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Cytotoxic Activity of Methanolic and Aqueous Extract of Eulophianuda

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ABSTRACT:
Eulophianuda belonging to family orchidaceae investigated to evaluates the cytotoxic property of the methanolic and aqueous extracts of Eulophianuda tuber using as three in-vitro models Allium cepa root, Brimeshirmplithality bioassay (BSLP) and MTT assay (A546 cell line). In the present bioactivity study, all of the extracts of Eulophianuda (methanol, water) showed positive results indicating that the test samples are biologically active.For brine shrimp lethality bioassay ten nauplii were placed intest tube filled with 5 ml total volume of artificial sea water and different concentrations (100- 1000 µg/ml) of methanolic and aqueous extract of Eulophianuda roots in a set of test tubes per dose. After 24 hours, live naupliiwerecounted and LC_{50} value was estimated. In Allium cepa root meristem model,onion bulbs were suspended inside 100 ml beakers at different concentration (1mg/ml and 10 mg/ml) of extract. The percentage root growth inhibition aftertreating with methanolic extract at 48 and 96 hrs was determined MTT assay have been utilised to measure the antitumor activity of Methanolic and aqueous extract of Eulophianuda by using A546 human cell line of Lung carcinoma. Brine shrimp lethality bioassay (LC_{50}= 450µg/ml and 600µg/ml), Allium cepa root meristemmodel and MTT assay (IC_{50} =2.2 µg/mland 9.8µg/ml) showed potent cytotoxic and antitumoractivity of methanolic and aqueous extract of Eulophianuda tubers.. Therefore, this plant has potential for the development of novel anticancer drug leads.
Keywords: Cytotoxic activity, brine shrimp lethality bioassay, Allium cepa root model, MTT assay EulophiaNuda,

I. INTRODUCTION:

Over the past decade herbal medicine have been acceptedуniversally, hence medicinal plants continue to play an important role in healthcare system of a large number of world's population. Infact there are several medicinal plants all over the world which arebeing used traditionally in the prevention and treatment of cancer. Plant derived compounds have played an important role in the development of several clinically useful anti-cancer agents. The study demonstrated the cytotoxic activity of methanolic and aqueous extract of Eulophianuda tubers reported by models Brine shrimp lethality bioassay (BSLB), Allium ceparoot tip meristem model andMTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide) assay.

Material And Method:

The tubers of Eulophianudawere collected from a supplier and authenticated by Dept. of Botany, R.T.M. Nagpur University, Nagpur, Maharashtra, India.The collected plant tubers were dried and pulverized into coarse material. The coarse plant material was used for preparation of extracts.

Invitro cytotoxic activity

I) Brine shrimp lethality bioassay

Brine Lethality bioassay was carried out to investigate the cytotoxicity of extracts of medicinal plant it can be used in laboratory bioassay in order to determine toxicity through the estimation of medium lethal concentration (LC_{50} values) which has been reported for series of toxins and plant extracts. This method, which determines the LC_{50} values of the active compound and extracts in saline medium in µg/ml has been used in research on medicinal plants carried out in different countries in order to evaluate toxicity, anticancer, and other biological actions, which in some cases have been related to pharmacological studies carried out for different chemical compounds as a screening method mainly for product of plant origin.

II) Aliumcepa root meristem model

Locally available Onion bulb (Allium cepa 50 ± 10 g) were obtained and grown in the dark over 100 ml tap water at ambient temperature until the roots have grown to approximately 2-3

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cm. The water was changed daily. For the root growth inhibition, the base of each of the bulbs was suspended on the extracts inside 100 ml beakers in the dark and the length of the roots of all onion bulbs with the best growth i.e. Length and Root number at 0, 48, 96 hrfor each concentration of extract and control was measured (in cm) with a ruler. The percentage root growth inhibition in relation to the negative control and the root growth after treating with different at 48 and 96 hr. extracts was determined.

III) MTT Assay

Cell proliferation activity of various extracts of Eulophianuda tubers carried out by MTT Assay, which estimated the effect of various extracts the growth of cell in vitro. Measured of cell viability and proliferation forms is used as basis for this in vitro assay.

II. RESULTS & DISCUSSION:

1. Brine shrimp lethality bioassay

In the present bioactivity study, extracts of Eulophianuda (methanol and aqueous) showed positive results indicating that; the test samples are biologically active. Plotting concentration versus percent mortality (% Mortality) for test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC_{50}, the concentration at which 50% mortality of brine shrimp nauplii occurred) were determined. LC_{50} value of methanol and aqueous extract was found to be 450 (µg/ml) and 650 (µg/ml) respectively. All the values were compared with standard cytotoxic agent cyclophosphamide, who's LC_{50} was found to be 300 (µg/ml).

