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Research Article

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Assessment of bioactivity of some Sudanese medicinal plants using brine shrimp (*Artemia salina*) lethality assay

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ABSTRACT

Medicinal plants constitute an important component of flora and are widely distributed in Sudan. The pharmacological evaluation of substrates from plants is an established method for the identification of compounds, which can lead to the development of novel and safe medicinal agents. Based of the ethno pharmacological literature, several species of medicinal plants used in traditional medicine in Sudan were collected. In the present study, ethanolic extracts of some medicinal plants were screened for their cytotoxicity using brine shrimp lethality test. Out of the 25 plants tested, Azadrichta indica (w.p.) LC50 45 ppm, (L) LC50 21; Aristolochia bracteolata(w.p.) with LC50. 50 ppm; (Sd.) LC50 185; (Aristolochic acid, LC50 19 ppm; while Savadoora persica,(L.), (Salvadoraceae) and Ocimum basilicum; (Labiatae); show no toxicity. The present study supports the previous that brine shrimp bioassay is simple, reliable, and convenient method for assessment of bioactivity of medicinal plants and leads support for their use in traditional medicine[1].

Key words: Artemia salina; brine shrimp lethality test; medicinal plants; cytotoxicity

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INTRODUCTION

The importance of medicinal plants and traditional health systems in solving the heath care problems of the word is gaining increasing attention. Because of this resurgence of interest, the research on plants of medicinal importance is growing phenomenally at international level, often to the detriment of natural habitats and mother population in the countries of origin. Most of the developing countries have adopted traditional medical practice as an integral part of their culture. Historically, all medicinal preparations were derived from plants, whether in simple form of raw plant materials or in the refined form of crude extracts, mixtures, etc. Recent estimates suggest that several thousands of plants have been known with medicinal application in various cultures [1] . There are well known drugs that are directly developed from plant species [2], for example Vinblastine and Vincristine from Catharanthus roseus, the first cures in human cancer. Beside the cytotoxic drugs as Aspirin (Analgesic, anti-inflammatory from Filipendula ulmavia. Benzoin (Oral disinfectant) from Slyrax tonkinensis, Morphine (Analgesic) from Papaver somniferum and Quinine (for malaria prophylaxia) from Cinchona pubescens. Some of these plants have been subjected to the isolation of the active ingredients (chemical compound), and their subsequent modification [3].

In continuation of our effort to verify the efficiency of traditional medicine we have collected several medicinal plants from central and other parts of the Sudan based on the ethno pharmacological information. In order to study toxicity of these medicinal plants we performed brine shrimp lethality bioassay, which based on the ability to kill laboratory cultured brine shrimp (Artemia salina nauplii). The brine shrimp bioassay was proposed by Michael *et al* [4], and latter developed by Vanhaecke *et al* [5]. The assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxin, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials [6, 7].

Brine shrimp assay is very useful tool for the isolation, of bioactive compounds from plant extracts [8]. The method is attractive because it is very simple, inexpensive, and low toxin amounts are sufficient to perform the test in micro well scale. In the present work, we report the cytotoxicity studies on various medicinal plant species collected from central and other parts of the Sudan, and the results obtained were described.

EXPERIMENTAL SECTION

2.1. Plant material

Authenticated medicinal plants were collected from different locations in Khartoum province, and western Sudan (Kordofan). The botanical identification was made by Dr. Maha Kordofani at the department of botany, University of Khartoum, and the voucher specimens were deposited at the herbarium of the botany department (U.of K.).

2.2. Preparation of extracts

The plant materials were dried under shade and grinded to a coarse powered. Powdered plant materials (each 100g) were individually defatted with petroleum ether (40-60), then extracted with 80% ethanol (1L) and then filtered. The filtrates were concentrated under vacuum using rotatory evaporator, and subjected for activity studies.

