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# Brine shrimp lethality and antibacterial activity of extracts from the bark of *Schleichera oleosa*

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ABSTRACT

**Objective:** To determine the antibacterial efficacy and brine shrimp toxicity of extracts (hexane, dichloromethane, ethyl acetate, methanol and water) obtained from the bark of *Schleichera oleosa*.

**Methods:** The powdered bark sample was Soxhlet extracted sequentially in hexanes, dichloromethane, ethyl acetate, methanol and water. Antibacterial evaluation was carried out by following the agar diffusion method and amoxicillin disc was used as a reference. Slightly modified Meyer's method was used to determine the toxicity of the extracts in brine shrimps.

**Results:** Among the nine bacterial strains tested, the methanolic and aqueous extracts showed promising antibacterial efficacy against *Serratia marcescens*, *Escherarichia coli*, *Bacillus subtilis* and *Micrococcus luteus*. None of the extracts were found significantly toxic to brine shrimps.

**Conclusions:** Strong antibacterial activity and low brine shrimp toxicity of methanolic and aqueous extracts can provide new antibacterial compounds.

## 1. Introduction

Use of plant materials as medicines by human was from the time of their origin and the records in South Asia dates back to 4 500 BC[1]. These days, a large number of population rely on plant-based medicines and several countries are interested to bring it as a mainstream medicine by integrating with modern drugs[2,3]. People in the villages use herbal medicine preferably due to its availability, low price and less side effects. Despite long history and wide-spread practice, the herbal medicine lacks authentication as per the standards of the modern science[4]. Scientific evaluations of such 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]. *Schleichera oleosa* (*S. oleosa*) (synonym: *Schleichera trijuga*) is a semi-evergreen, medium sized (15–32 m) tree, distributed up to 1 200 m in Southern Asia[11]. It is a monogenic plant in family Sapindaceae[12]. Traditionally, different parts of *S. oleosa* have been used for the treatment of different human disorders like pain, skin diseases, dysentery, snake bite and reported to have properties such as antioxidant, antiulcer, antifungal[13-19]. There have also been reports on antimicrobial activities of extracts as well as of pure compounds isolated from less polar fractions of this plant[20]. Here we report a comparative potency of extracts obtained in different solvents from the bark of *S. oleosa* from Nepal to inhibit Gram-positive and Gram-negative bacteria. Motivated with the strong inhibition of few bacterial strains by polar fractions, we also evaluated the toxicity of all extracts in brine shrimps.

## 2. Materials and methods

Bark of *S. oleosa* was collected from Srijana Community Forest, Kohalpur, Banke, Nepal in April 2014. The bacterial strains of *Salmonella typhimurium* (ATCC 14028) (*S. typhimurium*), *Serratia marcescens* (ATCC 13880) (*S. marcescens*), *Staphylococcus aureus* (ATCC 25992) (*S. aureus*), *Escherarichia coli* (ATCC 25922) (*E. coli*),

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*Pseudomonas aeruginosa* (ATCC 27853) (*P. aeruginosa*), *Klebsiella pneumonia* (ATCC 700603) (*K. pneumonia*), *Bacillus subtilis* (ATCC 6501) (*B. subtilis*), *Micrococcus luteus* (*M. luteus*), and *Enterobactor* were obtained from National Endemic Health Care Center, Teku, Kathmandu, Nepal. Brine shrimp (*Artemia salina*) eggs were purchased from Ocean Star International Inc., Snowville, UT, USA.

### 2.1. Preparation of extracts

The bark of *S. oleosa* was cut into small pieces, shed dried and powdered. About 120 g of powdered sample was Soxhlet extracted (16-24 h) sequentially with hexanes, dichloromethane, ethyl acetate, methanol and water. After evaporation of the solvents in reduced vacuum and water bath, we obtained 0.2, 0.1, 0.2, 16.6 and 11.35 g of extracts from hexanes, dichloromethane, ethyl acetate, methanol, and water respectively.

### 2.2. Antibacterial evaluation

Inhibitory activity of the extracts was performed by following the agar well diffusion method[21]. The strains of *S. aureus, B. subtilis, M. luteus, S. typhimurium, S. marcescens, E. coli, P. aeruginosa, Enterobacter* and *K. pneumoniae* were grown in nutrient broth to get required turbidity and swabbed uniformly on Muller Hinton agar (MHA) plates[22]. Wells in the MHA plates were made with the help of a cork borer of 6 mm size. 50 µL solution (obtained by dissolving 50 mg of extract in 1 mL of dimethyl sulfoxide) of each extract were loaded into separate wells for each bacterial strain. The MHA plates were made in duplicate and incubated at 37 ℃ for 24 h. A 25 µg amoxicillin disc was used as a reference for comparison.

### 2.3. Brine shrimp toxicity evaluation

Toxicity of the extracts was evaluated in newly-hatched nauplii of brine shrimps by slightly modifying the method reported by Meyer[23]. Samples were prepared in three dilutions in respective solvents used for extraction and transferred into the test tubes containing discs (made from filter paper). The test tubes were dried in vacuum to get rid of the organic solvents. The nauplii were obtained by hatching eggs of brine shrimp in artificial sea water (ASW) for 48 h in a lighting environment[24]. Ten brine shrimp nauplii with ASW were transferred to each test tube with the help of a disposable pipette. Final adjustment of ASW to 5 mL in each test tube provided three sets of concentrations (1 000, 100, and 10 µg/mL) of the extracts. Five test tubes were prepared for each dose level and blanks were made without extracts. Unlike Meyer, the food (dry yeast suspension) for the nauplii was not added in the test tubes. The test tubes were illuminated for 24 h and the number of survived nauplii was counted. Percentage deaths and $LC_{50}$ values for each dose level were obtained using Finney's probit analysis method[25].

