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Research Article

PHARMACOLOGICAL STUDIES ON FOUR ANTI-TUMOR MEDICINAL PLANTS GROWN IN SUDAN

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ABSTRACT

Medicinal plants have been indispensible in treating diverse forms of diseases. Therefore, searching for plant species and highlighting their applications in tradition medicine provide an overview of their curative activities. This study is carried out to assess the cytotoxicity of four medicinal plants namely, *Ambrosia maritima*L., *Ammivisnaga* L., *Aristolochiabracteolata*L. and *Lawsoniainermis* L., grown in Sudan, traditionally used as antitumor. Furthermore, the study extent to investigate their safety profile on RAW 246.7 normal cell line. Dichloromethane (DCM) and 80% methanolic (MeOH) plant extracts were screened for their cytotoxic activity via the Brine Shrimp Lethality Assay (BSLA). The potential cytotoxic extracts were estimated for their safety profile using MTT assay. The most active and less toxic extract was subjected to preliminary phytochemical screening. The plants extracts, except those of *A. visnaga*, showed lethality against brine shrimp (LC_{50}= 0.015 to 6.6 µg/ml). Of all of the MTT assessed extracts, *A. maritima* DCM, *A. bracteolata* seeds and branches MeOH extracts showed no detrimental effect on RAW 246.7 normal cell line. Preliminary phytochemical screening of *A. maritima* DCM extract revealed the presence of coumarins, flavonoids, sterols and tannins.

Keywords: Medicinal plants, Antitumor, Brine Shrimp Lethality Assay, RAW 246.7, Sudan.

INTRODUCTION

In Sudan, the assimilation of many plants species into traditional practice was reported a long time[1].Albeit, many species were not investigated systematically or in depth, yet traditional knowledge ascertains their appreciable diverse curative properties[2].In this context, four plants 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[3]. Traditionally, these species are reputed to cure gastrointestinal disturbances, diabetes, hypertension, malaria, tumor as well as many bacterial, fungal and viral infections[3,4]. Concerning the area of cancer, it is worth noting that, it's a leading cause of death worldwide[5]. Despite the existence of chemotherapeutic curable cancers[6], yet numerous cases still suffers from resistance to such therapy or drastic health deterioration pertaining with their side effects[7].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[8]to assess the cytotoxic properties of natural products[9,10]. This assay is a veritable tool adopted for detection, fractionation and isolation of antitumor compounds from plants extracts[11,12].Many herbal preparations, in spite of their natural originality, may exert pronounced toxicities[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[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 fractionwill be subjectedto 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 authenticatedby Dr. HaidarAbdalgadir 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[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 5µl, 50µl and 500µl of each test extract solution were distributed in 3 glass vials A, B and C, respectively. Brine shrimp (*Artemiasalina*) 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 shrimpsnauplii were added and the volume of the mixture was completed to 5ml with sea water to obtain concentrations of 10, 100 and 1000µg/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[14]. The monolayer RAW 264.7 cell line culture was mechanically scraped and detached, and then the cell count was adjusted to 1×10^4-10^5 cells/ ml using RPMI- 1640 media containing 10% fetal bovine serum (FBS). To each well of the 96 well microtitre plate, 100µl of diluted cell suspension was added. After 24 hours, when the monolayer formed, the supernatant was flicked off and 100µl 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 µg/ml. The microtitre plate was incubated at 37ºC in 5 % CO_2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, and/ or swelling. After the incubation period, 50µl of MTT solution (5mg/ml in phosphate buffered saline) was added to each well thenthe plates were gently shaken and incubated for 4 hours at 37ºC in 5% CO_2 incubator. Afterwards, the supernatant was removed, 100µl of neat DMSO was added, and the plates were gently shaken to solubilize the formed formazan. Theabsorbance was measured using ELISA reader at a wavelength of 570 nm. Triton X 100was 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 the all bio-assessed 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.Form the results obtained in table (2), the mean mortality percentagewas 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 DCMextract (AMW-1) 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}<1000µg/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-1revealed 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 promisingresults support the traditional uses. To this end, it is likely toendorse the activity to the presence of potential antitumor molecules. Further work is underway to isolate and characterize the active constituents.

