Pharmacological Studies on Four Anti-Tumor Medicinal Plants Grown in Sudan

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INTRODUCTION

In Sudan, the assimilation of many plant species into traditional practice was reported a long time. Albeit, many species were not investigated systematically or in depth, yet traditional knowledge ascertains their appreciable diverse curative properties. In this context, four plant species namely, *Ambrosia maritima* L., *Ammivisnaga* L., *Aristolochiabracteolata* L. and *Lawsoniainermis* L., grown in Sudan, were reported to treat vast myriad of diseases including infectious and neglected diseases. Traditionally, these species are reputed to cure gastrointestinal disturbances, diabetes, hypertension, malaria, tumor as well as many bacterial, fungal and viral infections. Concerning the area of cancer, it is worth noting that, it’s a leading cause of death worldwide. Despite the existence of chemotherapeutic curable cancers, yet numerous cases still suffers from resistance to such therapy or drastic health deterioration pertaining with their side effects. Thus, it has become imperative for scientists to tap another field of research and make benefit of the reputed medicinal plants’ curative properties with a hope to find new, safe and effective therapeutic regimen. Many bioassays are adopted to assess the diverse therapeutic properties including the antitumor activity of medicinal plants in...
question. Herein, the Brine shrimp Lethality Assay (BSLA) is an internationally accepted in vivo bioassay to assess the cytotoxic properties of natural products\textsuperscript{1,10}. This assay is a versatile tool adopted for detection, fractionation and isolation of antitumor compounds from plants extracts\textsuperscript{1,12}. Many herbal preparations, in spite of their natural originality, may exert pronounced toxicities\textsuperscript{13}. Hence, it is also desirable to include an assay on normal cell-line so as to elicit the safety profile during the pharmacological screening level. There are several toxicological assays in practice to determine the safety profile of the plant extracts. The assessment can be performed on whole laboratory animal, isolated tissue specimen or cell line, example, the Micro-culture Tetrazolium (MTT) assay\textsuperscript{14,15}. The present study aimed to assess the reputed antitumor activity of four medicinal plants, grown in Sudan, by the BSLA and to assess the safety profile of the BSLA active extracts on RAW 264.7 normal macrophage cell line. Moreover, the most promising fraction will be subjected to preliminary phytochemical screening to reveal their phytochemicals that may be associated with the cytotoxicity.

**MATERIALS AND METHODS**

**Collection and preparation of plants materials**

Four plant species, grown in Sudan, were obtained. Three species were collected from their natural habitat and the fourth was obtained from the local market (Table 1). The plants were taxonomically authenticated by Dr. Haidar Abdalgadir and their voucher specimens had been deposited at Medicinal and Aromatic Plant Research Institute (MAPRI) Herbarium, Sudan. Based on ethnomedicinal uses, the active morphological parts were cleaned from dirt, shade dried, grounded into coarsely powder crude material (Table 1).

**Extraction of plants materials**

Cold maceration extraction of the dried powdered materials (100g) was commenced with dichloromethane (DCM) then 80% methanol (MeOH). Maceration process was repeated, several times, for each solvent to complete the extraction process. The obtained extracts were filtered, air- dried at room temperature. The yield percentage of each dried extracts was calculated prior to bioassays.

**Bioassays**

**Brine shrimp lethality assay**

The BSLA was conducted according to the standard method\textsuperscript{16} with some modifications. A weight of 20 mg of each dried crude extract was dissolved in 2 ml distilled water containing 2% dimethyl sulfoxide (DMSO). Volumes of 5\(\mu\)l, 50\(\mu\)l and 500\(\mu\)l of each test extract solution were distributed in 3 glass vials A, B and C, respectively. Brine shrimp (Artemia salina) eggs were placed in a shallow well-aerated rectangular tank, filled with sea water. The tank was left at ambient temperature for 48 hours. To each vial A, B and C, ten brine shrimps nauplii were added and the volume of the mixture was completed to 5\(\mu\)l with sea water to obtain concentrations of 10, 100 and 1000\(\mu\)g/ml respectively. After 24 hours count, the survivor nauplii were counted and mortality percent (M\%) was calculated. Potassium dichromate was used as the reference standard and 2% DMSO in natural seawater was used as negative control for the cytotoxicity assay. The assay was performed in triplicate for each concentration and M\% and LC\(_{50}\) were statistically analyzed.

