Toxicity of *Sanguinaria canadensis* L. as Compared to *Aloe vera* L. against Brine Shrimp (*Artemia salina*) Using the Probit Methodology

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Abstract: Toxicity study of *Sanguinaria canadensis* L. was determined as compared to *Aloe vera* (L.) Burm. f. against brine shrimp (*Artemia salina*). Upon statistical analysis of obtained toxicity bioassay data through the method of probits, LC_{50} of *Sanguinaria canadensis* was estimated as 0.021 mg/ml, with (95% C.I : 0.0091-0.0485) whereas *Aloe vera* was found to be almost non-toxic showing relatively higher LC_{50} that is, 180783.7 mg/ml.

These results show that *Aloe vera* that is being used widely as an herbal medicine throughout the world, could be used safely for other various expected purposes for instance IPM etc. where the excessive amount is anticipated to drain into the sea ecosystem ultimately.

Keywords: *Sanguinaria canadensis* L., *Aloe vera* (L.) Burm. f., invitro toxicity assay, *Artemia salina*, Probit, LC_{50}.

INTRODUCTION

Sanguinaria canadensis L. (Papaveraceae) is a well known medicinal plant and it is an important part of Homeopathic materia medica for respiratory system ailments. Sanguinarine, an important phytochemical from it, has been shown to posses remarkable antimicrobial activity [1-3]. Likely, *Aloe vera* (L.) Burm. f. (Liliaceae) is famous for its use in cosmetology as emollient and for burn treatment. For some systemic ailments like constipation its leaf exudates (dried) has been used. Both the plants have reasonable shares in drug market [2, 4-7]. Furthermore, plant materials are being tested for pest management components as well [8].

Toxicity testing of any chemical is carried out when it is expected to get in contact with the living body, skin, mouth, lung or get way inside through ingestion by any mean. The estimation of LD_{50} is the best way of getting an idea of the toxicity profile of any chemical and natural compound [8]. The statistical method for calculating LD_{50} is the method of probits, which was first introduced by Chester Bliss (1932) and developed by Finney (1962). It transforms the sigmoid dose-response curve to a straight line that can then be analyzed by regression. It is also utilized in bioassays, quantal bioassays and tolerance study [9-12].

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Many of the chemicals are being tested for their possible toxic effects including drugs and medicines [13]. To minimize the associated effects of synthetic chemicals to the man, animals and the environment around the world, inclination is going towards the usage of natural products, instead. Keeping in view this changing trend of world, it is urged to get more knowledge in the field of toxicology of most common plants and their products and thus in this connection *Sanguinaria canadensis* L. was tested as compared to *Aloe vera* (L.). These tested plants are renowned medicinal plants [1-7], since to screen a wide range of crude plant extracts and cytotoxicity assay using larvae of brine shrimp is a cheap and quick method, therefore presently; the *Artemia salina* was used as a subject organism.

MATERIALS AND METHODS

Plant Material and Shrimps

Plant material and shrimps were reared, collected, processed and/or identified. Hydro-EtOH extract of *S. canadensis* while dried *A. vera* gel was utilized in the study.

Brine Shrimp Lethality Bioassay

The eggs of brine shrimps (*Artemia salina* (Leach)) are available in the pet shop as fish food easily. They are stored at 4°C and are allowed to hatch in the sea water before the cytotoxic assay. The count of viable larvae after their incubation with the test sample

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indicates the LD_{50} of the respective sample [14]. 50 mg of the brine shrimp eggs were sprinkled in the freshly prepared filtered saline water (38 g of sea salt /L of D/W, pH 7.4 also called brine solution) and are allowed to hatch and mature for two days. In the meantime 200 mg of the extract of plants were weighed accurately to three significant figures and in each, 20 ml of the volatile organic solvent was added. From each of them, then 0.5, 5, 50, 500 and 5000 µl was transferred to five vials to get 1, 10, 100, 1000 and 10000 µg/ml respectively, of each sample to be tested for the assay after evaporation of the solvent overnight. Other vials were supplemented with control. Then 5 ml of brine solution (sea water) was added and 10 matured larvae were transferred in each vial using Pasteur pipette. The vials were incubated at 25-27°C, under illumination for 24 hours. After incubation the larvae were observed and dead or alive organism/s were counted and noted down for calculating the LD_{50}.

