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Toxicity assessment of Egyptian *Pterocephalus sanctus* Decne. on *Artemia salina* (Leach.)

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Abstract: *Pterocephalus sanctus* Decne. is endangered and near endemic species belonged to Dipsacaceae family. An overview on the cytotoxicity properties of the selected species was conducted. The brine shrimp lethality bioassay was used to evaluate four of the *P. sanctus* extracts against the larvae of the *Artemia salina* Leach. The results revealed the potency of the hexane extract and could predict its cytotoxic EC_{50} to be ~20.5 µg/ml. The results were supported by the folk use and encourage further fractionation towards the toxicity properties.
Key words: *Pterocephalus sanctus*; *Artemia salina*; Toxicity.

Introduction:

Endemic and near endemic plants are most likely of limited investigations and consequently the availability of published reports are also restricted. The presented work could be in part a review on *Pterocephalus sanctus* Decne. as a module of such near endemic species to encourage the researchers for continuing their phytochemical and medicinal profile. An overview on the cytotoxicity properties of the selected species was also conducted.

While collecting the available data about *P. sanctus* Decne., I found an expected limited number of previously published reports. Seven are books or reports describing the plant origin and habitat[1,2,3,4,5,6,7]. The concerned species belongs to Dipsacaceae family. It is a very rare wild plant growing in Saint Catharine Mountains, Sinai, Egypt[2,5]. It is a sinaitic plant but it was also discovered in the neighboring geographical area; mount Hor and its adjacent peaks and valleys in Jordan[3] as well as Palestine and northwest Saudi Arabia[8]. However, it was found nowhere else in Egypt[6].

The plant taxonomy are listed in several data bases where four are displaying over the taxonomy, the origin and habitat[9,10,11,12]. Moreover, the mention of the selected species in the old books from year 1834-1918 is introduced in a very useful one[13].

The published research articles are eleven where most of them are describing its serious status to seek for a solution to conserve the plant from extinction due the overgrazing and climatic changes[14,15,16,17,18]. [19]reported 0.32 spatial similarity ratio with *Hypericum sinaicum* where [20]recorded 8% Blue butterflies of 897 roosting on the selected species and [21]presented a palynological study. I didn't find any biological reports rather the anti-bacterial by[22] and the anti-hepatotoxic that led to the isolation of sweroside as active agent[23]. Concerning the phytochemical contents of *P. sanctus*, the literature is also displayed few reports. Three iridoids namely; secologanin, loganin and sweroside[24], two flavonoids namely; C-glycoside; luteolin-6-C-β-D-glucoside-7-O-methyl ether and apigenin-6-C-β-D-glucoside-7-O-methyl ether[25] were isolated from the plant areal parts. The phytochemical contributions to the Dipsacaceae systematic were discussed by a review article[26] and mentioned the presence of the flavonoids and iridoids in the selected species.

In the present study, the toxicity of four extracts from *P. sanctus* was evaluated against *Artemia salina* (Leach.) larvae. *A. salina* is a crustacean belongs to the subclass Branchiopoda, order Anostraca[27,28,29] and famously known as brine shrimp. In 1956, [30]firstly introduced the brine shrimp as test organism. Then after, several reports on the use of this animal for environmental studies, the evaluation of crude drugs used in traditional medicines, detecting bioactivity of metabolites from fungal pathogens and marine products as well as screening for potential pesticides were all published[31,32].

2. Materials and Methods:

2.1 Plant material

2.1.1. Collection of plant material

The whole plant of *P. sanctus* was collected from Saint Catherine, Sinai, Egypt and kindly identified by Prof. Dr. Abd El-Raouf M., Suez Canal University, Ismailia, Egypt.

2.1.2. Preparation of *P. sanctus* extracts.

The ground-powdered herb of *P. sanctus* was subjected to 80% aqueous methanol (80% MeOH) extraction. The dried 80% MeOH extract was partitioned successively to finally obtained hexane, ethyl acetate (EtOAc) and butanol (BuOH) extracts.

2.2. Test organism (Brine shrimp larvae):

2.2.1. Hatching of the shrimp eggs

Both of shrimp eggs (Premium, Artemia-International) and the aquarium sea salt (Instant Oceans, Aquarium System, Inc.) were purchased from local ornamental aquarium fish stores. A saturated solution from the salt was prepared by dissolving 18 g in half liter distilled water. 4.4 g eggs were hatched in a V-shaped separating funnel with 250 ml of the prepared artificial sea-water. Direct light and warm are provided by 60 watt lamp. After 24 hours the larvae were collected.

2.2.2. Preparation of 1% Tween sea water (solubilizing aids):

Tween-80 (surfactant) has been used as solubilizing aids for the plant extracts. The *Artemia* larvae can survive at least for 24 hours in saline solution containing 5% Tween-80 (50 mg/ml)[31]. In the present study, 5 ml Tween-80 was mixed with half liter of the prepared sea water to prepare 1% Tween sea water.

2.3. Samples Preparation:

Stock solutions (S.S) of 100 mg/ml in DMSO were prepared from every tested extract. Potassium dichromate ($K_2Cr_2O_7$, Merck) as positive control was also prepared.

