SHORT COMMUNICATION

Brine Shrimp Bioassay of Ethanol Extracts of Sesuvium verrucosum, Salsola baryosma and Zygophyllum quatarense Medicinal Plants from Bahrain

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Ethanol whole plant extracts of three halophytic plants from Bahrain Sesuvium verrucosum, Salsola baryosma, Zygophyllum quatarense have been tested for their cytotoxic activity by the brine shrimp method. Only S. verrucosum showed a marked significant activity (LC50 = 102.7 μg/mL) Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: Bahraini medicinal plants; cytotoxicity; brine shrimp; Sesuvium verrucosum.

INTRODUCTION

The halophytic plants, Sesuvium verrucosum Raf. (vernacular name: Rokhama), Salsola baryosma (Roem. et Schult.) Dandy (vernacular name: Gaghraf) and Zygophyllum quatarense Hadidi (vernacular name: Harm) are important plant cover in the process of land reclamation (Phillips, 1988). In Bahrain they are important in folk medicine. S. verrucosum is used for the treatment of ear disorders, whereas S. baryosma is used against inflammations and as a diuretic (Al-Saleh et al., 1993). The latter plant has been tested and found to possess CNS depressant activity (Woo et al., 1977). The genus Zygophyllum to which Z. quatarense belongs is known to contain harmine, a plant toxin and a CNS stimulant (Duke, 1985), whereas the other species present in Bahrain, Z. simplex (El-Oqlah and Abbas 1994), is used as an antibiotic and a laxative (Al-Saleh et al., 1993). The plant extracts have been reported to contain mainly alkaloids in leaves and sterols and tannins to a lesser extent. S. baryosma and Z. quatarense have tested positively for alkaloids, coumarins and sterols (Rizk, 1986). However, there was no detailed pharmacological study on the cytotoxicity of these plants. The objectives of this study were to evaluate the cytotoxic effects of the ethanolic extracts of these plants using the brine shrimp test (BST) method as a broad measure of anti-tumour activity.

MATERIALS AND METHODS

Plant material. S.verrucosum (SV), S.baryosma (SB) and Z. quatarense (ZQ) were collected from around Sakhir Campus and Zallaq beach and authenticated at the Biology Department, College of Science, Isa Town, Bahrain. The whole plants were dried in shade, powdered and kept (at 20°C) in closed plastic containers.

Preparation of extracts. Powdered plants (30 g) were defatted with petroleum ether 30–40°C (200 mL), extracted continuously (Soxhlet extraction) with 96% ethanol (300 mL) and whole plant extracts were obtained by complete evaporation of the solvent (rotatory evaporator) at 40°C. The residue left after evaporation (2.0–2.5 g) was dissolved in chloroform (20 mL) followed by filtration and the filtrate was separated from droplets of water and dried (anhydrous MgSO4). Evaporation of the chloroform whole plant extract (CWPE) afforded a syrupy liquid, which was used directly in the BST experiment.

Brine shrimp bioassay. The BST bioassay experiment was performed according to the procedure described by Meyer (Meyer et al., 1982). Samples for the experiment were prepared by dissolving 50 mg of the CWPE of each plant in 5 mL of methanol. Appropriate amounts of this methanol solution (5μL, 50μL and 500μL to give concentrations of 10, 100 and 1000μg/mL, respectively) were transferred (Volac Micro Pipetter) to 2.1 cm discs of filter paper (Whatman Grade 1). The discs were dried in air, placed in 30 mL glass vials (Beatson Clark Glass), then dried further in vacuum for 1 h to remove methanol completely.

Control discs were prepared using only methanol. Five replicates were prepared for each dose level. To begin the bioassay brine shrimp eggs (obtained from Directorate of Fisheries, Bahrain) were hatched in a shallow rectangular dish (22 × 32 cm) under the same conditions described in the literature except that natural instead of artificial...
seawater was used. Ten shrimps were selected and transferred into each sample vial by means of a 23 cm disposable Pasteur pipette (MRS Scientific) and the final volume in each vial was adjusted to 5 mL using natural seawater. A drop of dry yeast suspension (S. I. Lesaffre) (3 mg in 5 mL seawater) was added as food to each vial. The vials were maintained under illumination. Survivors were counted with the aid of a stereomicroscope, after 6, 24 and 48 h, and the deaths at each dose level and control were determined. No deaths were observed to occur in the control after 48 h.

**Lethal concentration determination.** The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC$_{50}$) and 95% confidence intervals were determined from the 24 h and 48 h counts and the dose-response data were transformed into a straight line by means of a trendline fit linear regression analysis (MS Excel version 7); the LC$_{50}$ was derived from the best-fit line obtained.

