective drugs through semi-synthetic and total synthetic procedures. According to World Health Organization (WHO), about 74% of 119 plant-derived pharmaceutical medicines of biotechnology medicines are used in modern medicine in ways that correlate directly with their traditional uses [2]. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, almost all medical preparations and drugs were prepared from plants, it was may be a raw plant material or a refined from of crude extracts. Recent estimates suggest that several thousands of plants have been known with medicinal applications in various [3]. *Litsea glutinosa* (Lauraceae) is a medium-sized, deciduous or semi-evergreen tree. It is widely distributed in the forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar, Sylhet, Gazipur, Madhupur and Dinajpur. The mucilaginous bark is largely employed as a demulcent and mild astrigent in diarrhoea and dysentery. Freshly ground bark used as an emollient application to bruises and as a styptic dressing for wounds. It is also considered aprodisiac. The oil from the berries is used in rheumatism. The mucilaginous leaves are considered antispasmodic and emollient; useful in colic and impotence. In Rema-Kalenga barks and leaves are used in Diarrhoea and dysentery [4].

Ethanolic extract of the leaves possess good antibacterial activity [5]. *Cissus quadrangularis* (Vitaceae) is a large climber with succulent stem, much contracted at the nodes, quadrangular, the angles of the young branches winged and the leaves are 2.5-5cm long. The plant is found growing abundantly in Sundarbans, Chittagong, and occasionally planted. The stem is laxative, stomachic, tonic and analgesic; used in piles, tumours, loss of appetite, constipation and in complaints of the back and spine. Stem juice is used in otorrhoea, epistaxis, scurvy and irregular menstruation. Paste of stem is given in asthma; stem boiling in limewater is a useful stomachic. Stems, roots and leaves are used as a plaster over broken limbs. Burnt to ashes of the young shoots administered in dyspepsia and other bowel complaints. Leaves and young shoots are considered as powerful alteratives. EtOH (50%) extract of aerial parts is hypotensive and diuretic [6].

Materials and Methods

Plant material

Litsea glutinosa & *Cissus quadrangularis* were collected from local area of Chittagong district (Sitakunda), Bangladesh and authenticated by the Botanist Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh.

Preparation of extraction

The leaves were indirectly sun dried by shade and ground. The ground (500g) were soaked in sufficient amount of ethanol (1:4) for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent were evaporated under vacuum at room temperature to yield semisolid. Ethanolic leaf extract was then preserved in a refrigerator at 4 ºC till further use.

Chemicals and reagents

Methanol and Kanamycin (30 µg/disc) were purchased from Merck (Germany) and Oxoid (England) respectively. Two more chemicals were purchased from Sigma Aldrich (Munich, Germany), which were vincristine sulfate and 99.5% absolute ethanol. All other reagents were of analytical grade.

Phytochemical screening

Chemical tests were carried out on the ethanolic extracts of *Litsea glutinosa* and *Cissus quadrangularis* using standard procedures to identify the constituents as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

Preparation of Test Sample Solutions

20mg of each plant extract was added to 10ml Methanol. Sonicator was used to dissolve the extracts. The solution was then filtered. The method was described by Savithramma, Rao, & Surhulatha, 2011.

Test for Alkaloids

Preparation of Dragendroff's Reagent

Step-01 Solution A - 0.17gm Bismuth nitrate was added in 2ml Acetic acid and 8ml Distilled water.

Step-02 Solution B - 4gm Potassium iodide was added in 10ml Acetic acid and 8ml Distilled water.

Step-03 Dragendroff's Reagent - Solution A and B were added and mixed together. The mixture was then diluted with distilled water up to 100ml.

Procedure

2ml of filtrate in another was taken in a test tube and 1% Hydrochloric acid was added to it and mixed properly. 1ml from the mixture was taken and 6 drops of Dragendroff's reagent was added to it. This method is identified and examined by Harborne, 1998.

Test for Flavonoids

5 ml of dilute ammonia solution were added to 4ml of filtrate of each plant extract followed by addition of 1ml concentrated H_2S0_4 [7].

