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BIOASSAY GUIDED ASSESSMENT OF FRACTIONS OF METHANOLIC EXTRACT OF *CRINUM DEFIXUM* AGAINST BRINE SHRIMP LETHALITY ASSAY

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ABSTRACT

Methanol extract of leaf of *Crinum defixum* was subjected to extensive column chromatography, which resulted in 70 fractions. On the base of TLC analysis and brine shrimp assay, fractions were recombined in 13 major fractions. Majority of the fractions showed excellent cytotoxic activity more than 90%. Preparative TLC of one fraction resulted in a single fraction. On spectral analysis it consisted of one major peak and 3 small peaks.

KEYWORDS: Methanol extract, Brine shrimp assay, Column chromatography, *Crinum defixum.*

INTRODUCTION

Crinum defixum belongs to family Amaryllidaceae, popular for medicinal potential of their corm and bulbs.[1] Although Crinum genus is popular as ornamental plants with beautiful and elegant flowers, but they also possess medicinal properties such as analgesic, immunostimulating, antineoplastic, antiviral and antimicrobial effects.[2]

Alkaloids isolated from the bulbs of the have been used in various biological activities like anti-parasitic, cholinesterase inhibitory activity, anti inflammatory and anticancer activities.[3] In our previous study,[4] plant was explored for cytotoxic activity using brine shrimp assay using four extracts, out of which methanol extract was found to show good activity. Hence it was thought worthwhile to study the same extract in detail for isolation of cytotoxic principle from the plant.

MATERIALS AND METHODS

Plant Collection and Preparation of Solvent extract

Fresh leaves of *Crinum defixum* were collected from the medicinal germplasm garden of Regional plant resource center (RPRC), Bhubaneswar. Leaves were weighed and washed with running tap water to remove dust and impurities. After drying, weight of leaves was again taken for the determination of moisture content. Moisture content of the leaves was calculated by using the following formula

$$\text{Moisture content (\%)} = \frac{\text{Fw} - \text{Dw}}{\text{Fw}} \text{ X } 100$$

Where, Fw = Fresh weight of leaf sample

Dw = Dry weight of powdered leaf sample

Further, Leaves were dried in shade till complete drying followed by grinding in grinder (Lexus make) to make fine powder for the preparation of solvent extracts.

Solvent extraction

After extraction the extract was concentrated by using Buchhi(R-200) Rotavapour under vacuum at 45-50°C. Yield of the solvent extracts was also recorded. Concentrated extract were transferred to screw cap vials and extract yield was calculated by using the formula-

$$\textbf{Percentage yield of extract} = \frac{\textbf{Extract weight} \times \textbf{100}}{\textbf{Powdered weight}}$$

Cytotoxic activity

Brine shrimp (Artemia salina) mortality assay

Cytotoxic activity study was carried out by brine shrimp lethality assay using standard protocol.[5] Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. Brine shrimp (*Artemia salina*) eggs were hatched in artificial sea water, which was prepared using black salt 2 gm/ 200 ml distilled water. The eggs were incubated for 24 hours at temperature of about 28° C to get the desired growth of the larvae for biological evaluation. For each dose level 3 replicates were used. To each test tube of control, positive control and extracts, 20 numbers of brine shrimp and volume was made up to 10ml by adding salt water. Cytotoxic assay was carried out at three doses 500, 1000 and 2000µg/ml. Motility assessment of larvae was conducted at each hour up to four hours.

Motility readings were graded as below.

4+ = high motile

3+ = motile

2+ = sluggish

1+ = slow

Nil = no activity

After 24 hrs, the number of survived larvae in the control and experimental tubes were counted. From this data, the percentage (%) of inhibition of the brine shrimp was calculated for each concentration using the following formula

$$\% \text{ Inhibition} = \frac{\text{No of larvae (control)} - \text{No. Of larvae(experiment)}}{\text{No. of larvae in control}} \times 100$$

Column chromatographic separation of methonlic extract

Chromatographic separation of methanolic leaf extract and TLC based analysis was carried out to separate the pure compounds present. For open column chromatography silicagel160-120 mesh was used as stationary phase. Methanolic leaf extract up to.40gms was loaded in the column was run using Methanol: acetonitrile gradients. i.e. (90:10, 70:30, 50:50, 30:70, 20:80, 10:90, 100) Fractions were collected at regular intervals based on the colour of fractions. A total of 70 fractions were collected in the 1st column run. The process was repeated for 3 times.