**Micro-culture tetrazolium (MTT) assay**

The MTT colorimetric assay was conducted according to a standard method\textsuperscript{17}. The monolayer RAW 264.7 cell line culture was mechanically scraped and detached, and then the cell count was adjusted to \(1\times10^4\)-\(10^5\) cells/ml using RPMI-1640 media containing 10\% fetal bovine serum (FBS). To each well of the 96 well microtitre plate, 100\(\mu\)l of diluted cell suspension was added. After 24 hours, when the monolayer formed, the supernatant was flicked off and 100\(\mu\)l of fresh complete media was added to all wells. Afterward, serial dilutions of the crude extracts in 5\% DMSO were prepared to give concentrations of 125, 250 and 500 \(\mu\)g/ml. The microtitre plate was incubated at 37\(^\circ\)C in 5 \% CO\(_2\) incubator for 72 hour and cells were periodically checked for granularity, shrinkage, and/ or swelling. After the incubation period, 50\(\mu\)l of MTT solution (5mg/ml in phosphate buffered saline) was added to each well then the plates were gently shaken and incubated for 4 hours at 37\(^\circ\)C in 5 \% CO\(_2\) incubator. Afterwards, the supernatant was removed, 100\(\mu\)l of neat DMSO was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using ELISA reader at a wavelength of 570 nm. Triton X 100 was used as positive control. The assay was conducted in triplicate and the percentage growth inhibition was calculated and was statistically analyzed.
Statistical analysis
The data collected for the BSLA was statistically analyzed by using the Statistical Package for Social Sciences (SPSS) version 11.5 program. The LC$_{50}$ values were obtained with Finney computer program with 95% confident intervals$^{17}$. Whereas data obtained from MTT assay was statistically analyzed and the results were expressed as means ± standard deviation of the mean.

Preliminary phytochemical screening
Of all bioassessed plant’s extracts, only those most active extract, based on LC$_{50}$, was subjected to standard phytochemical screening methods$^{18,19}$ to determine the presence of secondary metabolite namely, alkaloids, anthraquinones glycosides, flavonoids, saponins, tannins, sterol and triterpenes.

RESULTS AND DISCUSSION
Brine shrimp lethality assay (BSLA) was performed against Artemiasalina nauplii. The cytotoxic activity of the tested plant extracts is manifested as mortality of brine shrimps. From the results obtained in table (2), the mean mortality percentage was significantly (p<0.01) affected by the plant extracts and concentration. Potassium dichromate, +ve control, reported a significantly higher mean of mortality percentage for brine shrimp, followed by $A$. maritima’s whole plant DCM extract (AMW71) obtaining values of 100% and 94.4% respectively, as compared to other mean mortality values. On the other hand, no mortality was observed in the control negative vials; indicating that the test samples were responsible for the brine shrimp lethality. According to the standard method$^{16}$, all plants extracts, except those of $A$. visnaga, were active in the BSLA with LC$_{50}$<1000 µg/ml. In comparison with other tested plants extracts, AMW-1 extract ranked as the most potent in terms of both mean mortality percentage (94.4%) and LC$_{50}$(0.015µg/ml). Regarding the MTT assay, a glance at the toxicological profile of the resultant 8 BSLA active extracts (Fig. 1), the tested extracts displayed variable performance on RAW 246.7 normal cells viability. Gratifyingly, the dichloromethane extracts of $A$. maritima (AMW-1), $A$. bracteolata seeds (AAS-1) and branches (ABB-1) were proved to have no detrimental effect on cell viability up to a dose of 500µg/ml (Fig. 1).

Preliminary phytochemical screening of AMW-1 revealed the presence of alkaloids, coumarins, flavonoids and sterols. These phytochemical classes are well known among the Asteraceae members and bear diverse pharmacological activities including antitumor$^{20,21}$.