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Table 1: Represents botanical names, families, localities and the targeted parts of the studied plants species

Code	Botanical name (Family)	Locality	Selected part
AMW	*Ambrosia maritima*L. (Asteraceae)	Nile banks	Whole plant
AVS	*Ammivisnaga*L.(Apiaceae)	Local market	Seeds
ABS	*Aristolochiabracteolata*L. (Aristolochiaceae)	Lowland plain	Seeds
ABL			Leaves
ABB			Branches
LIL	*Lawsoniainermis*L.(Lythraceae)	Northern territory	Leaves

Table 2: Mean mortality percentage and LC_{50} values of the tested plants extracts

Code	Solvent	Mortality %			LC_{50} (µg/ml)	Mean Mortality %
		10 µg/ml	100 µg/ml	1000 µg/ml		
AMW	DCM	90	93.2	100	0.015	94.4
	MeOH	80	93.2	100	0.260	91.1
AVS	DCM	13.0	23.12	43.21	2522.65	26.4
	MeOH	1.9	1.9	16.3	12480.2	6.7
ABS	DCM	63.2	83.26	90	1.644	78.8
	MeOH	90	90	100	0.077	93.3
ABL	DCM	56.6	73.3	93.3	6.605	74.4
	MeOH	63.2	90	100	4.521	84.4
ABB	DCM	76.6	83.2	93.2	0.135	84.3
	MeOH	83.3	90	100	0.237	91.1
LIL	DCM	80	90	96.6	0.193	88.9
	MeOH	73.3	90	100	1.704	87.8
Ctrl +ve						100
Ctrl-ve						0

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.

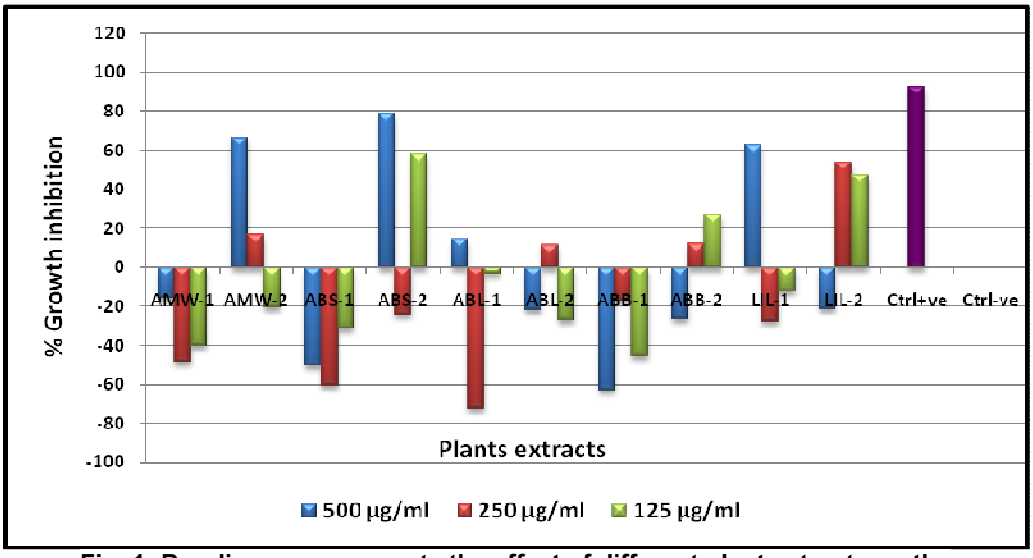

Fig. 1: Bar diagram represents the effect of different plant extracts on the percentage of growth inhibition for RAW 264.7 cell line

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.

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