## 3. Results

Antibacterial potencies of extracts were measured as clear zones of average inhibition to bacterial growth and expressed in millimeter (Table 1). Polar extracts (methanol and water) of bark of *S. oleosa* showed strong bacterial inhibition (Figure 1 and Table 1) to both Gram-positive and Gram-negative bacteria. Similar fractions from the leaves of *S. oleosa* were reported to inhibit few bacterial strains[13,26]. Both the extracts effectively inhibited *S. marcescens*, *E. coli*, *M. luteus* and *B. subtilis* while the inhibition was not clearly transparent for *S. aureus*. In the case of *S. marcescens*, *E. coli* and *M. luteus,* the inhibitions were stronger compared to the standard. For *Enterobacter*, mild inhibitions were observed by both of these extracts. The least polar hexane fraction did not show any inhibitions; only slight inhibitions were noticed by dichloromethane and ethyl acetate extracts for *S. aureus*. The alkaloids and saponin obtained from this plant were reported to have inhibition activity against *E. coli*[27]. Less polar compounds like triterpenoids obtained from outer bark of *S. oleosa* were evaluated for antibacterial potency[28]. However, our work showed that the compounds with stronger antibacterial activity from the bark of *S. oleosa* were polar in nature and isolation of pure compounds from polar fractions was motivating to get potential antibacterial compounds. We also evaluated the toxicity of these extracts in brine shrimps. The percent deaths and $LC_{50}$ values for each dose level are presented in Table 2.

**Table 1**

Antibacterial potency of extracts from the bark of *S. oleosa*.

| Bacteria | Bark fractions (zones of inhibition in mm) | | | | | |
|---|---|---|---|---|---|---|
| | Amoxicillin disc (25 µg) | Hexane | Dichloromethane | Ethyl acetate | Methanol | Water |
| *S. typhimurium* (-) | 43 | 0 | 0 | 0 | 0 | 0 |
| *S. marcescens* (-) | 10* | 0 | 0 | 0 | 18 | 18 |
| *S. aureus* (+) | 47 | 0 | 13* | 14* | 18* | 16* |
| *E. coli* (-) | 12* | 0 | 0 | 0 | 16 | 15 |
| *P. aeruginosa* (-) | 48 | 0 | 0 | 0 | 0 | 0 |
| *B. subtilis* (+) | 17 | 0 | 0 | 0 | 15 | 13 |
| *Enterobacter* (-) | 50 | 0 | 0 | 0 | Mild | Mild |
| *K. pneumoniae* (-) | 47 | 0 | 0 | 0 | 0 | 0 |
| *M. luteus* (+) | 12 | 0 | 0 | 0 | 16 | 15 |

Agar gel plates were made in duplicates. *: The inhibition was not clear.

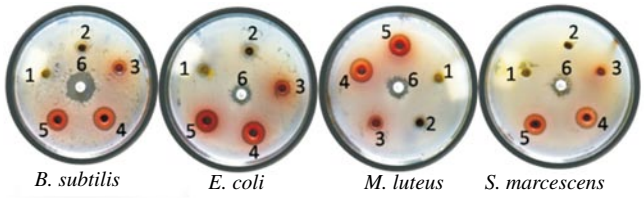


**Figure 1.** Bacterial inhibition by extracts obtained from the bark of *S. oleosa*.

1: Hexanes extract; 2: Dichloromethane extract; 3: Ethyl acetate extract; 4: Methanol extract; 5: Aqueous extract; 6: Amoxycillin disc (25 µg).

**Table 2**

Brine shrimp toxicity assay of extracts from bark of *S. oleosa*.

| Extracts | Percent of inhibition with dose levels | | | $LC_{50}$ (µg/mL) |
|---|---|---|---|---|
| | 10 µg | 100 µg | 1 000 µg | |
| Hexanes | 12 | 20 | 28 | $5.62 \times 10^5$ |
| Dichloromethane | 6 | 18 | 18 | $1.00 \times 10^8$ |
| Ethyl acetate | 16 | 16 | 30 | $1.58 \times 10^6$ |
| Methanol | 16 | 22 | 96 | $1.31 \times 10^2$ |
| Aqueous | 14 | 24 | 50 | $1.38 \times 10^3$ |

## 4. Discussion

Leaves of *S. oleosa* are used as a fodder for cattle and people eat the pulp of its fruit. The compounds isolated from such plants have higher chance to pass the toxicity barrier to emerge as a new drug.

The antibacterial potency of methanolic and aqueous fractions motivated us to evaluate the toxicity of its fractions. In the toxicity evaluation, different extracts possessed different toxicities to brine shrimps. The methanol fraction was found most toxic while the dichloromethane fraction was the least. The percent death of brine shrimps was found increasing with dose; however, none of the fractions were found significantly toxic ($LC_{50} \leqslant 30$ μg/mL) [18]. Thus the less toxic nature of extracts and their strong bacterial inhibition capacity for few strains have encouraged us to isolate pure compounds from the active fractions.

Methanolic and aqueous fractions of bark of *S. oleosa* were found effective to inhibit *S. marcescens*, *E. coli*, *M. luteus* and *B. subtilis*. Less toxic nature of these fractions provides motivation to purify active compounds from these extracts toward the development of new antibacterial drugs.

## Conflict of interest statement

We declare that we have no conflict of interest.

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