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# ANTIMICROBIAL ACTIVITY OF *LAGENARIA SICERARIA* CRUDE EXTRACTS OBTAINED FROM ITS FLOWERS

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**Keywords:**

*Lagenaria siceraria*, Antimicrobial activity, Antibiotic, Gram-positive, Gram-negative, *n*-hexane extract

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**ABSTRACT:** Many herbal remedies have been employed in various medical systems for the treatment and management of different diseases. The plant *Lagenaria siceraria*, a plant belonging to the family Cucurbitaceae, has been used in the different system of traditional medication for the treatment of diseases and ailments of human beings. The *n*-hexane extract of *Lagenaria siceraria* was screened for antimicrobial activity against a wide range of both gram-positive and gram-negative bacteria by the disc diffusion method. The results obtained were compared with that of a standard antibiotic, kanamycin. The *n*-hexane extract showed significant antimicrobial activity against *Escherichia coli* (17.25 mm), and *Salmonella typhi* (17.80 mm). But there was no antimicrobial activity against *Vibrio cholera*, *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus*.

**INTRODUCTION:** The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in the history of all civilizations. Our present study was designed to determine the antimicrobial activity of *Lagenaria siceraria* with *n*-hexane extract. The plant Lagenaria siceraria belongs to Cucurbitaceae family. Cucurbitaceae family is commonly mentioned as the bottle gourd, melon or pumpkin family is medium sized generally a climbing plants family composing 118 genera and 825 species having a wide distribution in the warmer regions of the world [1, 2].

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The bottle gourd is one of humankind's first domesticated plants, providing food, medicine and a wide variety of utensils and musical instruments. It is used as medicine in Bangladesh, India, China, European Countries, Brazil, Hawaiian island, *etc*. for its cardiotonic, general tonic and diuretic [3] properties. Further, the anti-diabetic [4], anti-hyperlipidemic [5], anti-hepatotoxic [6], analgesic [7], CNS activity [8], anti-cancer [9], cardioprotective [10], anti-inflammatory [11], immunomodulatory [12] and antioxidant [13] activities of its fruit extract have been evaluated. In many countries, this plant has been used traditionally as a single treatment for diabetes mellitus [14]. The usable parts are pulp, fruit, shoots, leaves, seeds. For this study flowers were investigated.

## MATERIAL AND METHODS:

**Collection and Identification:** The plant *Lagenaria siceraria* was collected from Noakhali Science and Technology University Campus, Sonapur, Noakhali, Bangladesh. The flowers of the

plant were collected followed by thorough washing with water several times. During collection, any adulteration was strictly prohibited. The plant sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. After identifying they gave an accession no. of 35, 399.

**Drying and Grinding:** The collected plant parts (flowers) were washed with water, separated from undesirable materials or plants or plant parts. They were air-dried under shade to protect from sunlight for one week after cutting into small pieces. The plant parts were ground into a fine powder with the help of a Hammer Mill. The fine powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

**Cold Extraction (Methanol Extraction):** About 284 gm of powdered material was taken in a clean, flat bottomed glass container (4litres) and soaked in 1300 ml of 90% methanol. The container with its contents was sealed and kept for 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper and the filtrate thus obtained was concentrated by using traditional spontaneous natural vaporization method at room temperature.

**Preparation of Mother Solution:** 5 gm of methanol extract was triturated with 100 ml methanol containing 10% of distilled water (90 ml methanol + 10 ml water). The crude extract went to the solution completely. This is the mother solution, which was partitioned off successively by n-hexane solvent.

***n*-Hexane Extraction:** The mother solution was taken in a separatory funnel. 100 ml of n-hexane was added, and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice. *n*-hexane fractions were collected together and evaporated at room temperature. The aqueous fraction was preserved for the next step.

**Antimicrobial Screening:** The antimicrobial screening which is the first stage of antimicrobial drug research is performed to ascertain the susceptibility of various fungi and bacteria to any agent. This test measures the ability of each test sample to inhibit the in vitro bacterial growth. Any of the following three methods may estimate this ability.

1. Disc diffusion method
2. Serial dilution method
3. Bioautographic method

Among the techniques mentioned above, the disc diffusion (Bauer *et al*., 1966) is a widely accepted in vitro investigation for preliminary screening of test agents which may possess antimicrobial activity.

**The Principle of the Disk Diffusion Method:** Solutions of known concentration (g/ml) of the test samples are made by dissolving the measured amount of the samples in the calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette.

Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are then kept at low temperature (4 °C) for 24 h to allow maximum diffusion.

During this time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffused out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel (Barry, 1976). As a result, there is a gradual change in test materials concentration in the media surrounding the discs.

The plates are then incubated at 37 °C for 24 h to allow maximum growth of the organisms. If the test materials have any antimicrobial activity, it will inhibit the growth of the microorganisms, and a clear, distinct zone of inhibition will be visualized surrounding the medium. The antimicrobial activity of the test agent is determined by measuring the diameter of the zone of inhibition expressed in millimeter. The experiment is carried out more than once, and the mean of the readings is required (Bayer *et al*., 1966).

**Experimental:**

**Apparatus and Reagents:**

- Filter paper discs
- Petri dishes
- Inoculating loop
- Sterile cotton
- Sterile forceps
- Spirit burner
- Micropipette
- Screw cap test tubes
- Nose mask and Hand gloves
- Laminar air flow hood
- Autoclave
- Incubator
- Refrigerator
- Nutrient Agar Medium
- Ethanol
- Chloroform

**Test Materials:**

**Test Materials of *L. siceraria*:** *n*-hexane extract.

**Test Organisms:** The bacterial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram-positive and gram-negative organisms were taken for the test, and they are listed in **Table 1**.