Test for Steroids

1ml of chloroform and 1 ml of concentrated H_2S0_4 was added to 4ml filtrate of each plant extract [8].

Test for Terpenoids

0.5ml Acetic anhydride and 0.5ml chloroform was added to 4mg of each plant extract [8].

Test for Reducing-sugars

1ml of distilled water was added to 0.5ml of filtrate of each plant extract. Then 5-8 drops of Fehling's solution A & B were added in equal amounts. The mixture was then heated [8].

Test for Tannins

About 0.5 g of the dried extract of *Litsea glutinosa* was boiled in 20 ml of distilled water and then filtered. A few drops of 0.1% ferric chloride were added [8].

Test for Anthraquinone

0.5gm of dried extract of *Litsea glutinosa* was boiled with 10ml concentrated H_2S0_4. The solution was then filtered while hot. The chloroform layer was pipette into another test tube and 1ml ammonia solution was added to the chloroform layer [7].

Test for Cardiac glycosides (Keller-Killani test)

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. 1 ml of concentrated sulphuric acid was then added to it [8].

Antimicrobial activity

Test Materials

In this present study, the antibacterial activity of ethanolic extracts of *Litsea Glutinosa* and *Cissus quadrangularis* was investigated in comparison with standard kanamycin (30 µg/disc) antibiotic agent against a number of pathogenic Gram-positive and Gram-negative bacteria and fungus.

Test Organisms

Both Gram-positive and Gram-negative strains of bacteria and fungus were used as the test organism to observe the anti-bacterial activity of the isolated compounds. These organisms were collected from the Microbiology research laboratory, Department of Pharmacy, East West University. The pure of which was previously collected from the Department of Microbiology, University of Dhaka.

Preparation of the media

The instant nutrient broth was prepared from purified powdered agar, which were weighed and then reconstituted with distilled water in a conical flask according to specification. It was then heated in a water bath to dissolve the agar until a transparent solution was obtained. In this way nutrient broth was prepared.

Antimicrobial activity testing by disc diffusion method

Antimicrobial activity was performed by using disc diffusion method [9-10]. Circular discs of 5 mm in diameter were made from Whatman No. 1 filter paper (China) by using a punch machine. The filter discs were autoclaved at 180 ºC for 30 min and then each disc was soaked with 50 µl of each of the two different plant extracts at the concentration of 400 µg/disc by using micropipette. All of the discs were aseptically placed over the plates of agar media containing the agar media inoculated with test microorganisms. At last the plates were incubated at 37º for 24 hours. After 24 hours, diameters (in mm) of the inhibition rings were measured. Kanamycin (30 µg/disc) was used as standard and it was compared with the two ethanolic plant extracts. Zones of inhibition with diameter less than 12 mm were considered as having low antimicrobial activity. Diameters between 12 and 16 mm were considered as moderately effective and diameters more than 16 mm were considered as highly active [11].

Anticancerous Activity

Anticancerous activity of plant extracts are evaluated by brine shrimp lethality bioassay, which is widely used for screening bioactive compounds [12, 13]. In this study, a simple zoological organism (*Artemia salina*) was used as a convenient monitor for the experiment. The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hrs to develop into larval shrimp called nauplii. The anticancerous assay was performed on the brine shrimp nauplii using the Meyer method. The test samples (extract) were prepared by dissolving them in DMSO (not more than 50 µL in 5 mL solution) plus seawater (3.8% NaCl in water) to attain concentrations of 10, 50, 100, 200, 300 and 500 µg/ml. A vial containing 50 µL DMSO diluted to 5 mL was used as a control. Standard vincristine sulfate was used as a positive control. Mature shrimps were placed into each of the experimental vials. After 24 h, the vials were inspected using a magnifying glass, and the number of surviving nauplii in each vial was counted. From these data, the percentage of lethality of the brine shrimp nauplii was calculated for each concentration using the following formula:

% Mortality = $(N_t)/No \times 100\%$

Where N_t = Number of dead nauplii after a 24-h incubation;

N_0 = Number of total nauplii transferred i.e., 10.

