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# Antibacterial potency screening of *Capparis zeylanica* Linn.

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ABSTRACT

**Objective:** To conduct the antibacterial potency and minimum inhibitory concentration of extracts (*n*-hexane, acetone, chloroform and methanol) obtained from the root, leaf and stem of *Capparis zeylanica*.
**Methods:** The powdered leaf, root and stem samples were Soxhlet extracted sequentially in *n*-hexane, acetone, chloroform and methanol. Antibacterial potency was evaluated by following the agar diffusion method and amoxicillin disc was used as a control.
**Results:** *In vitro* antibacterial activity against 12 bacteria was performed with crude extracts. Among them, all the bacteria showed the moderate activity but chloroform and methanolic extracts showed promising antibacterial potency against *Staphylococcus aureus, Sarcina lutea, Bacillus megaterium, Bacillus subtilis, Salmonella typhi* and *Shigella dysenteriae* (leaf > root > stem). This activity was evaluated using disc diffusion method with a standard antibiotic, 30 μg/disc of amoxicillin.
**Conclusions:** Strong antibacterial potency of chloroform and methanolic extracts provides new antibacterial compounds.

## 1. Introduction

Human beings have relied on natural products as a resource of drugs for thousands of years. Plant-based drugs have formed the basis of traditional medicine systems that have been used as traditional medicines for their primary health care[1]. There are 119 chemicals, derived from 90 plant species, which can be considered as important drugs in one or more countries[2-4]. These plant-derived medicines not only validate the traditional knowledge but also can contribute in the development of better allopathic drugs[5,6]. Bacterial ability to outsmart current drugs demands the continual supply of new drugs[7]. It has been a big challenge to find compounds with strong antibacterial potency and low toxicity[8,9]. Exploration of plant-based extracts and compounds for the importance of antibacterial potency can help to fulfill such demand[2,10]. *Capparis zeylanica* Linn. (*C. zeylanica*) (*Capparis horrida* Linn., *Capparis brevispina* DC.) is known as Indian caper belonging to family Capparidaceae. It is a rigid, wiry and much- branched shrub and widely distributed in Bangladesh, India, Sri Lanka and Malaysia[10]. Plants are 2–3 m in height. Almost all the parts *i.e.* root, bark, fruits, leaves and seeds are used for different purposes. The root and bark of the plant are bitter and useful as tonic, expectorant, anthelmintic, emmenagogue, analgesic and also used in rheumatism, paralysis, toothache, enlarged spleen[11]. In Unani medicine, the decoction of the root bark is prescribed as a deobstruent to liver and spleen and as an anthelmintic and anti-inflammatory agent[12]. The present paper reports a comparative potency of extracts obtained in different solvents from leave, root and stem of *C. zeylanica* to inhibit Gram-positive and Gram-negative bacteria.

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## 2. Materials and methods

### 2.1. Plant collection and identification

The plant specimen was collected from Rajshahi University Campus, Bangladesh. Identification of voucher specimen was confirmed at the Taxonomical Section, Department of Botany, University of Rajshahi, Bangladesh.

The bacterial strains of *Staphylococcus aureus* (ATCC-259233) (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Bacillus megaterium* (QL-38) (*B. megaterium*), *Bacillus subtilis* (QL-40) (*B. subtilis*), *Sarcina lutea* (*S. lutea*), *Streptococcus β-haemolyticus* (CRL) (*S. β-haemolyticus*), *Salmonella typhi* (*S. typhi*), *Shigella dysenteriae* (AL-35587) (*S. dysenteriae*), *Shigella shiga* (ATCC-26107) (*S. shiga*) *Shigella sonnei* (AJ-8992) (*S. sonnei*), *Shigella boyclii* (*S. boyclii*) (AL-17313) and *Escherichia coli* (FPFC-1407) (*E. coli*) were obtained from Molecular Pathology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh.