Statistical Analysis

The experimental data were analyzed using the probit methodology and chi-square test. Probit is a method which transforms a concentration-mortality curve to a straight line which helps in estimating the value of LC_{50}. Regression equation that was calculated by probit values would be used to measure the concentration that would give percentage mortality as low as zero to as high as hundred percent. Here, the Chi-square test was used to test the relationship between probit mortality and regression mortality [9-12].

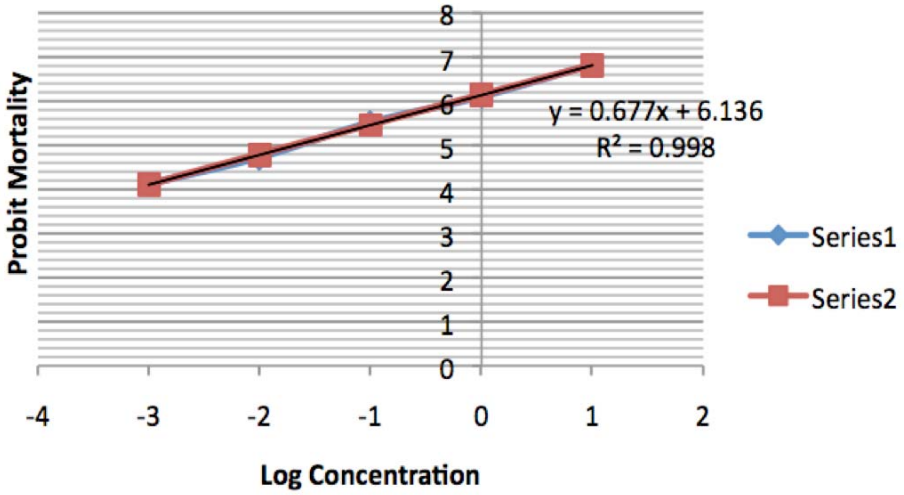
RESULTS

As given in Tables **1** and **3**, a gradual increase in percent mortality with increase in concentration has been observed. The results were subjected to probit analysis as shown in Tables **2** and **4** and Graphs **1** and **2**. The probit mortality equations for *S. canadensis* and *A. vera* were Mortality = 6.13694 + 0.6773logCon. and Mortality = 3.82794 + 0.2463logCon. for LD_{50} 0.021 mg/ml (95% C.I : 0.0091- 0.0485) and 180783.7 mg/ml, respectively. In both the graphs, series 1 denotes that the line was plotted between log concentration and probit values while, series 2 refers that the line was plotted between log concentration and mortality fitted equation.

Table 1: Toxicity of Sanguinaria Canadensis

Concentration (mg/ml)	Organisms exposed	Percent Mortality	Probit Value	Mortality=6.13694+0.6773logCon.
0.001	30	19.1	4.1258	4.10504
0.01	30	39.33	4.7285	4.78234
0.1	30	69.66	5.5158	5.45964
1	30	86.52	6.1031	6.13694
10	30	96.63	6.825	6.81424
Control	90	1	-	-

Chi- square =0.999.



Graph 1: Probit mortality curve for *Sanguinaria canadensis.*

Table 2: LC of *Sanguinaria canadensis*

Percent mortality	LC (mg/ml)	S.E.	Dose limit at 95% C.I. (mg/ml)
10	0.0003	2.35	0.0001 to 0.0015
30	0.0036	1.74	0.0012 to 0.0106
50	0.021	1.53	0.0091 to 0.0485
70	0.1229	1.56	0.0517 to 0.2919
90	1.6216	1.97	0.4289 to 6.1311
99	57.2859	3.14	6.0871 to 539.114

