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Assessment of In Vitro Cytotoxicity of *Diospyros Melanoxylon* Roxb. Leaf Using Brine Shrimp Assay.

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Abstract

Diospyros melanoxylon Roxb. is a species of flowering tree in family Ebenaceae known as Coromandel Ebony native to southern India and Sri Lanka. The preliminary cytotoxicity of petroleum ether extract, ethyl acetate extract and isolated compounds β-sitosterol and catechin was studied using Brine Shrimp Lethality Assay (BSLA). Ethyl acetate extract showed significant cytotoxicity with LC_{50} of 45.11µg/ml. Pet ether extract, β-sitosterol and catechin also showed significant cytotoxicity with LC_{50} 50.68, 80.20, 62.59µg/ml. The present study supports that brine shrimp assay is simple, reliable and convenient method for assessment of cytotoxicity of medicinal plants.

Key Words

Diospyros melanoxylon Roxb., Cytotoxicity, Brine shrimp lethality assay, $LC_{50.}$

Introduction

Coromandel Ebony or East Indian Ebony (*Diospyros melanoxylon* Roxb.) is a species of flowering tree in the family Ebenaceae that is native to southern India and Sri Lanka. The genus *Diospyros* consists of 240 species, 59 of which are distributed in India.[5] The leaves are used in cigarette (bidi) industry.[7] The plant and parts, especially the fruit has been used as an anti-inflammatory and antipyretic drug in many local traditional medicines.[3] Cytotoxic naphthalene derivatives were found in *D. assimilis* species.[6] But no cytotoxicity was found for *Diospyros melanoxylon* Roxb. Therefore the preliminary in vitro cytotoxicity of compounds like β-sitosterol and catechin isolated from leaves and bark was carried out.

Materials and methods

Collection and Authentication of plant material

The plant was collected from forests of Vankaneda, Dist. Sabarkantha, Gujarat, India. The fresh leaves were collected and then dried. The plant was identified and authenticated as *Diospyros melanoxylon* Roxb. by Dr. Bimal Desai, Department of Botany, Agriculture University, Navsari, Gujarat, India.

Preparation of extract

The air dried whole plant materials were powdered by using pulverizer and passed through sieve no. 20. Petroleum ether extract, ethyl acetate extract, isolated compounds β-sitosterol and Catechin were tested by this method.

Brine shrimp assay[1, 8]

In this test, brine shrimp (*Artemia salina*) eggs were hatched in artificial sea water in beaker (33 g/L sea salt) for 48 h. After 48 h of incubation, 10 nauplii were transferred to each sample test tube using pipette and artificial sea water was added to make volume 5 ml. For all samples, dose range was optimized at initial concentrations of 10, 100 and 1000 µg/mL of test material using ASW. Each concentration of test material (1 ml) was added in sample test tube in triplicate using micropipette. Survivors were counted after 24 h and accordingly final concentrations of test material were determined. Three replicates were prepared for each concentration of test material.

Lethality concentration determination

The lethal concentration of test material resulting in 50 % mortality of the brine shrimp (LC_{50}) after 24 h was determined by using probit method.[4]

Statistical analysis

LC_{50} values were determined according to the probit method reported by Ghosh, (2005)[2]. In this method the observed percentage mortality is converted into probit, and the values thus obtained are plotted against log dose. The LC_{50} value determined from

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the graph. Before plotting, the percentage dead for 0 and 100 are corrected. The probit values are plotted against log doses, and then the dose corresponding to probit 5 (50 %) is found out**.**

Results and Discussion

Preliminary in vitro cytotoxicity was determined by using brine shrimp assay. LC_{50} for isolated compounds and both pet ether extract and ethyl acetate extract was determined and it was found to be 62.59, 80.20, 50.68 and 45.11 µg/ml for catechin, β-sitosterol, pet ether extract and ethyl acetate extract respectively. This significant lethality of different extracts, β-sitosterol and catechin to brine shrimp is an indicative of the presence of potent cytotoxic components in extract. In pet ether and ethyl acetate extract higher cytotoxicity was due to the presence of triterpenoids and polyphenolics respectively as compared to single compounds like β-sitosterol and catechin.

Conclusion

The brine shrimp test represents a rapid, inexpensive and simple *in-vitro* bioassay for testing plant extract lethality which in most cases correlated reasonably well with cytotoxic and anti-tumour properties. The study first time explored the cytotoxicity of *D. melanoxylon* Roxb. leaves. This study is helpful for further evaluation of in vivo cytotoxicity.

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Table 1: Brine shrimp lethality assay (**LC_{50}**).

Sr. No.	Extract/Compound	Concentration tested (µg/ml)	r^2	LC_{50} (24 h) (µg/ml)
1	Catechin	25, 50, 100, 200, 250	0.9864	62.59
2	β-sitosterol	50, 100, 200, 400, 500	0.9483	80.20
3	Pet. Ether extract	5, 10, 20	0.9422	50.68
4	Ethyl acetate extract	5, 10, 20	0.9610	45.11

LC_{50} value is mean of six replicates

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