### 2.2. Preparation of the extracts

Leaves, root wood and stem wood were dried in shade and stored in cotton bags, and then finally powdered (500 g) with the help of grinder and, weighed and placed in conical flasks to add sufficient amount of chloroform, methanol, *n*-hexane and acetone to yield the first extracts of the leaves, root wood and stem wood by filtration through Whatman filter paper for 24 h separately. The filtered one was then allowed to vaporize in rotary evaporator until completely dried and kept in a refrigerator at 4 ˚C.

### 2.3. Antibacterial evaluation

Inhibitory activity of the extracts was performed by diffusion assay[13]. The nutrient agar gel created a concentration gradient. If the agar was seeded or streaked with a sensitive organism, the concentration of inhibition zone exceeded the minimum inhibitory concentration (MIC) for the particular organism. The test samples were dissolved in definite volumes of solvent to give the known concentrations (μg/mL) to solutions. The sterile (BBL, Cocksville, USA) filter paper (5 mm diam) discs were impregnated with known amounts of the test substances and dried. These test material discs were placed on plates containing nutrient agar medium and seeded with the test organisms. These plates were kept at a low temperature (4 ˚C) for 24 h to allow maximum diffusion. A number of events took place on the discs simultaneously: i) the dried discs absorbed water from the agar medium and the material under test was dissolved, ii) the test material diffused from the discs to the surrounding medium according to the physical law that controled the diffusion of molecules through agar gel and iii) there was a gradual change of test material concentration in the agar surrounding each disc (Figure 1).


**Figure 1.** Antibacterial activity test.

To determine the most optimal concentration of extracts used in this study, sterile filter paper discs (7.5 mm) were treated with 200 μL of the *n*-hexane, acetone, chloroform and methanol extracts, while the only solvents were used as control. The bacteria were inoculated on full-strength nutrient agar (Qualigens Fine Chemicals, Prod # 58673) by suspending loops in sterile de-ionized water. The bacterial suspension was then smeared on agar plates with a sterile glass rod to ensure that the entire surface of the agar had an even coating of the bacterial suspension. The test plates were divided into several areas and one filter paper disk was placed on each of the areas. The plates were then kept in an incubator (37 ˚C) for 12–18 h to allow the growth of the organisms. If any of the test materials had antimicrobial activity, it would inhibit the growth of microorganisms just giving a clear distinct zone (zone of inhibition). Biological activity of the *C. zeylanica* components on bacterial growth was quantified in this way by measuring the diameter of zones of inhibition (mm) reducing the size of the treated filter paper discs.

## 3. Results

### 3.1. Antibacterial activity of the leaf extracts

The leaf extracts (*n*-hexane, acetone, chloroform and methanol) were responsive to both Gram-positive and Gram-negative bacteria to *S. aureus, B. cereus, B. megaterium, B. subtilis, S. lutea, S. β-haemolyticus*, *S. typhi*, *S. dysenteriae*, *K. pneumoni*, *P. aeruginosa*, *S. boydii* and *E. coli* with clear inhibition of zones (Table 1 and Figure 1). The results showed that the highest inhibition zones were recorded for the control (30 μg/disc of amoxicillin) which were 30, 31, 28, 27, 30, 28, 29, 30, 27, 28, 30 and 28 mm.

**Table 1**
Antibacterial activity of leaf extract of *C. zeylanica* and the standard amoxicillin.

| Test organisms | | Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *n*-Hexane (200 µg/disc) | Acetone (200 µg/disc) | Chloroform (200 µg/disc) | Methanol (200 µg/disc) | Amoxicillin (30 µg/disc) |
| Gram-positive bacteria | *S. aureus* | 12 | 13 | 21 | 20 | 30 |
| | *B. cereus* | 15 | 10 | 10 | 10 | 31 |
| | *B. megaterium* | 12 | 10 | 12 | 18 | 28 |
| | *B. subtilis* | 16 | 17 | 16 | 18 | 27 |
| | *S. lutea* | 14 | 15 | 20 | 16 | 30 |
| | *S. β-haemolyticus* | 10 | 12 | 15 | 15 | 28 |
| Gram-negative bacteria | *S. typhi* | 12 | 15 | 22 | 14 | 29 |
| | *S. dysenteriae* | 15 | 10 | 20 | 12 | 30 |
| | *K. pneumoni* | 13 | 9 | 14 | 15 | 27 |
| | *P. aeruginosa* | 9 | 11 | 13 | 16 | 28 |
| | *S. boydii* | 10 | 14 | 15 | 14 | 30 |
| | *E. coli* | 10 | 12 | 16 | 16 | 28 |

