Comparative Toxicities of *Hydrastis Canadensis* L., *Berberis aristata* DC. and *Achillea millefolium* L. Against Brine Shrimps (*Artemia salina*) Using Dosage Mortality Curve: A Probit Approach

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Abstract: *Hydrastis canadensis*, *Berberis aristata* DC. and *Achillea millefolium* were compared for their toxicities against brine shrimps (*Artemia salina*). After applying statistical method of probits to the experimental data, *H. canadensis* was found to be the most toxic with the $LC_{50}$of 3.236 mg/ml, while, *B. aristata* showed moderate toxic potential showing that of 60.264 mg/ml and *A. millefolium* manifested the highest value, that is, 41297.5 mg/ml.

Keywords: *Hydrastis canadensis* L., *Berberis aristata* DC. and *Achillea millefolium* L., invitrotoxicity assay, *Artemia salina*, Probit, LC_{50}.

INTRODUCTION

Hydrastis canadensis L. (Ranunculaceae), commonly known as Golden seal, is famous for its being strong general tonic for mucous membrane. It is a part of Homeopathic materiamedica [1]. *Berberis aristata* DC. (Berberidaceae), commonly known as Indian barberry, is a part of Greeco-Unani system of medicine and famous for its use in liver infections, and topically for eye and ear infections [2]. *Achillea millefolium* L. (Asteraceae), popular with the name of yarrow, is used for cuts, burns, piles and internally as diuretic, cold, flu and inflamed joints [3].

Although, being of high interest in therapeutics, there are the reported toxicities of herbal medicines that emphasized on the toxicity analysis. Estimating Median Lethal concentration (LC_{50}) of any chemical or herbal compound or plant extract of medicinal value is the easy and fast way of analyzing it for use in the biological systems and environment [4]. Estimation of LD_{50} uses the statistical method of probits [5-8].

In this research exercise, an efficient, cost effective and easy method of brine shrimp lethality bio-assay was used whose results are comparable to the expensive cytotoxic bioassays [9]. Thus, the LD_{50} of the respective plant extracts were estimated.

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MATERIALS AND METHODS

Plant Material and Shrimps

Plant material and shrimps were reared, collected, processed and/or identified. Hydro-EtOH extract of *Hydrastis canadensis* L., EtOH extract *Berberis aristata* DC. and *Achillea millefolium* was utilized in the study.

Brine Shrimp Lethality Bioassay

It was in accordance to Meyer *et al.,* 1982 [9] and Karim *et al.,* 2015 [10].

Statistical Method

The Percent mortality were analysed statistically using Probit method and chi-square test. LC_{50} were calculated using the equation of probit mortality. Probit method transforms the dosage-mortality curve to a straight line which provides the value of LC50. The Chi-square test was used to test the relationship between probit mortality and regression mortality [5-8].

RESULTS

Tables **1**, **3** and **5** showed a steady increase in percent mortality with increase in concentration. The results were subjected to probit analysis as shown in Tables **2**, **4** and **6** and Graphs **1**, **2** and **3**. The probit mortality equations for *H. canadensis, B. aristata* and *A. millefolium* were 4.70654+0.567logCon., 4.507+ 0.278logCon. and 3.982+0.262logCon. for LD_{50} 3.236

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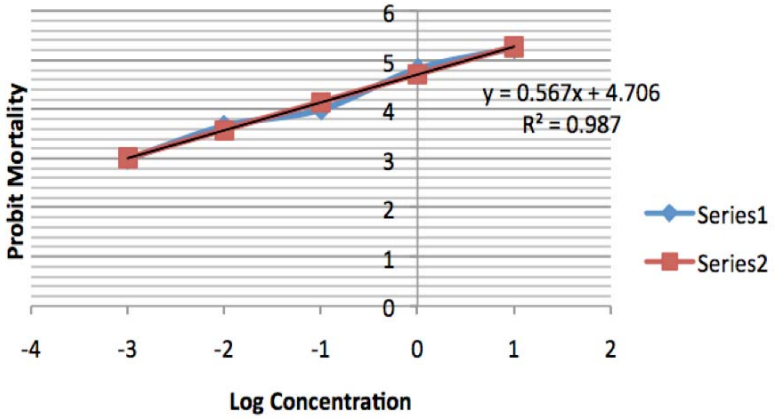
Table 1: Toxicity of Hydrastis Canadensis

Concentration (mg/ml)	Organisms exposed	Percent Mortality	Probit Value	Mortality=4.70654+0.5671logCon.
0.001	30	2.25	2.9859	3.00524
0.01	30	8.99	3.6592	3.57234
0.1	30	15.73	3.9931	4.13944
1	30	42.70	4.816	4.70654
10	30	59.55	5.243	5.27364
Control	90	1	-	-

Chi- square =0.999.