Caffeine (LC$_{50}$ = 306 mg/mL) (Meyer et al., 1982) purchased from Fluka was used as a positive control and methanol (500 mg/mL) as a solvent and a negative control in the bioassay experiments.

**Statistical analysis.** The results were expressed as the mean ± SE. Statistical significance of the mean mortality at each concentration was analysed using one-way analysis of variance (ANOVA) and compared using Duncan’s multiple range test. Values of $p \leq 0.05$ were taken to be statistically significant. All statistical analyses were carried out using Statigraphic Package Version 6.

**RESULTS AND DISCUSSION**

The brine shrimp test (BST) represents a rapid, inexpensive and simple bioassay for testing plant extract lethality which in most cases correlates reasonably well with cytotoxic and anti-tumour properties (McLaughlin, 1991).

Following the procedure of Meyer (Meyer et al., 1982) the lethality of the plant (SV, SB and ZQ) extracts to brine shrimp was determined and the values are expressed in Table 1.

Table 1 gives the results of the brine shrimp after 24 h exposure to whole plant extracts of Sesuvium verrucosum (SV), Salsola baryosma (SB), Zygophyllum quatarense (ZQ) and the positive control caffeine (CAF). Caffeine, compared with the negative control (methanol) which showed no mortality to brine shrimp, was moderately lethal, giving significantly higher mortality to the shrimp than the negative control. The plant extracts, of Salsola baryosma (SB) and Zygophyllum quatarense (ZQ) were not significantly different from that of the negative control. Also SB and ZQ showed no statistical differences in shrimp percentage mortalities between different concentrations within the same plant species, indicating no significant lethality. However, significant lethality to Artemia salina was observed with exposure to different dose levels of Sesuvium verrucosum (SV) extracts. The degree of lethality was directly proportional to the concentration of the extract ranging from significant with the lowest concentration (10 mg/mL) to highly significant with the highest concentration (1000 mg/mL) compared with methanol. Maximum mortalities took place at a concentration of 1000 mg/mL, whereas least mortalities were at 10 mg/mL concentration. In other words, mortality increased gradually with the increase in concentration of SV plant extract. Other plant species, however, showed no statistical differences in percentage mortalities between different concentrations within the same plant species, indicating no significant toxicity compared with SV. The Positive control compound caffeine (CAF) showed a less significant toxicity than SV.

A similar mortality effect was found after 48 h exposure to each plant extract.

The lethal concentration LC$_{50}$ of (SV) plant at 24 h was obtained by a plot of percentage of the shrimps killed against the logarithm of the SV plant extract concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis. LC$_{50}$ was obtained from the best-fit line slope and found to be LC$_{50}$ = 102.7 mg/mL (Fig 1). This significant lethality of Sesuvium verrucosum plant to brine shrimp is indicative of the presence of a

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**Table 1. One-way ANOVA of brine shrimp Artemia salina mortality (%) when subjected to different concentrations of three plant species after 24 h exposure**

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Plant SV</th>
<th>Plant ZQ</th>
<th>Plant SB</th>
<th>Standard CAF</th>
<th>ANOVA F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.00$^{(a)}$ ± 0.547</td>
<td>0.00$^{(b)}$</td>
<td>0.00$^{(b)}$</td>
<td>0.00$^{(b)}$</td>
<td>3.333$^{*}$</td>
</tr>
<tr>
<td>100</td>
<td>4.00$^{(a)}$ ± 1.00</td>
<td>0.00$^{(b)}$</td>
<td>0.00$^{(b)}$</td>
<td>0.00$^{(b)}$</td>
<td>16.00$^{*}$</td>
</tr>
<tr>
<td>1000</td>
<td>8.60$^{(a)}$ ± 0.510</td>
<td>0.40$^{(b)}$ ± 4.00</td>
<td>1.60$^{(b)}$ ± 1.166</td>
<td>0.60$^{(b)}$ ± 0.245</td>
<td>33.101$^{*}$</td>
</tr>
<tr>
<td>ANOVA F ratio</td>
<td>28.179$^{*}$</td>
<td>1.00$^{NS}$</td>
<td>1.882$^{NS}$</td>
<td>6.000$^{*}$</td>
<td></td>
</tr>
</tbody>
</table>

Mean value in each column followed by the same letter superscript are not significantly different at $p \leq 0.05$.

Mean value in each row followed by the same letter superscript and between ( ) are not significantly different at $p \leq 0.05$.

* Significantly different.
potent cytotoxic component which warrants further investigation. This order of magnitude of toxicity is comparable to the values for *Myrsine africana*, a plant from which McLaughlin (1991) isolated the more potent 2-hydroxychrysophanol.

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REFERENCES