Table 3: Toxicity of *Aloe vera*

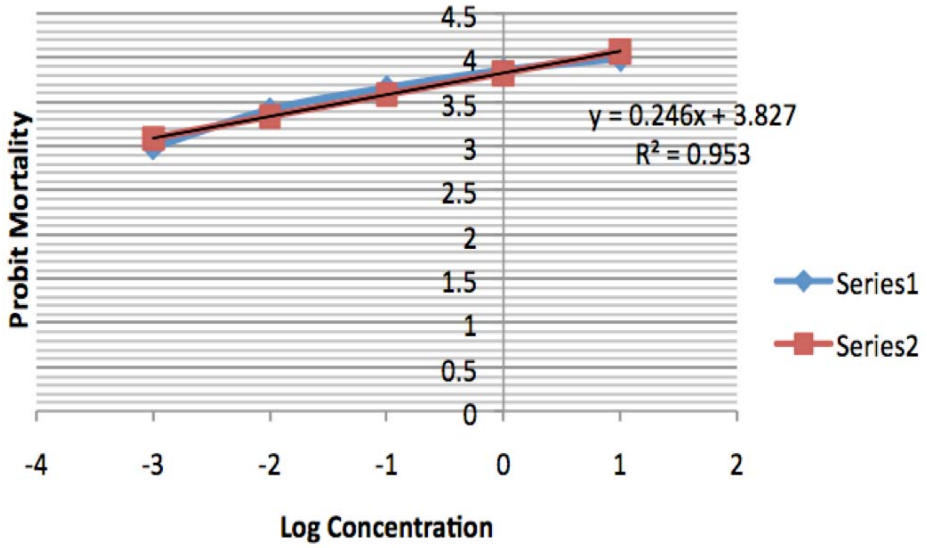
Concentration (mg/ml)	Organisms exposed	Percent Mortality	Probit Value	Mortality=3.82794+0.2463logCon.
0.001	30	2.25	2.9859	3.08904
0.01	30	5.62	3.4107	3.33534
0.1	30	8.99	3.6592	3.58164
1	30	12.36	3.8593	3.82794
10	30	15.73	3.9931	4.07424
Control	90	1	-	-

Chi- square = 0.999.

Table 4: LC of *Aloe vera*

Percent mortality	LC (mg/ml)	S.E.*	Dose limit at 95% C.I. (mg/ml)*
10	0.364	0.66	-1.74 to 0.86
30	877.37	1.83	-0.65 to 6.54
50	180783.7	2.94	-0.51 to 11.02
70	37264990	4.075	-0.42 to 15.56

*Values are in logarithm.



Graph 2: Probit mortality curve for *Alo evera*.

DISCUSSION

Medicinal plants have been in the life of humans since ages. Now the people are more interesting in getting aware of the possible toxicities of any herbal extract as never before as there had reported such cases of toxicity that ultimately banned the product to be sold. Drug toxicity is a key reason for drug attrition. Identifying potential toxicity at an early stage in drug discovery can save both time and development costs, and reduces the likelihood of late stage failure [8, 14, 15].

Although having potential medicinal value, *S. canadensis* is considered a toxic plant [1-3] and it was also manifested by this research exercise as it showed very low value of LD_{50} in contrast to the edible *A. vera.* The results of this study were in line with another attempt by Jancula Daniel *et al.*, 2007 in which aqueous extract of root of *Sanguinaria canadensis* showed significant toxicity against aquatic organisms [16]. Although, considered almost non-toxic, Cock and Sirdaarta, 2011, evaluated *A. vera* toxicity on *Artemia napulii,* and they concluded that the gel of the plant may contain some cytotoxic material and it may manifest toxicity in the concentration as low as 4.3 % with 24 h LC_{50} of 4.6% ± 0.3 [17].

There are methods that can assess the toxicity profile of any chemical agent and that can test the possible cytotoxic, mutagenic, tumorogenic or cancerogenic potential of it but the cost, efficient handling, high laboratory demands might be the hindrance. The estimation of LC_{50} using such small animals like brine shrimps are the quick process to get an overview of the nature of the substance tested. Nevertheless, plant materials are being tested for pest management components as well. These results show that *Aleo vera* could be used safely for the various expected purposes including IPM etc. where the excessive amount is anticipated to drain into the sea ecosystem ultimately [8].

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