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Brine shrimp toxicity of various polarities leaves and fruits crude fractions of *Ziziphus jujuba* native to Oman and their antimicrobial potency

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ABSTRACT

Ziziphus jujuba (*Z. jujube*) is a herb used traditionally for the treatment of different aliment since ancient times, however, the scientific information on this plant is still insufficient. The pure active chemical compounds and crude extracts of *Z. jujube* can be used in food, formulated pharmaceutical products, natural antioxidant, antibiotics, anticancer agent and preservatives. Therefore, the objective of this work is to determine the cytotoxic and antimicrobial activities of different polarity of crude fractions of locally grown *Z. jujube* by applying brine shrimp lethality and agar gel disc diffusion bioassay. The cytotoxic result reveals that all prepared crude fractions from the leaves and fruits were lethal to all shrimp larvae at a concentration of 1000 μg/ml. Among the leaves crude fractions, butanol extract showed the highest cytotoxic activity with LC_{50} value of 8.69 μg/ml, whereas the lowest activity was in chloroform crude extract with LC_{50} value of 45.12 μg/ml. Similarly, in fruits, the highest activity was in ethyl acetate extract with an LC_{50} value of 11.62 μg/ml and the lowest was in water extract with an LC_{50} value of 149.28 μg/ml. The methanol and its corresponding fractions were displayed moderate antimicrobial activity against Gram (+ and −) bacteria at various concentrations with a zone of inhibition range of 0–17 mm. Therefore, the active polar crude fraction of *Z. jujuba* or its pure component could be candidates for use as safe, effective and comparatively cheap antibiotics and anticancer agents.

1. Introduction

Large numbers of population all over the world still rely on complementary and alternative medicine for the treatment of incurable diseases, including cancers and various types of infectious diseases. Herbal products and their formulated drugs (including antibiotics) represent more than 60% of all drugs in clinical use in the world. About 25% of the total drugs comes from the higher plants. Today, the world population is nearing five billion at a growth rate, which is likely to reach 7.5 billion by 2020. According to the WHO report, 80% of people all over the world used complementary and alternative medicine for some aspect of principal health care system. Recently, scientists are involved to investigate new bioactivity from several natural resources, including medicinal plants and to isolate the principal ingredients with new chemotherapeutic drugs against different life threaten diseases such as cancer, AIDS, SARS and transferable diseases etc. (Fodouop et al., 2015; Sasidharan et al., 2011; Dias et al., 2012). The demand of plant related drugs is continuously increasing more and more to protect life threaten diseases. The biological and phytochemical screenings on natural resources including plants having their medicinal values are going on nationally and internationally (Fodouop et al., 2015; Sasidharan et al., 2011; Dias et al., 2012). Fruits, vegetables, medicinal plants and their formulated products with pharmacological properties have been shown to be rich sources of ingredients with potential to prevent incurable diseases (Kumar et al., 2010; Asma et al., 2016; Upadhyay, 2016). Sustainable development involves cost effective, reduced waste management, environmental protection, high market demand of plant extracts. In our present study, several organic solvents are used for the extraction of phytochemicals from *Z. jujuba* however, there is a need to extract by using sustainable and cost effective solvents which are safe for human consumption. In addition, the active and safe crude fraction of *Z. jujuba* or its isolated pure components could be candidates for use as safe, effective and comparatively cheap antibiotics and anticancer agents.

Z. jujuba is a medicinal plant belonging to Rhamnaceae family. It has been used in many disorders since long times all over the world. The plant is widely found in tropical and subtropical areas (Al-Reza et al., 2010). In the Arabian Gulf, it is known as Sidr (Erenmemisoglu et al., 1995; Kim, 2009). The plant height is about 5–10 m with branches. Several bioactive compounds were already isolated and

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identified from the fruits of Chinese *Z. jujube* (Wu et al., 2014). Chinese and Korean people have used traditionally its fruits as antifungal, antibacterial, antiulcer, anti-stress, anti-inflammatory and sedative (Souleles, and Shammas, 1998; Khare, 1995). They have also been used as antispastic, contraception, hypotensive and against chronic constipation, cardiotonic, antinephritic, antioxidant, immunostimulant, and for wound healing (Bashir et al., 2011; Dang et al., 2014; Asma et al., 2016). However, Omani communities, the selected plant is used traditionally to treat swollen joint, dandruff, antifungal, acne, fever, burns, constipation, cardiotonic, diarrhea and as an antiseptic since ancient times (Asma et al., 2016). Limited work has been done on the selected species worldwide and as yet no work has been carried out on the Omani *Z. jujuba*. Therefore, our aim of this present project is to evaluate cytotoxic and antimicrobial activities of leaves and fruits crude fractions of *Z. jujube* by using BSL and disc diffusion bioassay..

2. Materials and methods

2.1. Materials

The chemicals such as dimethyl sulphoxide, acetone, hexane, chloroform, methanol and butanol were bought from Fisher Scientific Company, UK. Sodium sulfate was bought from Scharlau, European Union. Filter paper was purchased as discs (Whatmann, GE Healthcare Companies, China, Catalogue number 1001090). Shrimp egg, amoxicillin, sodium chloride, petti dishes and other chemicals were bought from Sigma-Aldrich Company, Germany.