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

Table 2: LC of *Hydrastis canadensis*

Percent mortality	LC (mg/ml)	S.E.	Dose limit at 95% C.I. (mg/ml)
10	0.018	2.14	0.004 to 0.079
30	0.391	1.66	0.145 to 1.055
50	3.236	1.88	0.936 to 11.189
70	26.779	2.50	4.441 to 161.481
90	587.803	4.13	36.401 to 9491.877

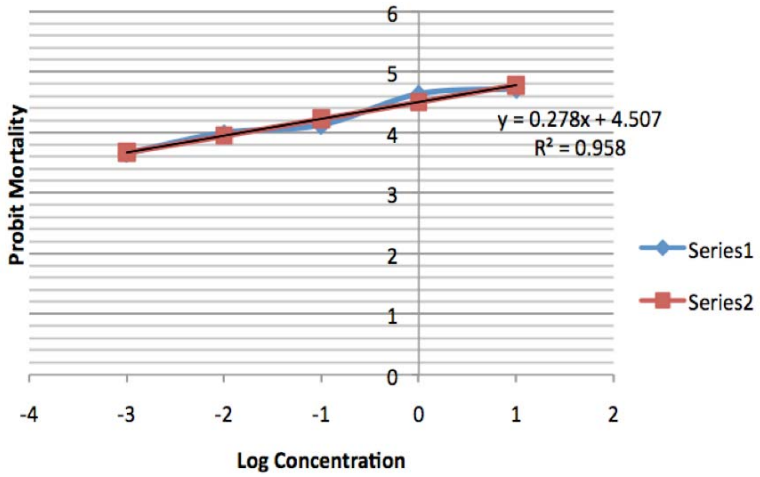
Table 3: Toxicity of *Berberis aristata*

Concentration (mg/ml)	Organisms exposed	Percent Mortality	Probit Value	Mortility=4.507+0.278logCon.
0.001	30	8.99	3.6592	3.67222
0.01	30	15.73	3.9931	3.95066
0.1	30	19.10	4.1258	4.2291
1	30	35.36	4.6389	4.50754
10	30	39.33	4.7285	4.78598
Control	90	1	-	-

Chi- square = 0.999.

Table 4: LC of *Berberis aristata*

Percent mortality	LC (mg/ml)	S.E.	Dose limit at 95% C.I. (mg/ml)*
10	0.001	6.18	0.000 to 0.049
30	0.785	2.88	0.099 to 6.227
50	60.246	7.46	1.172 to 3096.243
70	4621.208	25.80	7.907 to 2700683



Graph 2: Probit mortality curve for *Berberis aristata*.

Table 5: Toxicity of *Achillea millefolium*

Concentration (mg/ml)	Organisms exposed	Percent Mortality	Probit Value	Mortility=3.982+0.262logCon.
0.001	30	2.25	2.9859	3.19658
0.01	30	8.99	3.6531	3.45856
0.1	30	12.36	3.8448	3.72054
1	30	15.73	3.9931	3.98252
10	30	19.1	4.1258	4.2445
Control	90	1	-	-

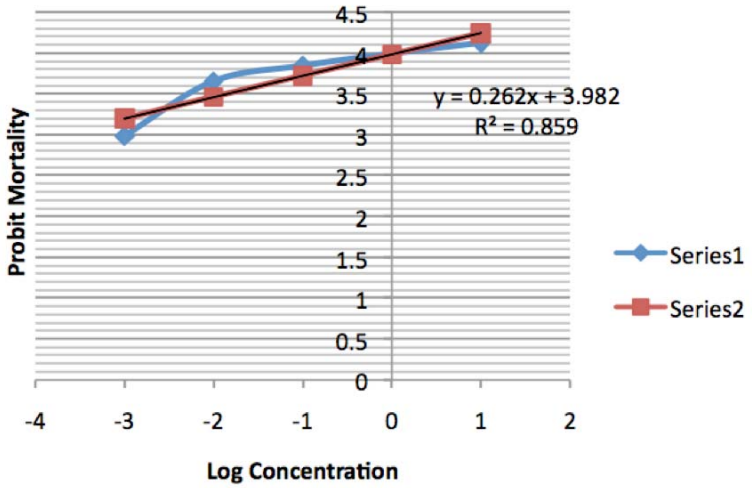
Chi-square = 0.999.

mg/ml (95% C.I: 0.936 to 11.189), 60.264 mg/ml and 41297.51 mg/ml, respectively. In all 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.

DISCUSSION

Floras are in the life of creatures since eternities. Man has been using them for food, for medicines, for shelter (in the form of wood), for clothing's etc. although are of valuable importance, some may be toxic if ingested or even if being in contact to the life [4]. Knowing this nature of herbs, we are urged to get fully equipped with the knowledge of their toxicity profile before being use as a medicine or food; in order avoid any unbearable circumstances.

Thus, the three medicinal plants were analyzed. *Hydrastis canadensis* showed tumorogenic properties in an animal study utilizing male and female mice and rats [11] which is in line to this research in which it showed to be the most toxic plant of all the extracts tested. Although, berberine is phototoxic [12] but *Berberis aristata* extract found safe with LD_{50}>5000 mg/kg body weight in mice [13] which is in accordance to this research in which it showed higher LC_{50}.



Graph 3: Probit mortality curve for *Achillea millefolium*

Table 6: LC of *Achillea millefolium*

Percent mortality	LC (mg/ml)	S.E.*	Dose limit at 95% C.I. (mg/ml)*
10	0.072	0.67	-2.44 to 0.163
30	188.984	1.45	-0.55 to 5.1
50	41297.51	2.45	-0.18 to 9.41
70	9024475	3.49	0.12 to 13.79

*Values are in logarithm.

Although, we found greater LC_{50} but in another study, *Achillea millefolium* showed LC_{50} of 41.9mg/ml (4.19%) against another organism small cabbage Pierisrapae L. (Lepidoptera: Pieridae) [14].

The most toxic of all the herbs tested was the one that was the part of Homeopathic system of medicine, while the other two that were not that part were almost non-toxic. In Homeopathic some poisonous plants are also being used but they are diluted (potentised in homeopathic terms) in such a low value of the extract that are not able to produce any side effects [1]. *B. aristata* was more toxic than *A. millefolium* may be due to the presence of berberine, a isoquinoline alkaloid, in it.

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