**TABLE 1: LIST OF TEST BACTERIA**

| Gram-positive Bacteria | Gram-negative Bacteria |
|---|---|
| *Bacillus cereus* | *Escherichia coli* |
| *Bacillus subtilis* | *Salmonella typhi* |
| *Staphylococcus aureus* | *Vibrio cholera* |

**Culture Medium and Their Composition:** The following media is used normally to demonstrate the antimicrobial activity and to make subculture of the test organisms, and they are listed in **Table 2**. Nutrient agar medium (DIFCO) is used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

**TABLE 2: NUTRIENT AGAR MEDIUM**

| Ingredients | Amounts |
|---|---|
| Bacto peptone | 0.5 gm |
| Sodium chloride | 0.5 gm |
| Bacto yeast extract | 1.0 gm |
| Bacto agar | 2.0 gm |
| Distilled water q.s. | 100 ml |
| pH | 7.2 at 25 °C |

**Preparation of Medium:** To prepare the required volume of this medium, the calculated amount of each of the constituents was taken in a conical flask, and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25°C) was adjusted at 7.2-7.6 using NaOH or HCl. 10 ml and 5 ml of the medium were then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs pressure at 121°C for 20 min. The slants were used for making the fresh culture of bacteria and fungi that were in turn used for sensitivity study.

**Sterilization Procedures:** To avoid any contamination and cross-contamination by the test organisms, the antimicrobial screening was done in Laminar Hood, and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121 °C and a pressure of 15-lbs/sq inch for 20 min. Micropipette tips, cotton, forceps, blank discs, etc. were also sterilized.

**Preparation of Subculture:** In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh, pure cultures. The inoculated strains were then incubated for 24 h at 37 °C for their optimum growth. These fresh cultures were used for the sensitivity test.

**Preparation of the Test Plates:** The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms.

The bacterial and fungal suspension was immediately transferred to the sterilized petri dishes. The petri dishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

**Preparation of Discs:** Three types of discs were used for antimicrobial screening.

**Standard Discs:** These were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for the comparison of the response produced by the known antimicrobial agent with that of the test sample. In this investigation, Kanamycin (30 g/disc) standard disc was used as the reference.

**Blank Discs:** These were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

**Preparation of Sample Discs with Test Samples:** A measured amount of each test sample was dissolved in a specific volume of solvent to obtain the desired concentrations in an aseptic condition. Sterilized metrical filter paper discs were taken in a blank petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

**Preparation of Sample Dishes with a Test Sample of *Lagenaria siceraria*:** The *n*-hexane extract extracts were tested for antimicrobial activity against a number of both grams positive and gram-negative bacteria.

**The Test Sample for *n*-hexane:** The amount of sample per disc was 30 µg per disc.

**Preparation and Application of the Test Samples:** The test samples were weighed accurately, and calculated amounts of the solvents were added accordingly using a micropipette to the dried samples to get desired the concentrations. The test samples were applied to previously sterilized discs using adjustable micropipette under aseptic conditions.

**Diffusion and Incubation:** The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 4 °C for about 24 h upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37 °C for 24 h.

**Determination of Antimicrobial Activity by Measuring the Zone of Inhibition:** The antimicrobial potency of the test agents is measured by their activity to prevent the growth of the microorganisms surrounding the discs which give a clear zone of inhibition. After incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

**RESULTS AND DISCUSSION:** The zones of inhibition produced by the n-hexane extract were found to be 17.25-17.80 mm, at a concentration of 30 g/disc. The n-hexane extract showed significant antimicrobial activity against *Escherichia coli* (17.25 mm), and *Salmonella typhi* (17.80 mm). But no antimicrobial activity against *Vibrio cholera*, *Bacillus cereus*, *Bacillus subtilis*, and *S. aureus*.

**TABLE 3: ANTIMICROBIAL ACTIVITY OF THE *n*-HEXANE EXTRACT OF *LAGENARIA SICERARIA***

| Bacterial strains | The diameter of the zone of inhibition in mm | |
|---|---|---|
| | n-Hexane 30 µg/disc | Kanamycin 30 µg/disc |
| Gram-negative | | |
| *Escherichia coli* | 17.25 | 16.25 |
| *Vibrio cholera* | - | 18.50 |
| *Salmonella typhi* | 17.80 | 15 |
| Gram-positive | | |
| *Bacillus cereus* | - | 18.40 |
| *Bacillus subtilis* | - | 17.60 |
| *Staphylococcus aureus* | - | 17 |

**CONCLUSION:** Medicinal plants are the local heritage with global importance. The world is endowed with a rich wealth of medicinal plants. Medicinal plants also play an important role in the lives of rural people, particularly in remote parts of developing countries with few health facilities. This plant named *Lagenaria siceraria* is also utilized for the treatment of some common disease. The pharmacological investigation for anti-microbial activity of n-hexane extract showed microbial growth inhibitory effect. The *n*-hexane extract showed significant antimicrobial activity against *Escherichia coli* (17.25 mm), and *Salmonella typhi* (17.80 mm). But no antimicrobial activity against *Vibrio cholera*, *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus a*ureus.

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## CONFLICT OF INTEREST: Nil

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