The LC_{50} (median lethal concentration) was determined from the log concentration versus percent mortality curve [14].

Results

Table 1: Results of Different Chemical Group Test of the Ethanolic Leaf Extracts of *L. glutinosa* and *C. quadrangularis*

Phytochemical content	*L. glutinosa*	*C. quadrangularis*
Alkaloids	Present	Present
Flavonoids	Present	Present
Steroids	Present	Absent
Terpenoids	Present	Present
Reducing Power	Absent	Absent
Tannins	Present	Absent
Anthraquinones	Present	Absent
Cardiac Glycoside	Absent	Absent

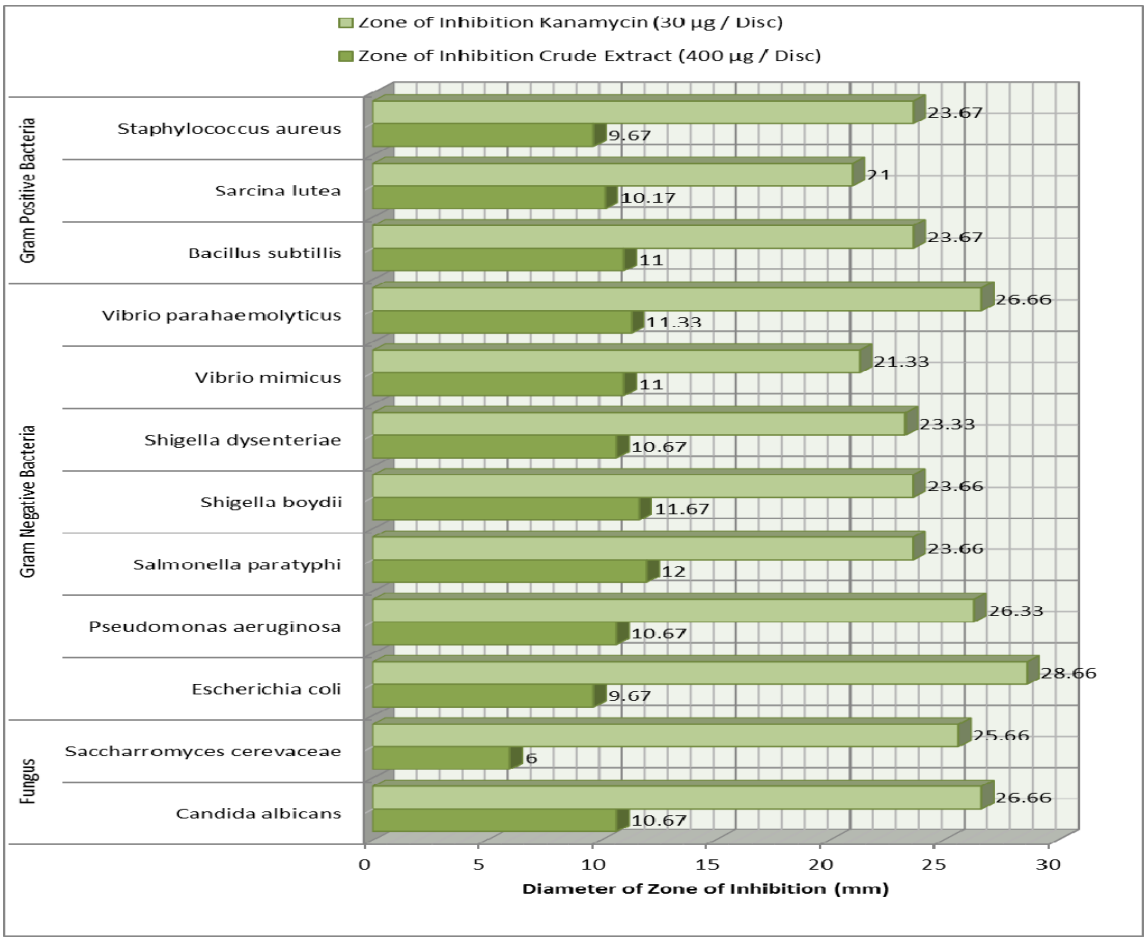

Fig 1: Graphical Presentation of Antimicrobial Activity of *Litsea glutinosa*

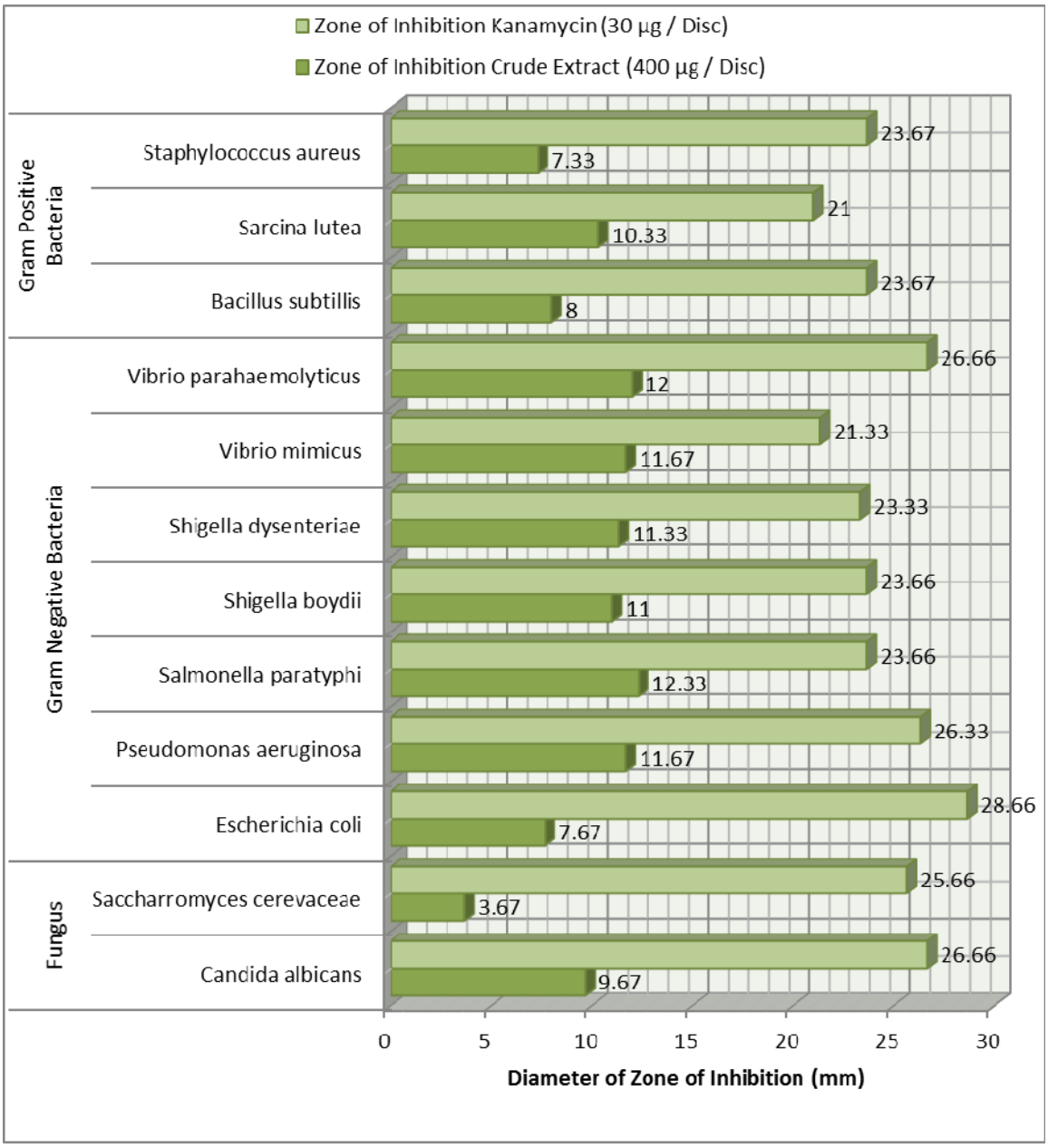

Fig 2: Graphical Presentation of Antimicrobial Activity of *Cissus quadrangularis*

