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Toxicological profiles of the leaf extracts of *Wrightia arborea* & *Wrightia tinctoria*

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Abstract

The research work was conducted with the leaf solvent extracts of *Wrightia arborea* and *Wrightia tinctoria* to make toxicological profiles by employing Brine Shrimp Assay method (BSA) (*Artemia Salina LEACH*).. The LC_{50} values were determined for both the plant solvent extracts respectively in µg/ml of active compounds and extracts. It was found that the leaf ethanolic and methanolic extracts were toxic for the Brine Shrimp Naupli. The results indicated that *Wrightia tinctoria* leaf ethanol (70%) extract and methanolic extract showed LC_{50} values of 471.604 and 517.038 µg/ml respectively .While the *Wrightia arborea* leaf ethanol (70%) extract and methanolic extracts showed LC_{50} values of 498.213 and 531.082 µg/ml respectively. The remaining solvent extracts showed no toxicity (as found more than 1000 µg/ml) in BSA method.

Key Words: Wrightia tinctoria, Wrightia arborea, Brine shrimp Assay (BSA), Toxicological profiles.

1. Introduction

Traditional medicine should be able to play an even greater role in the modern primary healthcare system of the developing countries. The natural ingredients of traditional medicine are believed to be more acceptable to the human body with out producing any toxicity on compared to modern synthetic drugs. Thus the most important factor needed is to derive the maximum benefit from the traditional system of medicine for providing adequate health care services to rural people[1]. Many new natural compounds are isolated, characterized and published without any biological testing whatsoever. Even compounds extracted ,from plant extracts would be toxic at higher concentrations Brine Shrimp Lethality Bioassay in a bench top bioassay method for evaluating the toxicity anticancer, antimicrobial and pharmacological activities of natural products.[2,3,4]

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By this method, natural product extracts, fractions as well as pure compounds can be tested for their bioactivities. The BSA method is indicative of cytotoxicity and a wide range of pharmacological activities of the compounds.[5,6] The eggs of brine shrimp, *Artermia salina* LEACH, are readily available at low cost as a food for tropical fish, and they remain viable for years in the dry state. Upon being placed in a brine solution the eggs hatch within 48 hours, providing large numbers of larvae (nauplii). Brine shrimp have been previously utilized in various bioassay systems. Among these applications have been the analyses of pesticide residues [7, 8] mycotoxins[9, 10] stream pollutants, anesthetics dinoflagellate toxins, morphine – like compounds[11], toxicity of oil dispersants, co carcinogenicity of phorbol esters[12] and toxicants in marine environments. Most workers have made use of the hatched nauplii, although inhibition of hatching of the eggs has also been studied [13, 14].

2. Experimental

2. 2. **Plant Material**

The leaves of *Wrightia tinctoria* and *Wrightia arborea* was authenticated at the Botanical Survey of India and voucher specimen was deposited at the herbarium of the same.

2. 3. **Preparation of Extract**

The leaves of *Wrightia tinctoria* and *Wrightia arborea* family Apocyanaceae were washed dried and ground to a fine powder and soxhleted with ethanol (70%) methanol, water petroleum ether dichloromethane, ethyl acetate and chloroform. The percentage yield of all the extracts were calculated as given in the table.

2. 4. **Preparation of Simulated Sea Water**[2]

38 gm of sea salt was weighed by rough balance and dissolved in 1 liter of double- distilled water in a small tank and then filtered off to get a clear solution. This simulated sea water was used for hatching of brine shrimp.

Simulated sea water was taken in a shallow rectangular dish with a plastic divider which had several 2mm holes, which was clamped in a dish to make two unequal compartments. The eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. The shrimps were allowed for two days to hatch and mature as nauplii (larvae).

2. 5 **.Preparation of test sample**

Samples were prepared by dissolving 50 mg of each extract in 5ml of dimethyl sulfoxide (DMSO) and various concentrations like 0,100,200,400,500 and 1000µg/ml solutions were prepared from the stock solution with the help of a micropipette having 100 units equal to 1 ml.

2. 6. **Bioassay**

In each of the vial containing different concentration of test samples, 10 brine shrimp nauplii were transferred with the help of 9 inches disposable pipette and simulated sea water was added to make 5ml. The nauplii can be counted macroscopically in the stem of the pipette against a lighted background. A drop of dry yeast suspension was added as food to each vial.

The vials were maintained under illumination. Survivors were counted, with the aid of 3x magnifying glass after 1,6,12 and 24 hours. The percentage deaths at each dose and control were determined after 12 hours were taken as standard and from this the percentage mortality/lethality was calculated[18]. By using Abbott's formula % death = [(test- control)/Control] x 100 and the LC_{50} were determined from a dose response graph.