### 3.2. Antibacterial activity of the root extracts

The root extract showed almost identical zone of inhibition (*n*-hexane, acetone, chloroform and methanol) to above studied bacteria (Table 2). However, methanol extract showed the highest activity (18 mm of *S. typhi*). The inhibition zones for the standard amoxicillin (30 µg/disc) were 30, 31, 28, 27, 30, 28, 29, 30, 27, 28, 30 and 28 mm respectively but the highest one was 31 mm.

**Table 2**
Antibacterial activity of root extract of *C. zeylanica* and the standard amoxicillin.

| Test organisms | | Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *n*-Hexane (200 µg/disc) | Acetone (200 µg/disc) | Chloroform (200 µg/disc) | Methanol (200 µg/disc) | Amoxicillin (30 µg/disc) |
| Gram-positive bacteria | *S. aureus* | 11 | 10 | 13 | 12 | 30 |
| | *B. cereus* | 12 | 15 | 15 | 10 | 31 |
| | *B. megaterium* | 10 | 14 | 16 | 15 | 28 |
| | *B. subtilis* | 12 | 12 | 12 | 16 | 27 |
| | *S. lutea* | 13 | 12 | 10 | 10 | 30 |
| | *S. β-haemolyticus* | 14 | 10 | 14 | 13 | 28 |
| Gram-negative bacteria | *S. typhi* | 8 | 9 | 14 | 18 | 29 |
| | *S. dysenteriae* | 11 | 10 | 12 | 13 | 30 |
| | *K. pneumoni* | 8 | 12 | 11 | 13 | 27 |
| | *P. aeruginosa* | 10 | 14 | 13 | 10 | 28 |
| | *S. boydii* | 12 | 10 | 12 | 13 | 30 |
| | *E. coli* | 8 | 13 | 16 | 14 | 28 |

### 3.3. Antibacterial activity of the stem extracts

The stem extracts also exhibited bacterial activities (*n*-hexane, acetone, chloroform and methanol) to above mentioned both Gram-positive and Gram-negative bacteria (Table 3). Both chloroform and methanol extracts showed the highest activity (15 mm) but the lowest (8 mm) in *S. aureus*.

**Table 3**
Antibacterial activity of stem extract of *C. zeylanica* and the standard amoxicillin.

| Test organisms | | Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *n*-Hexane (200 µg/disc) | Acetone (200 µg/disc) | Chloroform (200 µg/disc) | Methanol (200 µg/disc) | Amoxicillin (30 µg/disc) |
| Gram-positive bacteria | *S. aureus* | 8 | 9 | 12 | 10 | 30 |
| | *B. cereus* | 10 | 10 | 9 | 9 | 31 |
| | *B. megaterium* | 9 | 11 | 10 | 10 | 28 |
| | *B. subtilis* | 10 | 9 | 12 | 11 | 27 |
| | *S. lutea* | 9 | 10 | 10 | 9 | 30 |
| | *S. β-haemolyticus* | 11 | 12 | 11 | 12 | 28 |
| Gram-negative bacteria | *S. typhi* | 9 | 10 | 13 | 12 | 29 |
| | *S. dysenteriae* | 12 | 8 | 12 | 10 | 30 |
| | *K. pneumoni* | 9 | 12 | 15 | 13 | 27 |
| | *P. aeruginosa* | 14 | 10 | 10 | 15 | 28 |
| | *S. boydii* | 12 | 11 | 13 | 9 | 30 |
| | *E. coli* | 10 | 9 | 14 | 12 | 28 |

### 3.4. MICs against test bacteria

Depending on the intensity of activity, only chloroform extracts of the root wood were subjected to evaluate the minimum inhibition zones. The results indicated that the MIC values of the chloroform extract of the root wood were 128 µg/mL against *E. coli,* 64 µg/mL against *S. dysenteriae* and *S. shiga* and 32 µg/mL against *S. β-haemolyticus*. The findings indicated that root wood extract of *C. zeylanica* was more potent and relatively good antibacterial agents. This probably explained the use of the extract of this plant in traditional medicines against a number of infections. So more comprehensive studies were solicited for their effective use, specially in medicine and agriculture.