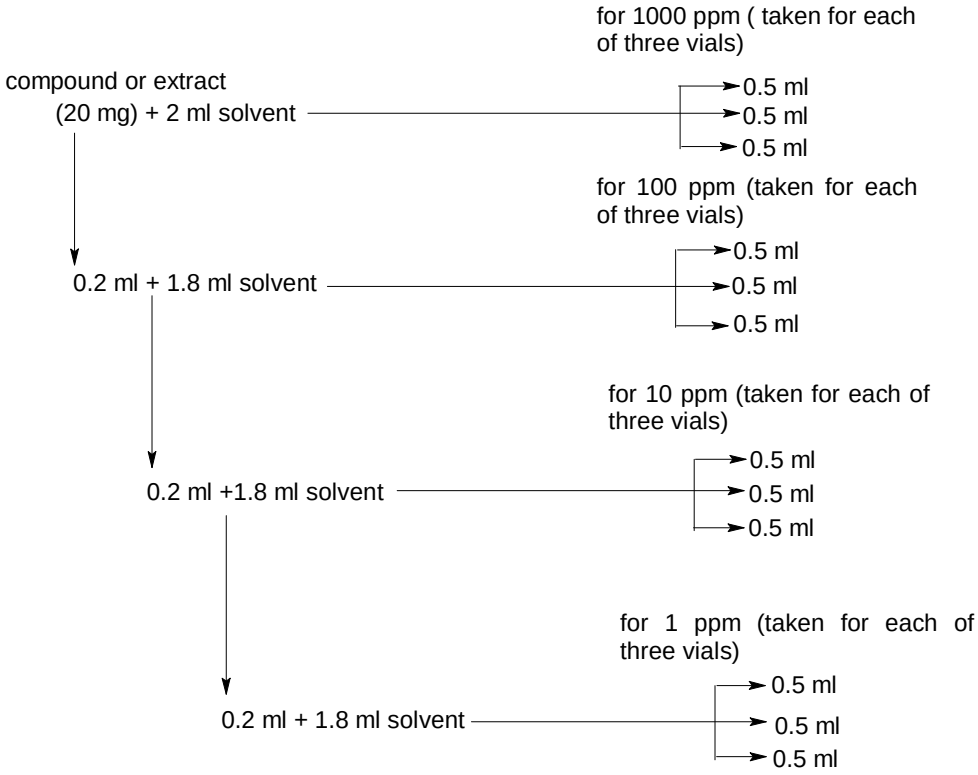

Figure 1. Flow Chart for Alternative Dilution Procedure for Brine Shrimp Bioassay

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2.3. Cytotoxicity bioassay

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of some Sudanese medicinal plant. Brine shrimp (Artemia salina) were hatched using brine shrimp eggs in a shallow rectangular dish (22 × 23) filled with artificial seawater, which was prepared using sea salt 38g/L and double distilled water. After 48 hours, the active phototropic nauplii were collected by pipette. Samples for the experiment were prepared by dissolving 20 mg of each plant extract in 5ml of ethanol.

Appropriate amount of this ethanol solution (5ul, 50uL, 500uL. to give concentration of 10, 100, and 1000 ppm respectively), were transferred to vials dried ready for experiment.

	Table 1 : Brine Shrimp test, toxicity of plant extracts under study					
	Plant	Family	Part of the sample	Traditional uses	LC_{50} Ppm 24h.	95% Confidence Interval
1	*Nerium oleander*	Apocynaceae	L.	Treatment of cardiac illness Oleander has been used, asthma, diabetes mellitus, corns, scabies, cancer, and epilepsy.	398	266-558
2	*Acanthosperum hisbidum*	Asteraceae	Sd. WP.	To treat vomiting, cephalgias, headaches, abdominal pains, convulsions, cough, eruptive fever, snake bites, jaundice, epilepsy, constipation, blennorrhoea, diarrheas, hepato-biliary disorders & malaria	126 1224	56-219 --
3	*Azadirachta indica*	Meliaceae	Sd. L.	The macerations of the leaves are used against snake scorpion bites , as anathematic, eczema and skin diseases	45 21	33-107 12-40
4.	*Aristolochia bracteolata*	Aristolochiaceae	Sd. W. A. Acid	Whole plant poultices are used as antitumor.	185 50 19	130-336 27-86 --
5	*Calotropis procera*	Asclepiadaceae	L. R. Latex	Deduction against jaundice, anti reumatic.	159 393 110	125-315 271-566 55-219
6	*Kiglea africana*	Bignoniaceae	R. Fr.+ sd.	Antitumor.	593 124	331-855 78-199
7	*Salvadora persica*	Salvadoraceae	L.	Fruits edible and used as a carminative, as antidote carminative.	>1000	--
8	*Citrullus colocynthis*	Cucrbitaceae	Fr. Sd.	Root is used to treat swelling, Seeds anti-diabetic, & gonorrhoea.	189	171—216
9	*Ocimum basilicum L.*	Labiatae	L.	The infusion of the leaves are used against jaundice, antulcer, seeds demulcent. is toxic to *Leishmania.*	>1000	--
10	*Mangefra indica*	Anacardiaceae	L. Park Kernel	Antiviral	365	254-516
11	*Trigonella foenum*	Fibaceae	Sd.	Anti-diabetic, Anti-tumor.	60	41-102
12	*Bosica senegalenesis*	Capparidaceae	Sd.	Maceration used as anthelmintic & as eye wash.	384	277-532
13	*Guiera senegalenesis*	Combretaceae	Sd.	The root treat lpros, Y,the leaves are anti-pyrtic	289	211-435
14	*Anona squamosa*	Annonaceae	Sd.	Anti-diabetic	232	189-423
15	*Vinca rosea*	Apocynaceae	L.	Anti-diabetic, anti-cancer.	326	203-549
16	*Vernonea amagdilina*	Asteraceae	L.	Anti-oxidant,Anti-diabetic,Antimalaria,reduces fever.	300	199-498
17	*Ziziphus spina-christi L.*	Rhamnaceae	L.	Decoctions of the bark are used against intestinal spasms. Anti-bacterial.	620	488-743
18	*Annona squamosa*	Annonaceae	Sd.	Anti-diabetic	510	367-693
19	*Lemon grass*	Poaceae	L.	Anti-cancer, anti-bacterial, antifedent.	290	151-348
20	*Moringa oleifera*	Moringaceae	Sd. L.	The whole plant is used for the treatment of asctes &rheumatism.	900 >1000	--
21	*Matricaria*	Asteraceae	Fl.	anti-inflammatory, antispasmodic, , laxative,	>1000	--
22	*Datura stramonium*	Fabaceae	L.	Anti-asthmatic, antispasmodic, narcotic and mydriatic properties.	39	17-99
23	*Thevetia peruviana*	Apcynaceae	L.	It is a plant toxin insecticide for termites. seed oil was used to make a 'paint' with ant.ifungal, antibacterial.	641	456-947
24	*Polygonium glabrium*	Polygonaceae	L.	Used as anthelimentic, anti-parasitic.	354	238-465
25	*Annona squamosa*	Annonaceae	Sd.	Anti-diabetic	150	115-195
	Caffeine (standard)				363 306 *	