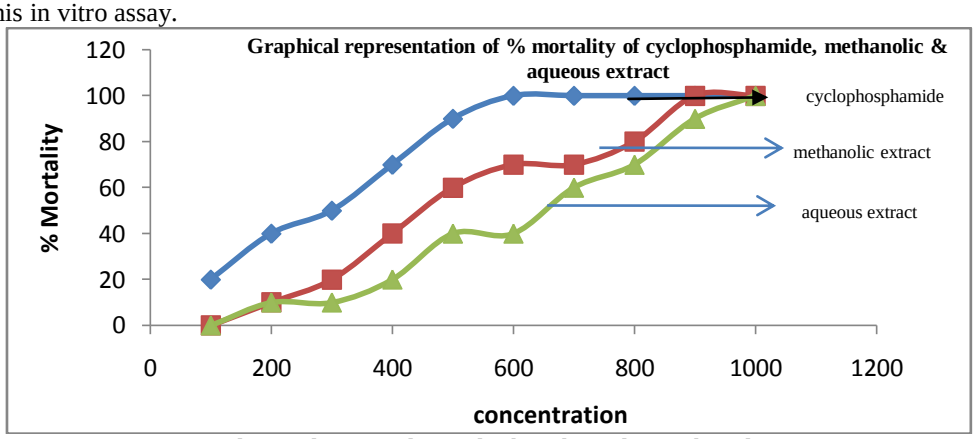

Figure No.1 % mortality and LC_{50} valuecyclophosphamide, methanolic& aqueous extract

2. Allium cepa root meristem model

Incubation of Bulbs in different concentration of cytotoxic agents produced a growth retardation effect that was associated with a decrease in root number. Both extracts specially the Methanolic extract and cyclophosphamide arrested the root growth. Methanolic extract has shown the maximum growthretarding effect at 10mg/ml when compared to standard drugs. The root length after 0, 48, 96 hr. with significance at 10 mg /ml was found to be 3.78 ± 0.71 (n = 21), 2.16 ± 0.50 (n = 12) and 2.21 ± 0.71 (n = 8).

Sr. No	Group	0 hrs		48 hrs		96 hrs	
		Root no.	Root length (cm)	Root no.	Root length (cm)	Root no.	Root length (cm)
1.	Control	14	3.55±0.61	23	3.67 ± 0.64	25	4.11 ± 0.89
2.	Cyclophospham ide	12	2.72±0.75	08	1.78 ± 0.55	04	1.07±0.09 **
3.	Methanol Extract	16	2.36±0.64	12	2.27 ± 0.41	08	1.84 ±0.46**
4.	Aqueous Extract	13	2.18±0.67	13	2.41 ± 0.89	12	2.05±0.58 *

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Table No.1: Allium cepa root length and root number attained following incubation with different extract of Eulophianuda with concentration 10mg/ml

*indicates significant difference at P<0.05 when compared with control

** indictes significant difference at P<0.01 when compared with control

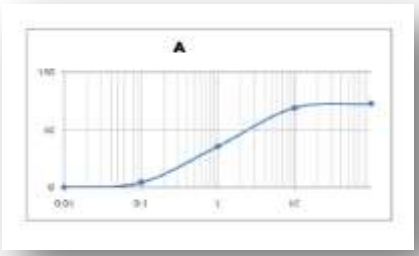
Data Analysed by one way ANOVA Dunnett's test.

3. MTT Assay:

Cell Proliferation activity of various extracts of Eulophianudacarried out by MTT Assay, which estimated the effect of various extracts on the growth of cell in vitro. Measurement of cell viability and proliferation forms is used as basis for this in vitro assay. Analogous to the results obtained in previous models, methanol extract was found to be active with IC_{50} value of 2.2 µg/ml and also aqueous extract was found to be active with IC $_{50}$ value of 9.8 µg/ml

Name of Extract	Methanol extract	Water extract
IC_{50}	2.2	9.8
Status	Active	Active

Table No.2Results for IC_{50} values of respective extracts of Eulophianuda



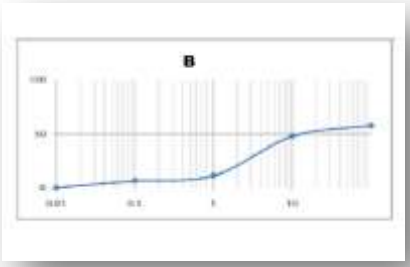


Figure No:3Dose response curves for Methanol Extract against A549.Figure No:4 Dose response curves for Water Extract against A549.

III. CONCLUSION:

From the observation and results of cytotoxic assay of the extracts it was found that the methanol and aqueous extract of tubers had shown significant cytotoxic activity in three reported models (Brine shrimp, Allium cepaand MTT assay) data showsconsistent results and potential for cytotoxic activity of methanol and aqueous extract. Based on the possible relationship between all these models used and plant bioactivity, this work could serve for further pharmacological research viz, isolation of constituents from the extracts and finding out the constituents responsible for activity.

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