3. Results and discussion

Among the seven different solvent leaf extracts of *Wrightia tinctoria* and *Wrightia arborea the ethanolic* (70%) and methanolic leaf extracts showed an LC_{50} of 471.604 µg/ml and 517.038 µg/ml for *Wrightia tinctoria* and 498.213 µg/ml and 531.082 µg/ml for *Wrightia arborea* .All the other solvent extracts taken for there two leaves showed no toxicity in BSA method.

The percentage mortality LC_{50} was also calculated by constructing graphs considering log concentration versus percentage mortality. Though after 24 hours, all the samples showed a significant lethality but these were not accepted for the safest and more accurate result. Because, after 24 hours, some nauplii may die normally as their life span is from 24 to 48 hours. The toxicity that was found in the other vials of different concentrations might be due to the toxic property of the plant extracts.[19] *Wrightia arborea Wrightia tinctoria* may contain some cytotoxic constituents that may be soluble in methanol and (70%) ethanol than other solvents used A further research is warranted to investigate the composition and nature of active constituents in these plants leaf extracts.

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Table 1. Results of Brine Shrimp Lethality Bioassay for *Wrightia tinctoria*

Drug Group	Sample Conc. (µg/ml)	Log Conc.	No. of Animals Dead n=3			% Lethality for each Group			Mean% lethality/ mortality ± S.D.	LC_{50} (µg/ml)			Mean LC_{50} ± S.D.
70% Ethanol	0	0	0	0	0	0	0	0	0	518.605	454.011	442.197	471.604±41.130[a]
	100	2.000	1	2	2	10	20	20	16.66±5.774				
	200	2.301	3	3	3	30	30	30	30±0.000				
	400	2.602	4	4	5	40	40	50	43.33±5.774				
	500	2.698	5	6	7	60	60	70	60±10				
	1000	3.000	9	10	9	100	100	90	93.33±5.774				
Methanol	0	0	0	0	0	0	0	0	0	562.275	482.741	506.098	517.038±40.880[b]
	100	2.000	1	1	2	10	10	20	13.33±5.774				
	200	2.301	3	2	3	30	20	30	26.66±5.774				
	400	2.602	3	5	4	30	50	40	40±10				
	500	2.698	4	5	5	40	50	50	46.66±5.774				
	1000	3.000	9	9	9	90	100	90	93.33±5.774				

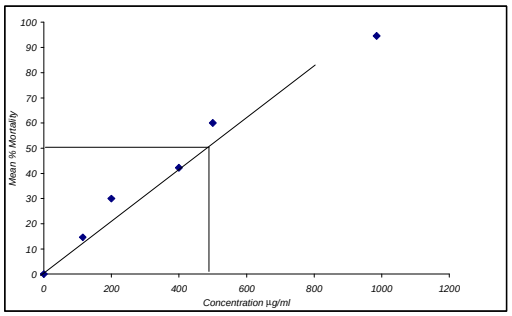
n=5; The results are expressed as mean ±S.D. of three parallel measurements. Values with different superscripts a, b, c, d are not significantly different p value>0.05; whereas a,c & b, d are significantly different p value<0.05. (One way ANOVA done by Dunnett's test.

Table 2. Results of Brine Shrimp Lethality Bioassay for *Wrightia arborea*

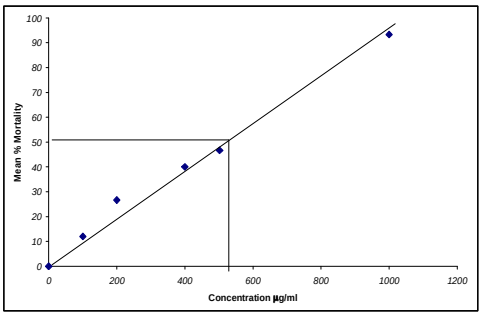
Drug Group	Sample Conc. (µg/ml)	Log Conc.	No. of Animals Dead n=3			% Lethality for each Group			Mean% lethality/ mortality ± S.D.	LC_{50} (µg/ml)			Mean LC_{50} ± S.D.
70% Ethanol	0	0	0	0	0	0	0	0	0	519.792	506.098	468.750	498.213 ± 26.49^{c}
	100	2.000	1	2	2	10	20	20	16.66±5.774				
	200	2.301	2	3	2	20	30	30	23.33±5.774				
	400	2.602	4	4	4	40	40	50	40±0				
	500	2.698	4	5	6	40	50	70	50±10				
	1000	3.000	10	90	10	100	90	90	96.666				
Methanol	0	0	0	0	0	0	0	0	0	500.00	470.370	622.876	531.082 ± 80.865^{d}
	100	2.000	1	2	1	10	20	10	13.33±5.774				
	200	2.301	2	2	2	20	20	20	20±0				
	400	2.602	4	5	3	40	50	30	40±10				
	500	2.698	5	5	4	50	50	40	46.66±5.774				
	1000	3.000	9	10	8	90	10	80	90±10				

