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Evaluation of *In-Vitro* Cytotoxic Activity of Aqueous and Methanolic Extracts of *Wrightia tinctoria*



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ABSTRACT

Number of Studies has confirmed various traditional medicinal uses of the plant *Wrightia tinctoria*. While effects of the extracts of *Wrightia tinctoria* on Brine shrimp lethality using their different concentrations has not been previously reported. Present study shows that the standardized aqueous and methanolic extracts of leaves of *Wrightia tinctoria* exhibited significant cytotoxic activity. The findings of present work provide promise for the development of new molecules of treat cancer.

INTRODUCTION:

The plants belonging to the genus Wrightia are widely distributed throughout the world.¹⁻³ *Wrightia tinctoria* plant are used in folk medicine for treatment of several diseases.¹⁻³ The review of the literature suggests that good number of preclinical have confirmed the medicinal use of various *Wrightia tinctoria* species that have been mentioned in traditional medicine.¹⁻³

Although *Wrightia tinctoria have* various ethnopharmacological uses, the plant have not been investigated for cytotoxic activity. So the present study *in-vitro* lethality test was carried out, as this test has been successfully used as a preliminary study for cytotoxic and antitumor agents.

MATERIALS AND METHODS:

Plant Material

The leaves *of Wrightia tinctoria* were collected from Jalgaon in the month of August. The plant was identified and authenticated by T. Chakraborty Joint Director, Botanical Survey of India, Western circle, Pune.

Extracts of Wrightia tinctoria:



The leaves of *Wrightia tinctoria* was collected, shade dried and then pulverized in grinder. The powered leaves utilized for extraction procedure was passed through 60-120 mesh to remove fine powder and coarse powder was used for extraction.

The extraction was started with non-polar solvent like methyl alcohol. Extraction was carried out by continuous hot extraction method using Soxhlet apparatus till all constituent are removed. The completion of extraction was confirmed by taking sample out of siphon tube on TLC plate and placing it in iodine chamber. Absence of colored spot on plate indicated complete extraction. After completion of extraction procedure methyl alcohol was distilled off and concentrated extract was air dried. The extract was stored in desiccators. The marc was refluxed for about 3 hours with distilled water to obtain aqueous extract. Both the extracts was used in the concentration range of 10, 50, 100, 150,300 μ g/ml.

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Brine shrimp lethality bioassay / Cytotoxicity assay⁴

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds. The bioassay was carried out against a simple zoological organism, brine shrimp naupli. The brine shrimp lethality bioassay was carried out on the aqueous and methanolic extracts of *Wrightia tinctoria* using standard procedure. Briefly, brine shrimp (*Artemiasalina Leach*) eggs were hatched in a hatching chamber filled with fresh sea water. The chamber was kept under illumination using a fluorescent bulb for 48 hrs for the eggs to hatch into shrimp larvae. 30 mg of each extract were separately dissolved in 3 ml of DMSO, and from these 300, 150, 100, 50 and 10 μ g/ml were prepared by serial dilution. Each concentration was tested in triplicate, giving a total of 15 test-tubes for each sample. A control containing 5 ml of DMSO solvent was used for each solvent. The final volume of the solution in each test-tube was made up to 5 ml with water immediately after adding shrimp larvae. The test-tubes were maintained under illumination. Survivors were counted after 24 h and the percentage death at each dose was determined and LC50 values were calculated after 24 hrs of incubation, the test tubes were inspected using a magnifying glass and the number of survivors was counted. The concentration-mortality data were analyzed statistically for the determination of LC50 values.⁴

RESULTS AND DISCUSSION

Brine shrimp lethality bioassay (Cytotoxicity assay)

The results of the brine shrimp lethality after 24 hrs exposure to the extract samples and vincristine sulphate are summarized in Table I. Following the procedure of Meyer, the lethality of *Wrightia tinctoria* extracts of the brine shrimp was evaluated. In the present study, aqueous and methanolic extracts of *Wrightia tinctoria* revealed prominent cytotoxicity with the LC50 values of 169.58 μ g/ml and 249.44 μ g/ml respectively. Both the extracts of the *Wrightia tinctoria* showed significant results indicating that the test samples are biologically active. Plotting of log of concentration (log C) versus percent mortality (% Mortality) for all test samples showed an approximate linear correlation.

CONCLUSION

The *Wrightia tinctoria* extracts have a potential to be a candidate for the investigation of cytotoxic compounds. The findings of present work would give baseline information on the

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most promising plant species that could be use as a basis for the development of new tools of great therapeutic importance to overcome microbial infections and cancer.

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Figure 1: In-vitro Cytotoxicity assay of Wrightia tinctoria extracts

(Values are means \pm SEM from three readings)



Figure 2: LC₅₀ of Wrightia tinctoria extracts