2.2. Bacterial strains

The selected Gram (+ and −) bacterial strains like *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli*), *Proteus spp* (*Proteus spp*) and *Haemophilus influenzae* (*H. influenzae*) was collected from local hospital on February 28, 2015.

2.3. Collection of plant materials

Z. jujuba leaves and fruits samples were collected from Sur, Sultanate of Oman. It is located in the northeastern part of Oman. The samples were collected on September 13, 2014 at 10 am. The collected samples were carried to the Pharmacy Research Lab, University of Nizwa, for further processing. Morphological features and database of this plant are present in the website (https://en.wikipedia.org/wiki/Jujube).

2.4. Preparation of plant samples

Z. jujuba leaves and fruits samples were washed with water and cut into small pieces for drying. The samples were kept at room temperature for 7 days. Both the samples were ground separately into a coarse powder by using a laboratory mill. Then, the coarse powdered samples (75 g) were extracted separately with methanol (150 ml) for 72 h by using a Soxhlet extraction method (Hossain et al., 2013, 2017). After extraction, the extracting solvent was evaporated by using a rotary evaporator at 22 °C under reduced pressure to get the methanol crude fraction (7.78 g). The methanol crude fraction (10 g) was defatted with water and subjected to sequential extraction with different solvents of increasing polarity to afford hexane (1.25 g), ethyl acetate (2.67 g), chloroform (1.72 g), and butanol (0.32 g), respectively. The whole extraction process is being repeated twice. All polarities crude fractions were combined together and concentrated by using a rotary evaporator. In addition, the remaining water part was evaporated by using a rotary evaporator to afford water fraction (0.43 g).

2.5. Antimicrobial activity assay

Antimicrobial activity of different polarities leaves and fruits crude fractions was performed using a standard disc diffusion assay on nutrient agar against the selective Gram (+ and −) bacterial strains. One Gram (+) (*S. aureus)* and three Gram (−) bacteria *(E. coli, Proteus spp* and *H. influenzae*) were used for the determination of antimicrobial activity against different polarities fractions at different concentrations as described by Hossain et al. (2014). Four concentrations, 2, 1, 0.5 and 0.25 mg/ml of each fraction were prepared with dimethyl sulphoxide (DMSO) in this experiment. Positive control discs contained of amoxicillin antibiotic (3 mg/ml) whereas the negative control discs were discs with DMSO solvent. Different concentrations of different crude fractions and positive control were loaded on the inoculated agar plates. Plates were incubated micro aerobically at 37 °C for 24 h until the culture developed. Antibacterial activity was expressed in terms of the diameter of the zone of inhibition against the tested bacteria and it was measured in millimeter using scale. The antimicrobial assay was repeated twice.

2.6. Cytotoxic activity assay

Cytotoxic activity of different polarity leaves and fruits crude fractions of *Z. jujuba* were estimated by the BSL method as described earlier by Weli et al. (2014). The shrimp eggs were hatched at the covered chamber of the duo compartment plastic container in artificial sea water for 24 h. After hatching, the active nauplii were separated from the eggs and these were used for the cytotoxic activity of the crude fractions. Different polarity crude fractions of leave and fruit samples (5 mg) were placed in vials and dissolved in 10 ml of dimethyl sulfoxide (DMSO) to get a stock solution of 1000 μg/ml. Different volumes were taken from the stock solution and diluted in NaCl solution to make final concentrations of 500, 100 and 10 μg/ml, respectively. Ten brine shrimp nauplii were taken in each vial and final volume (5 ml) was adjusted by artificial sea water. 1% DMSO solvent was used as a negative control. After 24 h, the numbers of surviving nauplii in each vial were counted using a magnifying glass. The percentage of lethality of shrimp was calculated for each concentration of the sample.

3. Results

The semisolid crude extract was obtained by the evaporation of methanol solvent. The obtained methanol crude fraction was defatted with water and then sequential fractionated with hexane, chloroform, ethyl acetate and butanol to afford hexane, ethyl acetate, chloroform, and butanol and residual methanol fractions respectively.

3.1. Antimicrobial activity

The antimicrobial activity of the leaves and fruits crude fractions was performed by the disc diffusion assay (Hossain et al., 2014; Rehab and Hossain, 2016a, 2016b). The activity of methanol crude fraction and its subfractions of *Z. jujuba* resulted in a range of zone inhibition patterns against different selected Gram (+ and −) microbes. Both leaves and fruits crude fractions showed antibacterial activity against the selected Gram (+ and −) bacteria strains at the concentration of 2 mg/ml, 1 mg/ml, 0.5 mg/ml and 0.25 mg/ml. Amoxicillin antibiotic (3 mg/ml) was used as positive control whereas 1% DMSO solvent was used as negative control. All the leaves and fruits crude fractions of *Z. jujuba* has shown significant antibacterial activity against all employed cultured bacteria strains within the range of 0–17% as shown in Table 1.

3.2. Cytotoxic activity

All the leaves and fruits crude fractions have displayed moderate