TOXIC ACTIVITY OF DIFFERENT EXTRACTS AND FRACTIONS ON BRINE SHRIMP, *Artemia salina*.

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Summary

Bioactive compounds are frequently toxic in high doses. Thus, *in vivo* lethality in a simple zoological organism as the eggs of brine shrimp, *Artemia salina*, might be used as a rapid and simple monitor during the fractionation of known bioactive plant extracts. We used *A. salina* whereby plant extract, fractions and also algae extract were tested at 10, 100 and 1000 ppm (µg/ml) in vials containing ten nauplii each one of three replicates, and the results were analyzed with the Reed-Muench method. Of four plants and one algae evaluated only fractions from acetone:water extract from *Cuphea aequipetala* showed a LC_{50} values less than 200 ppm, while the extract of *Tillandsia recurvata, Solanum torvun, Solanum hispidun* and *Sargassum vulgaris* were not toxicity ($LC_{50} \geq 1000$ ppm).

Keywords: *Artemia salina*, *Cuphea aequipetala, Tillandsia recurvata, Solanum torvun Solanum hispidun*, *Sargassum vulgaris*

Plants have been used in the treatment of cancer for more than 3500 years (1), nevertheless the scientific studies began in 1950 with vincristine and vinblastine discovery from *Catharantus roseus* (2).

Although marine organism do not have a history of use in traditional medicine, the oceans, covering more than 70 % of the earth's surface, represent an enormous resource for the discovery of potential chemotherapeutic agents (3). The systematic investigation of marine environments as source of novel biologically active agents began in the mid-1979s (4) where most prominent of these was Bryostatin 1, isolated from the bryozoan, *Bugula neritina*, which now has shown some promising activity against melanoma (3).

There are *in vivo* and *in vitro* methods to know and evaluate the anticancer potential of compounds. Some of then are *in vitro* studies are cellular and molecular methods to assay cytotoxicity activity (5). The cellular cytotoxicity assay employs human cancer cell lines (6), this technique requires special space and equipment as well as personal trained. Other methods used to perform this kind of studies are the Brine Shrimp toxicity and crown gall tumor inhibition (7), both bioassays are rapids, inexpensive, safe and statistically reliables.

In this paper we investigate the toxic effect of extracts o fractions from Solanum *torvun*, *Solanum hispidun*, *Cuphea aequipetala* y *Tillandsia recurvata*, plants used in Mexican traditional medicine against tumour and seaweed, *Sargassum vulgaris*.

Material and methods

Table 1 shows the taxonomic and ethnomedical information about plants and algae used to obtain extracts and fractions.

Table 1. Ethnomedical and other information of medicinal plants and algae studied.

Common name	Place of collection or acquisition	Voucher number	Ethnomedical uses	References
Tillandsia recurvata	**Texcoco, Edo. de México**	**UAMIZ58422**	**Breath affections, antiabortion, diuretic and to treat syphilis.**	**8**
Cuphea aequipetala	**Mercado de Sonora. México, D.F.**	**UAMIZ56709**	**To treat infections mucocutaneous and gastrointestinal. Against certain cancers.**	**8**
Solanum torvun	**Tuxtla Gutiérrez, Chiapas**	**Pending**	**To treat infections, rheumatism, analgesic. When vaginal blending occurs outside menstrual cycle. Against certain cancers.**	**8, 9**
Solanum hispidum	**Pauhatlán, Puebla**	**UAMIZ55771**	**Several skin mycosis conditions. Against certain cancers.**	**8, 10**
Sargassum vulgaris	**Antón Lizardo, Veracruz**	**Pending**	**unknown**	

Plant extracts and their fractions.

a) ***Tillandsia recurvata***. The whole plant was cleaned, dried, ground and extracted by maceration with chloroform: methanol (1:1) for 72 h. The liquid was obtained and it presented two phases, which were separated (polar phase and no polar phase). The plant was macerated with water again using the same experimental condition. The dissolvent were eliminated and stocks solutions were made at 10 mg/ml dimethylsulfoxide (DMSO) at 50%.

b) Solanum torvun and ***Solanum hispidum.*** The leaves were cleaned, dried, ground and extracted successively with hexane, chloroform, methanol and water by maceration. The extracts were concentrated *in vacuo* meanwhile the aqueous extract was concentrated by water bath. We used the aqueous extract to obtain a stock solution with concentration of 10 mg/ml in dimethylsulfoxide (DMSO) at 50%.

c) ***Sargassum vulgaris***. The algae was dried, ground and extracted by macerationtion and the extracts were obtained and stock solution prepared as we described in previous paragraph.

d) *Cuphea aequipetala* extract and their fractions.
The plat powder was macerated with acetone: water (7:3) during 48 h (3 times); the extract was filtered and concentrate under reduced pressure until acetone was eliminated and this liquid was concentrated at room temperature to one third of the total volume and a first precipitate was formed and separated from the residual liquid. This liquid was extracted with dichloromethane and ethyl acetate. The aqueous phase gave two precipitators that were separated and the residual liquid was eliminated at room temperature and newly was dissolved in water and added sodium dodecylsulfate (SDS) at final concentration of 1%, and was formed one precipitate (**P_1-SDS**), which was fractionated using a column chromatography with cellulose:celite (1:1) as stationary phase and eluted with different proportions of acetone (Me_2CO) and ethanol (ET-OH). We obtained twelve fractions whose solvent were evaporated and stock solutions were prepared.