TLC based analysis of fractions

All the fractions were subjected to TLC using solvent Toluene: Acetic acid(9:1). On the basis of TLC profile similar fractions were combined. All the combined extracts were subjected to cytotoxic activity. Based on the cytotoxic activity one fraction was selected for further fractionation using preparative TLC.

Preparative TLC of fraction one of Column chromatography was conducted using solvent tolune. Preparative TLC plate of merck was activated at 60°C for 1min and fraction was loaded with the help of microtip. After solvent run up to 16.5 cms height PLC sheet was removed and the spraying reagent(5% methanolic H_2SO_4) was sprayed on sides followed by heating. Only one yellow band was observed which was scraped from the plate and dissolved in methanol.

Spectral analysis of the isolated fraction was studied and peaks were recorded. Fraction was also subjected to cytotoxic activity. Results are discussed.

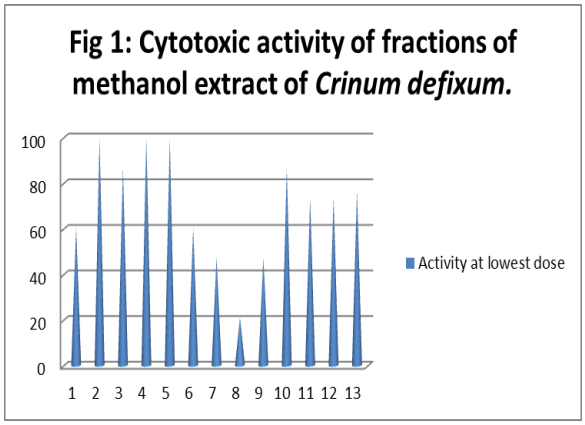
RESULTS AND DISCUSSION

Moisture content was quite high for the leaf it was 86.16% and yield of methanol extract was 49.6% and rest was residual mass. On the basis of TLC analysis 70 fractions were reassembled into 13 major fractions. Thin layered analysis of the fractions is shown in Table 1. It can be observed that majority of fractions possess more than one compound, Only fractions 8, 12 and 13 showed single band.

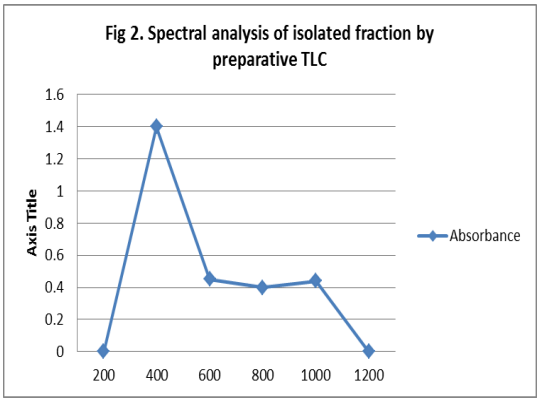
Table 1: TLC based analysis of fractions of the methanolic extract of *Crinum defixum*.

Fraction	Rf Values	No. of bands
1	0.48,0.5	2
2	0.3,0.36,0.38,0.42,0.45,0.47,0.51,0.52	8
3	0.12,0.3,0.38,0.49,0.53	5
4	0.12,0.3,0.37,0.49,0.52,0.54	6
5	0.41,0.45,0.74,0.78	4
6	0.42,0.45,0.79	3
7	0.13,0.32,0.37,0.47,0.5	5
8	0.48	1
9	0.11,0.52,0.54	3
10	0.47,0.54	2
11	0.37,0.4	2
12	0.45	1
13	0.5	1

All the above fractions were subjected to cytotoxic activity using brine shrimp assay. As can be seen in Fig 1. Fractions2, 4 and 5 exhibited highest activity suggesting that all the molecules are acting synergistically. However, all the bands were in close proximity hence could not be separated.



Preparative TLC of fraction 2 was conducted and one major band was isolated. One major spectral analysis peak was obtained at 400nm where as minor peaks were obtained at 600, 800 and 1000(Fig2). Thus, fraction is not a single entity but a mixture of molecules. Thus methanolic leaf extract of the plant is a good candidate for the isolation of cytotoxic principle and a number of molecules could be isolated which is beyond the scope of this project.



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