CONCLUSION
It is clearly evident from the above findings that $A$. maritima DCM extract rank the top in the BSLA. Other tested extracts, except those of Ammivisnaga, reported significant cytotoxic activity (LC$_{50}$<1000 µg/ml). The dichloromethane extracts of $A$. maritima, $A$. bracteolata seeds and branches displayed no detrimental effect upon toxicological studies, and these promising results support the traditional uses. To this end, it is likely to endorse the activity to the presence of potential antitumor molecules. Further work is underway to isolate and characterize the active constituents.

ACKNOWLEDGMENTS
Sincere thanks for Dr. Waleed S. Koko, Department of Microbiology and Parasitology, Medicinal and Aromatic Plants Research Institute (MAPRI), The National Centre for Research, Sudan, for the providing the cell line. We are greatly indebted to Professor Hassan M. Ali, Department of Pharmacology-National University, Sudan, who has facilitated the purchase of Brine Shrimp from the United States of America. Grateful thanks to Dr. HaidarAbdalagadir taxonomist, Herbarium-MAPRI for the identification of plants species and his great help and cooperation in the cytotoxicity assay. The author, also, acknowledges Mr. Mudathir Seddig Elhassan, Department of Phytochemistry, MAPRI for their help in the plants’ extraction.

<table>
<thead>
<tr>
<th>Code</th>
<th>Botanical name (Family)</th>
<th>Locality</th>
<th>Selected part</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMW</td>
<td>Ambrosia maritima (Asteraceae)</td>
<td>Nile banks</td>
<td>Whole plant</td>
</tr>
<tr>
<td>AVS</td>
<td>Ammivisnaga (Apiaceae)</td>
<td>Local market</td>
<td>Seeds</td>
</tr>
<tr>
<td>ABS</td>
<td>Aristolochiabracteolata (Aristolochiaceae)</td>
<td>Lowland plain</td>
<td>Seeds</td>
</tr>
<tr>
<td>ABL</td>
<td></td>
<td></td>
<td>Leaves</td>
</tr>
<tr>
<td>ABB</td>
<td></td>
<td></td>
<td>Branches</td>
</tr>
<tr>
<td>LIL</td>
<td>Lawsoniainermis (Lythraceae)</td>
<td>Northern territory</td>
<td>Leaves</td>
</tr>
</tbody>
</table>

Table 1: Represents botanical names, families, localities and the targeted parts of the studied plants species
Table 2: Mean mortality percentage and LC\textsubscript{50} values of the tested plants extracts

<table>
<thead>
<tr>
<th>Code</th>
<th>Solvent</th>
<th>Mortality %</th>
<th>LC\textsubscript{50} (µg/ml)</th>
<th>Mean Mortality %</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>10 µg/ml</td>
<td>100 µg/ml</td>
<td>1000 µg/ml</td>
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<tr>
<td>AMW</td>
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<td>90</td>
<td>93.2</td>
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<td></td>
<td>MeOH</td>
<td>80</td>
<td>93.2</td>
<td>100</td>
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<tr>
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<td>DCM</td>
<td>13.0</td>
<td>23.12</td>
<td>43.21</td>
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<tr>
<td></td>
<td>MeOH</td>
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<td>1.9</td>
<td>16.3</td>
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<tr>
<td>ABS</td>
<td>DCM</td>
<td>63.2</td>
<td>83.26</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>MeOH</td>
<td>90</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>ABL</td>
<td>DCM</td>
<td>66.6</td>
<td>73.3</td>
<td>93.3</td>
</tr>
<tr>
<td></td>
<td>MeOH</td>
<td>63.2</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>ABB</td>
<td>DCM</td>
<td>76.6</td>
<td>83.2</td>
<td>93.2</td>
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<td>MeOH</td>
<td>73.3</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

Error = 0.175, C.V (%) = 5.3

Key:

- Ctrl +ve = Potassium dichromate
- Ctrl -ve = Ten brine shrimps nauplii were used in natural sea water without any plant extract.

Fig. 1: Bar diagram represents the effect of different plant extracts on the percentage of growth inhibition for RAW 264.7 cell line

Key:

1= Dichloromethane extract 2= Methanol extract

Ctrl +ve = Triton X 100 (dilution 1:200)

Ctrl -ve = RPMI-1640 media containing RAW 264.7 cell line in 5% fetal bovine serum (FBS) in without any drug.

REFERENCES


