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Evaluation of in vitro cytotoxic activity of different extracts of *Acslepias curassavica* leaf

G. Aiswarya *[1] and K. Venkatta Ramana[2]

[1]JDT Islam College of Pharmacy, Vellimadukunnu, Kozhikode, Kerala, India.
[2]ASN College of Pharmacy, Burripalam Road, Thenali, Andhra Pradesh, India

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ABSTRACT

Asclepias curassavica like most of the medicinal plants has highest significance for its valuable secondary metabolites. The successive extraction has been done by using hot continuous percolation method. The different extracts of the plant shows different level antitumour activity. Plant extracts that inhibit the growth of tumour cells without harming the host may have potential application as therapeutic agents.

Keywords: *Asclepias curassavica*, cytotoxic activity and Antitumor

INTRODUCTION

Asclepias curassavica (L.) (Tropical milkweed) is an erect, evergreen shrub belonging to the sub family *Asclepiadoideae*, family Apocynaeceae[1]. The sub family *Asclepiadoideae* constitutes many medicinally important plants comprising more than 250 genera and 3,000 species, of which 43 genera and 243 species are present in India. It has a woody based stem with milky sap leaves 6-14 x 1-3.5 cm, decussate, lanceolate, puberculous along nerves, acute at both ends. In general, *Asclepiadoideae* plants are the source of cardiac glycosides and contain highly valuable potential products for curing many diseases. Resinoid (galitoxin), a toxic principle in poisonous species is found in the milky latex of its stem. Several glycosides (cardiac glycosides) and an alkaloid have been isolated. Root extracts of this plant are widely used in South America as an emetic and laxative. A decoction of the plant is used as an abortifacient. Roots are known as 'Pleurisy root" and used as an expectorant for pneumonia, lung problems, employed to treat warts, fever, etc. Plant is used as anti-ovulatory, astringent, cardiotonic and used for abdominal tumor, haemorrhages, and headache. The plant contains highly potential esterified polyhydroxy pregnane glycoside that shows antitumour and anticancer property. The use of plants as medicines has paved way for the isolation of active compounds[2]. Medicinal plants are of great interest as pharmaceutical industries depend on plants for the production of secondary metabolites[3]. India has about 8,000 species of known medicinal plants and about 1,000 plants have been used in the traditional system of medicine like Ayurveda, Unani and Sidha, while tribals use 7,500 plant species for medicinal purposes using the current global rates of species extinction at around 10 to 12 per cent of the plants (800-1,000 species) are likely to be threatened[4]. The phytochemicals produced from the medicinal herbs are curative constituents for several diseases. Natural products derived from higher plants may form the source to search for novel drugs based on their new modes of pharmacological action. This action may be due to the presence of carbohydrates, carbohydrate derivatives, gums, mucilages, pectins, various forms of glycosides, tannins, phenolic compounds, lipids, fixed and volatile oils, resins, alkaloids etc. The significance of phytochemical analysis and its importance for the establishment of alternative medicine was expressed[5]. Study of literature showed that a large number of taxa in the family *Asclepiadoideae* are medicinally important and contain different kinds of secondary metabolites. Glycosides (steroidal, pregnane), flavonoids are the major compounds reported in this family. The production of an essential oil containing 2-hydroxy 4- methoxy benzaldehyde from the in vitro derived roots of Hemidesmus indicus has been carried out[6]. The batch culture of H. indicus roots had been conducted for the continuous production of roots and root specific compounds[7]. Selected medicinal plants of *Asclepiadaceae* family have been screened[8]. Recently, the antimicrobial activity of root extracts in *Asclepias curassavica* (L). was reported[9]. Here in this investigation, the leaf

***Corresponding Author Address:** G. Aiswarya, JDT Islam College of Pharmacy, Vellimadukunnu, Kozhikode, Kerala, India; E-mail: aiswaryageorge_d@yahoo.com*

extracts are studied for potential anticancer activity that information can be employed for further studies.

EXPERIMENTAL

Collection and authentication of plant Material: The fresh leaves of the plant *Asclepias curassavica* L.belongs to fmily *Asclepiadaceae* were collected from botanical garden, university of Calicut, Keralastate, India and authetified by Dr.P.S. Udayan, Taxonomist, SreeKrishna College, Kerala and the voucher specimen number was 841 and was deposited in Sree Krishna College, Guruvayur Herbarium,Trissur district of Kerala state.. The collected leaves were washed, shade dried and pulverized with mechanical pulverizer for size reduction and passed through #60 and that powder was used for preparation of extract.

METHOD

Extraction procedure: The powdered plant material was successively extracted by using Soxhlet extractor. 100g of powdered drugs were placed in a central compartment of Soxhlet assembly. 650 ml of solvent was placed in a lower compartment and a reflux condenser is attached above the central compartment. The vapour passes through the side-arm up into the reflux condenser. Here the vapour liquified and drips into the plant material to be extracted. The warm solvent percolates through the materialband the extracts gradually collects in the central compartment. Once the height of the extract reached the top of the siphon, the entire liquid flowed through this and back to the lower solvent container. The process is then repeated. The extract collected in the lower vessel, gradually becoming more and more concentrated. The vapour rising from the heated extract is pure solvent vapour and so the liquid dripping into the material from the condenser is essentially pure solvent, though derived from the extract. After complete extraction the lower vessel was removed, solvent recovered and the extract is concentrated and percentage yield was calculated. The solvents were recovered by using simple distillation method. The charged drug from the central compartment was removed, dried, recharged and extracted with N-hexane. By using Soxhlet extractor exhaustive extraction with a series of solvents of increasing polarity was done. Solvents used with increasing polarity: Petroleum ether, Benzene, Chloroform, Methanol and finally water. The percentage yield of of extracts produced is given in table no1.

Table 1: Percentage yield of Leaf extracts of *Crateava magna* with different solvents

Extracts	% yield of Extract (w/w)
Petroleum Ether Extract	2.70
N-Hexane Extract	0.446
Chloroform Extract	4.012
Methanolic Extract	0.914
Aqueous extract	0.723

Evaluation Cytotoxicity: The test compounds were studiedfor short term in vitro cytotoxicity using Dalton's lymphoma ascites cells (DLA). The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by trypan blue exclusion method. Viable cell suspension ($1x10^6$ cells in 0.1ml) was added to tubes containing various concentrations of test compounds and the volume was made up to 1ml using phosphate buffer saline. Control tude contained only cell suspension. These assay mixture was incubated for 3 hours at 370C. further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The number of stained and unstained cells were counted separately.

RESULTS AND DISCUSSION

The different Extracts of *Asclepias curassavica* were subjected to in- vitro cytotoxic activity by using Dalton's lymphoma ascites cells (DLA). The study was shown significant cytotoxic activity for all the extracts at different concentrations. The percentage od cell death is shown in the table2. More percentage of cell death was shown by N-hexane and Chloroform extracts at concentration 200 µg/ml.

Table2: Percentage cell death

Extracts	Percentage cell death				
	10 µg/ml	20 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
Petroleum ether extract	0	6	11	22	53
N-Hexane	24	40	46	70	82
Chloroform	11	40	48	70	100
Methanol	8	12	19	26	39
Water	0	0	0	8	10

DISCUSSION

The different Extracts of *Asclepias curassavica* were subjected to in- vitro cytotoxic activity by using Dalton's lymphoma ascites cells (DLA). The study was shown significant cytotoxic activity for all the extracts at different concentrations and more cytotoxic activity is shown by the chloroform extract at a concentration of 200µg/ml. However, further investigations using carcinoma cell line are necessary to isolate the active compounds responsible for activity.

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