n=5; The results are expressed as mean ±S.D. of three parallel measurements. Values with different superscripts a, b, c, d are not significantly different p value>0.05; whereas a,c & b, d are significantly different p value<0.05. (One way ANOVA done by Dunnett's test.

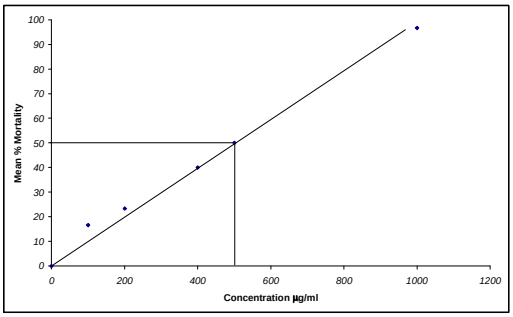
Determination of LC_{50} for *Wrightia tinctoria* 70% ethanolic extract



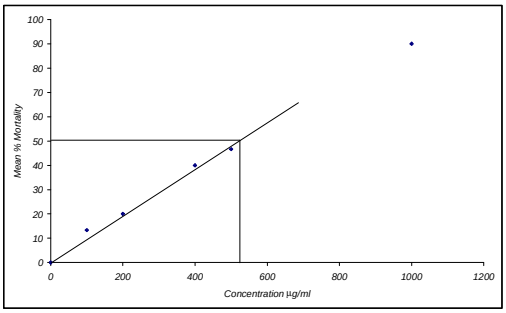
Determination of LC_{50} for *Wrightia tinctoria* methanol extract



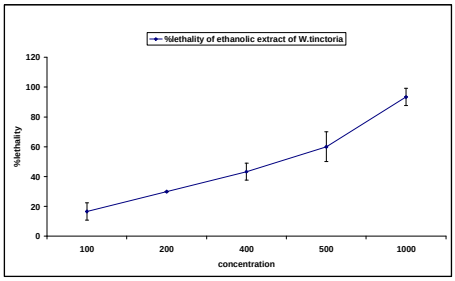
Determination of LC_{50} for *Wrightia arborea* 70% ethanolic extract



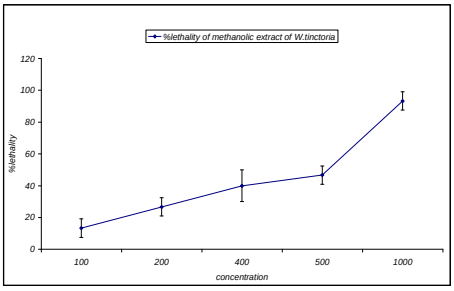
Determination of LC_{50} for *Wrightia arborea* 70% ethanolic extract

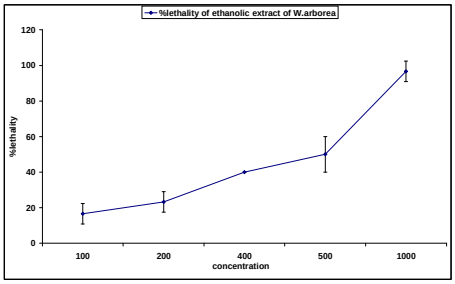


Determination % Lethality of 70% ethanolic extract of *Wrightia tinctoria*

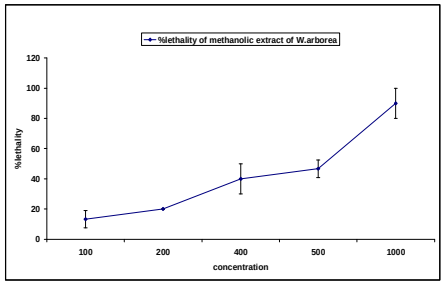


Determination % Lethality of methanolic extract of *Wrightia tinctoria*





Determination % Lethality of 70% ethanolic extract of *Wrightia arborea*



Determination % Lethality of methanolic extract of *Wrightia arborea*

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