The brine shrimp lethality bioassay
The brine shrimp eggs (0.50 g) were put in one liter of saline artificial sea (Instant Oceanic, Marineland Labs, USA) at 3.5 %, in reservoir with air and artificial light, during 48 hours. After this time, we deposited 10 nauplii with the stock solutions of extracts or fractions to obtain final concentrations of 1000, 100 and 10 ppm (μg/ml), in saline artificial solution, and incubated for 24 hours under artificial illumination at room temperature. After this time, we counted the number of dead nauplii, considered as dead if they were lying immobile at the bottom of the well. To obtain the chronic lethal 50 concentration (LC_{50}) we utilized the Reed Muench (12) method by

graphicated accumulated nauplii survivors and deaths on the same set of axes. The two curves intercept at the 50% lethal dos required for the animal population. Potassium dichromate was used as standard toxicant and dissolved in seawater, to obtain concentrations of 1000, 100 and 10 ppm. The negative control solution was DMSO solution at final concentration of 5% that is the maximum concentration of DMSO present in the solution evaluated at concentration of 1000 ppm. Each extract or fractions were assayed by triplicate in three independents events.

Results

Table 2, summarizes the effect (n=9) of extracts from *Tillandsia recurvata*, *Solanum torvun*, *Solanum hispidum* y *Sargassum vulgaris* on the viability of nauplii meanwhile Table 3 shows the effect of the fractions from *Cuphea aequipetala* on the viability nauplii.

Table2. Effect of extracts from *Tillandsia recurvata, Solanum torvum, Solanum hispidum* y *Sargassum vulgaris* on the viability of *A. salina* larvae.

Plants /algae	Extract/phase/compound	LC_{50} (ppm)
T. recurvata	$CHCl_3$:Methanol (1:1), no polar phase	>1000
	$CHCl_3$:Methanol (1:1), polar phase	>1000
	Aqueous	>100
S. torvun	Aqueous	>1000
S. hispidum	Aqueous	>1000
Sargassum vulgaris	Hexane	>1000
	Diclorometane	794
	Methanol	>100
	Aqueous	>1000
	K_2CrO_4 (Control)	29.21

Table 3. Effect of different fractions of P_1-SDS from *Cuphea aequipetala* on viability of *A. salina* larvae and type and proportion of solvent used to elute.

Fraction/compound	Mobil phase	Characteristics	LC_{50} (ppm)
I	*Me_2CO 100%	yellow	48.23
III	Me_2CO:**EtOH (7:3)	yellow	34.14
IV	Me_2CO:EtOH (7:3)	yellow	31.62
V	Me_2CO:EtOH (7:3)	yellow	34.14
VI	Me_2CO:EtOH (7:3)	yellow	30.43
VII	Me_2CO:EtOH (7:3)	white	32.85
K_2CrO_4 (Control)			29.21

*Me_2CO= acetone. EtOH = ethanol

Discussion

There are several simple ways to treat the quanta data derived from a test series, such as logarithmic-probit method of Miller and Tainer, the DeBeer procedure, and the Litchfield and Wilcoxon method (13). We used, the Reed-Muench method which assumes that an animal that survived a given dose also would also have survived any lower dose and, conversely, that an animal that dies with a certain would have also died at any other higher dose (12). In our studies we used DMSO (dissolvent) at 5%, as negative control solution, at the same concentration in 1000 ppm solution, as used in the nauplii culture and not affected their viability, it is agree with scientific literature where describes that the brine shrimp larvae can tolerate up 11 % of this compound (12). The positive control was a heavy metal salt as a toxicant were the chronic LC_{50} is in the region of 26.23 a 31.03 ppm, valor that is agree with other authors (12). MaLaughlin (11) has reported that the results obtained with *A. salina* are quantitative and reproducible, and the activities parallels cytotoxicities, as general observation is that ED_{50} values for cytotoxicities will fall one order of magnitude (10 times) lower than LC_{50} values for brine shrimp. According to the standards of the National Cancer Institute (NCI), ED_{50} values of $\leq$ 20 μg/ml for no pure compounds are considered active (14), so we take a level for the median lethal concentration (LC50) as 200 ppm. According with this value only *Cuphea aequipetala* fractions are toxic on *A. salina*, plant has showed to have selective cytotoxic on cancer cells lines (15).

The P_1-SDS was obtained using SDS, and it was fractioned by chromatographic procedures. We considered that SDS no was present in assayed fractions because they had yellow color. It is important to make sure that SDS is not present in any fraction because its LC_{50} is 25 ppm (12), so we will done further experiments to know what kind of compounds are present in each fraction.

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