LC_{50}= Lethal Concentration calculated by Probit analysis .W.P (whole plant);L.(leaves);Sd.(seeds).
** Meyer et al 1982*

Ten nauplii were selected and transferred into each sample vial by means of a 23 cm disposable Pasteur pipette, and the final volume in each vial was adjusted to 5ml using artificial seawater, a drop of dimethoxysulphoxide (DMSO). A drop of dry east suspension (3mg in 5ml artificial seawater) was added as food to each vial. The vials were maintained under illumination .Survivors were counted with the aid of magnifying glass, after 6,12, and 24 hours, and the deaths of each dose level, positive and negative control were determined. No deaths were observed to occur in the negative control after 24 hours.

2.4. Lethal concentration determination

The lethal concentrations of plant extracts resulting in 50% mortality of the brine shrimp LC50 and 95 confidence intervals were determined from the 24 hours counts and the dose response data were transferred into LC50 was derived from the best-fit line obtained.

Caffeine (LC50 = 306ug /ml) was used as appositive control and ethanol (1000 ug/ml) as a solvent and a negative control in the bioassay experiments.

2.5. Statistical analysis

The lethal concentration of plants' extracts resulting in 50% mortality of the brine shrimp LC50 and 95% confidence intervals were determined from the 24hrs counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis; the LC50 was derived from the best-fit line obtained

RESULTS AND DISCUSSION

The brine lethality assay represents a rapid, inexpensive, and simple bioassay for testing plant extracts bioactivity, which in most cases correlates reasonably well with cytotoxic and anti-tumor properties [9]. In the present study, the brine shrimp lethality of extracts of 25 medicinal plants used in traditional medicine was determined using the procedure of Meyer et al [10]. The LC50 values of the brine shrimp obtained for extracts of these medicinal plants and that of the positive control, caffeine, are given in Table 1. Alcoholic extract of Azadirchta indica showed most prominent activity with LC50 (L.21, Sd.45 ppm), the extracts of Aristolochia (Aristolochiaceae), Datura stramorium (Fabaceae), Trigonella foenum (Fibaceae) 19,50,39,60 ppm respectively. Acanthosperum hispidum (Asteraceae) (Sd..), Calotropis procera (Asclepiadaceae), Kiglea Africana, (Bignoniaceae), Citrullus colocynthis (Cucubitaceae), Anona squamosa Sd.(Anonaceae) 126, 110,159, 124,189, 510, respectively. Salvadora persica (Salvadoraceae), Ocimum basilicum L. were found to have lower LC50 >1000 as noted in Table 1. The degree of lethality was found to be directly proportional to the concentration of the extracts. Maximum mortalities took place at a concentration of 1000 ppm. Whereas least mortalities at 10 ppm.concentration. The LC50 values of the plant extracts were obtained by a plot of percentage of the shrimp nauplii killed against the concentration of the extracts and the best-fit line was obtained from the by means of regression analysis. This significant lethality of several plant extracts to brine shrimp is an indicative of the presence of potent cytotoxic components which warrants

CONCLUSION

Although the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. In the course of our studies, the brine shrimp lethality assay actually has proven to be a convenient system for monitoring biological activities of several plant species that are used in the traditional medicine. Out of the several plants screened for toxicity against the brine shrimp, some species showed LC50 values less than 100 ppm. In addition, these interesting results lend further support to their traditional use. Even though, the present study on these crude extracts is an addition to the scientific literature, detailed investigations on individual plants for pharmacological activities and active ingredients could provide leads to interesting pharmaceuticals of plant origin.

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