**Table 4**
MICs of the chloroform extract of root wood against four pathogenic bacteria.

| Test tube No. | Nutrient broth medium added (mL) | Root wood extract (µg/mL) | Inoculum added (µL) | *S. β-haemolyticus* | *S. dysenteriae* | *S. shiga* | *E. coli* |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 512 | 10 | - | - | - | - |
| 2 | 1 | 256 | 10 | - | - | - | - |
| 3 | 1 | 128 | 10 | - | - | - | - |
| 4 | 1 | 64 | 10 | - | - | - | + |
| 5 | 1 | 32 | 10 | - | + | + | + |
| 6 | 1 | 16 | 10 | + | + | + | + |
| 7 | 1 | 8 | 10 | + | + | + | + |
| 8 | 1 | 4 | 10 | + | + | + | + |
| 9 | 1 | 2 | 10 | + | + | + | + |
| 10 | 1 | 1 | 10 | + | + | + | + |
| Cm | 1 | 0 | 0 | - | - | - | - |
| Cs | 1 | 512 | 0 | - | - | - | - |
| Ci | 1 | 0 | 10 | + | + | + | + |
| Results of MIC values in (µg/mL) | | | | 32 | 64 | 64 | 128 |

+: Growth; -: No growth.

## 4. Discussion

The antibacterial activities were found very much promising. Among all the extracts (*n*-hexane, acetone, chloroform and methanol) of the

leaves, root wood and stem wood of *C. zeylanica,* only chloroform extracts of the root wood were subjected to evaluate the minimum inhibition zones just depending on the intensity of activity. The MIC value of the chloroform extract of the root wood was 128 μg/mL against *E. coli*, and 64 μg/mL against *S. dysenteriae* and *S. shiga*, and 32 μg/mL against *S. β-haemolyticus*. The results support to the work of *C. zeylanica* leaf extracts

The coarse material of *C. zeylanica* roots was successively extracted with petroleum ether, chloroform and ethanol using Soxhlet and macerated to form water extract. All extracts were screened for its antibacterial activity using agar well diffusion method. The microorganisms used for antibacterial activity were *Bacillus pumillus* (NCIM-2752), *S. aureus* (NCIM-2901), *B. subtilis* (NCIM-2063), *E. coli* (NCIM-2256), *Klebsiella pneumoniae* (NCIM-2957), *Proteus vulgaris* (NCIM-2027). Gentamicin (5 mg/mL) and clotrimazol (5 mg/mL) were used as standards. The extracts showed that antimicrobial activity were subjected to minimum inhibitory concentration assay by two-fold dilutions method. Petroleum ether, chloroform, ethanol and water extract exhibited *in vitro* antibacterial activity[14].

The present findings of *C. zeylanica* extracts demonstrate that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases[14]. These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of the plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources[15]. Our earlier study have demonstrated that the reliable antibacterial potentiality of *Clerodendrum infortunatum*[16].

Bangladesh being the homeland of this famous plant might have a bright future of earning foreign currency by exporting different products or preparations of this plant. The root wood extract offered the highest toxicity to the majority of the test agents and the multicellular test organisms, followed by the stem extracts. So, roots could be an export item, as well as the root products and easy formulation of the products is necessary while the folk use of the *Capparis* products given hints in this regard. So, we believe that *C. zeylanica* will be possible source for new antibacterial substances against important pathogens of medically and veterinary importance.

## Conflict of interest statement

We declare that we have no conflict of interest.

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