2.4. The brine shrimp lethality bioassay:

The modified method of[33] was employed in this study. The assay was performed on 24 to 30 hours old larvae (nauplii) where ten nauplii were transferred to labelled clear glass tubes. A parallel series of $K_2Cr_2O_7$, dilutions (400 - 800 µg/ml for acute and 20-40 µg/ml for chronic toxicities) as well as the vehicle were matched with every test extract. From every prepared concentration, 50 µl was added into corresponding labeled tube containing about 5 ml T-saline to reach the final concentrations; 10, 100 and 1000 µg/ml. The number of dead nauplii is counted in every tube after 6 hours to determine the acute LC_{50} and after 24 hours for the chronic LC_{50}. Five replicates were prepared for each concentration. Ten nauplii were added to every tube where all were maintained then after under illumination. Survivor larvae were counted after 6 and 24 and lethality percent were recorded per concentration.

Statistical analysis:

Percentage lethality was calculated as number of dead larvae divided by initial number; 10 multiplied by 100. GraphPad Prism® software was used to calculate the LC_{50} (concentration that kills 50% of the larvae after 24 hour incubation) using non-linear regression; concentration-response curve fit.

Results and discussion:

Larvae of the brine shrimp were separately incubated 24 hours with every prepared extract to determine the chronic toxicity where the results are displayed in Figure 1. However, the acute toxicity was not recorded by any of the examined extracts. The chronic LC_{50} (concentration that caused 50% lethality) was determined for every extract and is given in Figure 2. Hexane was recorded the least value (205.7µg/ml). It is the only extract that could be considered toxic according[31]. He reported that the tested sample possesses chronic toxicity when its lethal concentration (LC_{50}) is ≤ 12 times the chronic LC_{50} of potassium dichromate ($K_2Cr_2O_7$). In the present study, the LC_{50} of $K_2Cr_2O_7$ was recorded as 36µg/ml, accordingly, the active sample should be 432µg/ml or less and consequently, the only potent extract is the hexane. [33]reported that ED_{50} (effective dose) values for cytotoxicity assays would be 10 times lower than chronic LC_{50} values of brine shrimp lethality assay. We could predict the value of the cytotoxicity to be ~ 20.5µg/ml. This value encourages further investigation towards the hexane fractionation and recruits its cytotoxicity properties.

The folk uses could also emphasize further investigation towards the toxicity properties such as anti-microbial and /or anti-cancer properties. Pterocephalus species viz. *P. perennis* have been used traditionally, all over Greece, as anti-septic and astringent. *P. bretschneideri* and *P. hookeri* are used in Chinese traditional medicine for the cure of rheumatism, influenza and fever[23].

The species is currently endangered due to overgrazing and climatic changes. It is not enough to only investigate the endemic and near endemic species but a synchronized efforts must triggered towards conserving those naturally treasures plants by increasing the awareness of indigenous people to avoid overgrazing. Challenges to cultivate the plant in artificial optimized conditions should be addressed as well as the field of plant tissue culture to produce the active substances should be considered.

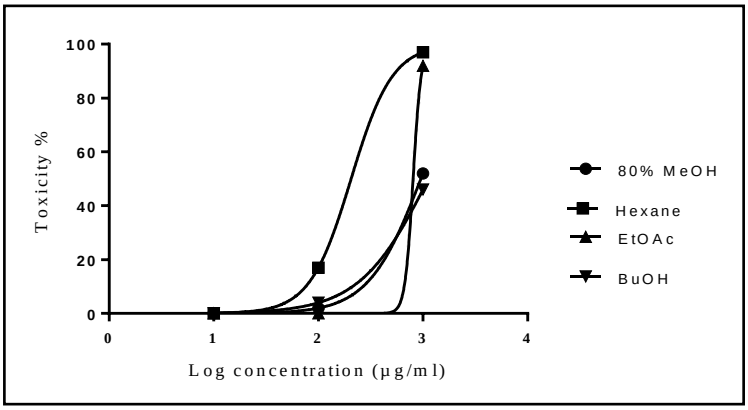

Fig 1: Dose-Response curves of *P. sanctus* extracts on *A. salina* larvae

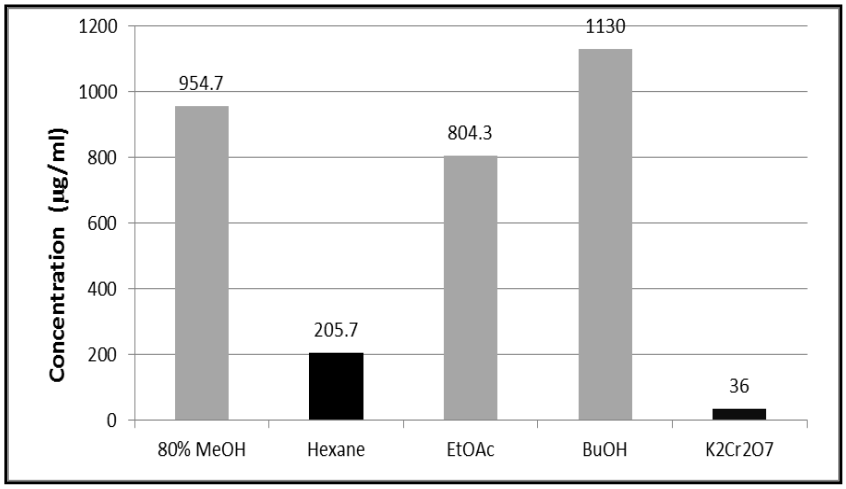

Fig 2: LC_{50} of *P. sanctus* extracts against *A. salina* larvae compared to $K_2Cr_2O_7$

Conclusion:

The results are considered the first insight on the toxicity properties of *P. sanctus* against the larvae of the brine shrimp. As far as I know, This is the first review on the concerned species.

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