



CYTOTOXIC ACTIVITY OF ISOLATED FRACTIONS FROM METHANOLIC EXTRACT OF *ASYSTASIA DALZELLIANA* LEAVES BY BRINE SHRIMP LETHALITY BIOASSAY

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ABSTRACT

The cyto-toxicity activity of methanolic extract and isolated product of *Asystasia dalzelliana* leaves were assayed by brine shrimp lethality bioassay. Methanolic extract upon the column chromatography yielded five fractions named (AD-01, AD-02, AD-03, AD-04, and AD-05). Among the five fractions tested AD-02 fraction (LC₅₀-32.12µg/ml) showed good and AD-04 (LC₅₀-35.00µg/ml), AD-05(LC₅₀-35.41µg/ml) showed moderate cytotoxic activity compared with standard vincristine sulfate (LC₅₀ of 0.52 µg/ ml). The crude methanolic extract showed LC₅₀ value at 48.12µg/ml. This research confirms that the cytotoxic properties of *Asystasia dalzelliana* leave extract and isolated products. The plant also used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hither to unmet needs. Furthermore, isolated product can be subjected to characterization by NMR, IR, and Mass Spectroscopy for their structural elucidation and mechanism involved in the activity..

Keywords: *Asystasia dalzelliana*, Brine shrimp, Fractions.

INTRODUCTION

Natural products have historically served as a source for cancer chemotherapy agents. The ethnomedicinal value of plants provides evidence of their biological activity that can be further utilized for the drug discovery process. Medicinal plants have been used for years in daily life to treat diseases all over the world. Drugs derived from unmodified natural products or drugs semi-synthetically obtained from natural sources corresponded to 78% of the new drugs approved by the FDA between 1983 and 1994. This evidence contributes to support and quantify the importance of screening natural products¹. Plants have a long history of use in the treatment of cancer. Drug discovery from plants is a multi-disciplinary approach which combines various botanical, ethno-botanicals, phytochemical and biological and chemical separation techniques². However, despite these observations, it is significant that over 60% of currently used anti-cancer agents are derived from natural sources, including plants, marine organisms and micro-organisms³. *Asystasia dalzelliana* commonly known as violet *Asystasia* (Marathi: Neelkanth) belongs to family *Acanthaceae*. It is a perennial branched herb, about 60-100m length, quadrangular stem, swollen at nodes. Leaves are opposite, elliptic-oblong acute apex truncate at base; petiole 2cm long⁴. The whole plant is used in Indian folk medicine as antioxidant, anti-inflammatory, anti venom a novel whose pharmacology yet to be proved⁵.

The brine shrimp (*Artemia salina*) lethality bioassay is rapid, simple and no aseptic techniques are required, easily mastered, inexpensive and requires small amounts of test material (2-20 mg). The bioassay has a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity^{6,7}. This in-vivo lethality test has been successively employed for providing a frontline screen that can be backed up by more specific and more sophisticated bioassays once the active compounds have been isolated. The objective of this research work was to investigate the in vitro cytotoxic activity of methanolic extract and isolated products of *Asystasia dalzelliana* leaves.

MATERIAL AND METHODS

Plant material

The fresh leaves were collected from Bidar (District of Karnataka state, India) and authenticated by Dr. Siddamallaya Regional Research Institute, Bangalore. The voucher specimen (RRR/BNG/SMP/2009-10/717) was deposited in the same institute. Prior to use, it was ensured that the leaves were free from contamination, sand and no microbial growth.

Preparation of methanolic extract

Dried leaves of *Asystasia dalzelliana* were macerated with methanol (1x4) for 24 hrs at room temperature and filtered. Filtrates were combined, concentrated under vacuum at temperature not more than 70 °C and dried in vacuum drier.

Fractionation Isolation and separation

50g of methanolic extract was chromatographed on a silica gel (60-120 mesh) column using gradient elution starting with n-hexane and ending with methanol to obtain 5 fractions. Fractions were concentrated to 1/10th of their volume and kept for crystallization at room temperature for 7 day. Three out of five fractions showed crystal formation and named as AD-01 (90% hexane in ethyl acetate), AD-02 (60:40 methanol and ethyl acetate), AD-03 (50:50 methanol and ethyl acetate) AD-04 (75:25 methanol and ethyl acetate), AD-05 (100% methanol). Further purification was done by re-crystallization. The compounds were confirmed for the three single constituent by running TLC, different solvent methods were used and finally the spots were separated as single in ethyl acetate: formic acid: glacial acetic acid: water (10:11:11:2.6) and were confirmed by measuring spots at 254nm.

