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

Laxman Pokhrel¹,²*, Bigyan Sharma¹,², Gan B. Bajracharya¹

¹Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal
²Kathmandu Institute of Applied Sciences, Banasthali, Kathmandu, Nepal

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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*, *Escherichia 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 4500 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 1200 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 25923) (*S. aureus*), *Escherichia coli* (ATCC 25922) (*E. coli*),
Pseudomonas aeruginosa (ATCC 27853) (P. aeruginosa), Klebsiella pneumonia (ATCC 700603) (K. pneumonia), Bacillus subtilis (ATCC 6501) (B. subtilis), Micrococcus luteus (M. luteus), and Enterobacter 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 (16-24 h) sequentially with hexanes, dichloromethane, ethyl acetate, powdered. About 120 g of powdered sample was Soxhlet extracted against bacterial growth and expressed in millimeter

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 duplicates and incubated at 37 °C 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 LC50 values for each dose level were obtained using Finney’s probit analysis method[25].

Table 2

| Extracts       | Percent of inhibition with dose levels | LC50 (μg/mL) |
|----------------|---------------------------------------|--------------|
| Hexanes        | 10 μg 20 28                           | 5.62 × 10^3  |
| Dichloromethane| 100 μg 18 18                          | 1.00 × 10^3  |
| Ethyl acetate  | 1000 μg 16 30                         | 1.58 × 10^3  |
| Methanol       |                                       | 1.31 × 10^2  |
| Aqueous        | 14 24 50                              | 1.38 × 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 (LC50 ≤ 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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