The two different leaf extracts showed different zone of inhibition at the concentration of 400 µg/disc against different gram positive, gram negative bacteria as well as fungus. This experiment was performed by using disk diffusion method to evaluate the antimicrobial activity of these leaves. *L. glutinosa* showed its highest activity against a gram negative strain *S. paratyphi* which exhibited a ring of 12 mm in diameter and only 6 mm zone of inhibition was found against the fungus which is *S. cerevaceae* at 400 µg/disc. In the case of *C. quadrangularis*, highest activity was found against *S. paratyphi* which showed a ring of 12.33 mm in diameter and lowest activity (3.67 mm in diameter) was found against *S. cerevaceae* at 400 µg/disc. On the contrary, standard Kanamycin showed its highest zone of inhibition against *E. coli* which is 28.66 mm in diameter and lowest zone of inhibition was demonstrated against *S. lutea* (21 mm in diameter) at 30 µg/disc.

Table 2: Percent of mortality of the extract at six concentrations

Concentration (ug/ml)	Log C	% of mortality		
		LG	CQ	Vincristine sulphate
10	1	10	10	40
50	1.699	10	10	80
100	2	09	10	100
200	2.301	08	09	100
300	2.477	08	08	100
500	2.699	05	06	100
LC_{50}		530.05	639.68	12.59

Table 2 represents the percentage of mortality of brine shrimp caused by the plant extracts at six different concentrations (10 to 500 µg/mL) of the extracts. It was precise that the percentage of lethality was directly proportional to the concentrations of extracts. LC_{50} values of ethanol extracts of *L. glutinosa* and *C. quadrangularis* obtained in this present

experiment are 530.05 µg/mL and 639.68 µg/mL respectively. In the case of vincristine sulphate (positive control), the LC_{50} value was 12.59 µg/mL. However, no mortality was obtained from the negative control.

Discussion

Traditional medicine is playing a pivotal role to alleviate different types of diseases in this modern era. It has been estimated that there are approximately 500,000 species of plants available on Earth [15] and among them only a few percentage (1% to 10%) are used as food supplement by humans and other animal species together. In addition, just more than 10% of plants are used for medicinal purposes. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived [16]. Approximately 25% of modern drugs used in the United States have been derived from plant origins (WHO, 2008). The discovery of effective antibiotics has decreased the disastrous impact of infectious diseases as well as enriched the quality of life. Nevertheless, the efficacy of many antibiotics is being endangered by the emergence of microbial resistance to existing chemotherapeutic agents because of their inappropriate use. The use of some antibiotics is associated with side effects, including allergy, immune suppression, and hyper-sensitivity. Lots of poor people are deprived from modern medicine because of high cost. For all these reasons, it is high time to identify new, safe, and cost-effective antimicrobial agents that would help to alleviate the problems of infectious diseases. Plant-derived natural products represent an attractive source of antimicrobial agents because they are natural and affordable, especially for rural societies [17-20]. Toxicity profile of plant materials is mainly an important criterion to experts and medical practitioners [21-23], and cytotoxic brine shrimp lethality (LC50, 24 h) test was conducted in this experiment to know about the toxicity of the plant extracts. Derived equation from Parra [24], showed a great correlation ($r = 0.85$; $P < 0.05$) between the LC50 of brine shrimp lethality test and the severe oral toxicity assay in mice. Based on derivation of the correlation, cutoff value for cytotoxicity is determined by LC_{50} which should be less than 10 µg/ mL (LD_{50} between 100 and 1 000 mg/kg) [24-26].

Conclusion

The result of phytochemical screening revealed that, ethanolic leaf extract of *L. glutinosa* posses alkaloids, steroids, flavonoids, terpenoids, tannins, anthraquinones *& C. quadrangularis* contain alkaloids, flavonoids and terpenoids. Ethanolic leaf extracts of *L. glutinosa* and *C. quadrangularis* possess moderate antimicrobial activity. Those plants are also exhibited minimal anticancerous activity.

Conflict of interest statement

Authors have none to declare.

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