Brine shrimp lethality bioassay^{8,9}

The toxic potentiality of the plant crude extract and fractions were evaluated using Brine Shrimp lethality bioassay method where 4 graded doses (25µg/ml, 50µg/ml, 100µg/ml, and 200µg/ml) for isolated fraction and for methanolic extract 4 graded doses (200µg/ml, 400µg/ml, 800µg/ml, and 1000µg/ml) were used. Brine shrimps (*Artemia salina*) nauplii obtained from natural remedies as a gift sample were used as test organisms. For hatching, eggs were kept in brine with a constant oxygen supply for 48 hours. The nature nauplii were then used in the experiment. DMSO (Di-methyl sulfoxide) was used as a solvent and also as a negative control. The median lethal concentration LC₅₀ of the test sample after 24 hours was calculated and compared with reference standard vincristine sulfate. The experiment was triplicated for concordant values.

RESULTS AND DISCUSSION

The brine shrimp lethality bioassay (BSLA) has been used routinely in the primary screening of the crude extracts to assess the toxicity towards brine shrimp, which could also provide an indication of possible toxicity of the test materials. A number of novel antitumor and pesticidal natural products have been isolated using this bioassay. As summarized in table 1, the toxicity exhibited by the

crude methanolic (MeOH) extract as well as the isolated product fractions of the plant showed potent activity against positive control (vincristine sulphate). The toxicity of the MeOH extract and its fractions on the BSLA increased in the order of AD-02>AD-01>AD-04>AD-05>AD-03 > MeOH were 32.12, 34.44, 35, 35.41, 38.33 and 48.12 $\mu\text{g}/\text{mL}$ in their LC_{50} , respectively. Moreover, this significant lethality of the crude plant extracts (LC_{50} values less than 100 ppm or $\mu\text{g}/\text{mL}$) to brine shrimp is indicative of the presence of potent cytotoxic and probably insecticidal compounds which warrants further investigation. AD-02 fraction (LC_{50} -32.12 $\mu\text{g}/\text{ml}$) showed more cyto toxicity activity compared with standard vincristine sulfate (LC_{50} of 0.52 $\mu\text{g}/\text{ml}$). Other cytotoxicity tests and specific bioassays may be performed on the isolated bioactive compounds on cell lines for further studies.

Table 1: Shows cytotoxic activity of methanolic extract, isolated fractions and positive control vincristine sulphate (VS) on brine shrimp

Sl No	Sample	LC_{50} $\mu\text{g}/\text{ml}$
1	Methanolic extract	48.12
2	AD-01	34.44
3	AD-02	32.12
4	AD-03	38.33
5	AD-04	35.00
6	AD-05	35.41
7	Vincristine Sulfate	0.52

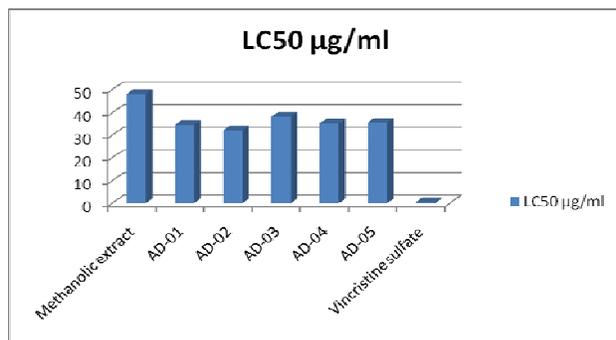


Fig. 1: Shows LC_{50} of methanolic extract, isolated fractions and vincristine sulphate

CONCLUSION

In conclusion, our finding confirms the cytotoxicity activity of methanolic extract and isolated fraction of *Asystasia dalzelliana* leaves. These could be of particular interest in relation to find out its unexplored efficacy and mechanism of action with characterization of isolated fraction by IR, NMR and Mass Spectroscopy. This research concludes the role of isolated fraction from *Asystasia dalzelliana* leaves in the discovery of lead compounds for the development of conventional drugs for the treatment of most human diseases.

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