

***In vitro* cytotoxicity of extracts and fractions from areial parts of *Calotropis procera* against CNS cancer cell line**

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(Accepted : February, 2009)

Calotropis procera (Ait.) R.Br., a popular medicinal species in Indian system of medicine, is widely used for the treatment of various diseases. In the present investigation the cytotoxicity of the three extracts and their fractions from aerial parts of *Calotropis procera* was evaluated against central nervous system (SNB-78) human cancer cell line. The results showed that hydro-alcoholic extract has more potential at three different concentrations of 10, 30 and 100µg/ml, among all the three extracts viz., alcoholic, hydro-alcoholic and aqueous extracts. On evaluation of the various fractions, n- butanol fraction of both hydro-alcoholic and alcoholic extracts had shown antiproliferative activity against human cancer cell line of central nervous system (SNB-78) at three different concentrations of 10 and 30µg/ml. Aqueous extract was found to be least active.

Key words : *Calotropis procera*, Aerial parts, Extracts, Fractions, Cytotoxicity, Human cancer cell line.

INTRODUCTION

Since medieval times, plants have been the source of medicines for the treatment of diseases. Regardless of the availability of a wealth of synthetic drugs, plants remain – even in the 21st century – an integral part of the health care in different countries, especially the developing ones. Developing countries have a rich flora of medicinal plants that can be potential sources of new drugs and new biologically active substances (Kirtikar and Basu, 1993). International efforts and co-operation is needed to exploit these vast sources and biological evaluation of these medicinal plants.

Calotropis procera (Ait.) R.Br. (*Asclepiadaceae*) is a shrub, reaching 15 feet height and found in various parts of India. The principle constituents are starch, mucilage, a bitter principle (mudar) and a small quantity of acrid resin. Mudar is an alternative, tonic and diaphoretic, and large doses emetic. It is said to have employed with the benefit in numerous obstinate cutaneous diseases, syphilitic affections, dysentery, diarrhea and chronic rheumatism (Robert and Henry, 2002). *C. procera* is known to contain cardio active glycoside calotropine which has shown an antitumor effect in vitriol on human epidermoid carcinoma cells of the rhinopharynx, it also acts as expectorant and diuretic (Khanzada *et al.*, 2008). The root extract of *C. procera* has been found to produce a strong cytotoxic effect on COLO 320 tumor cells (Smit *et al.*, 1995). The chloroform-soluble fraction of its roots, ethanolic extract

of its flowers and aqueous and organic extracts of its dried latex also exhibit a strong anti-inflammatory activity in animal model of acute and chronic inflammation (Arya and Kumar, 2005). This study aims to evaluate the cytotoxic potential of *Calotropis procera* against human colon cancer cell line.

MATERIALS AND METHODS

Collection of plant material:

Aerial parts of *Calotropis procera* was collected locally from Parmandal area of Jammu in the month of December and was authenticated at source by Dr. B.K. Kaphai taxonomist of the institute. A voucher specimen has been deposited at the herbarium of the Institute vide IIM collection No.17600, Acc. No. 194731.

Processing of plant material:

Dried powdered plant material of the *C. procera* was soaked in absolute alcohol and was extracted in Soxhlet extractor and then concentrated to dryness under reduced pressure. Similarly, hydro-alcoholic (1:1) extract was prepared by percolating another lot of dried powdered material, plant material with 50% of alcohol (in water) and process was repeated. For the preparation of aqueous extract, the dried powdered plant material was heated with distilled water on steam bath for 2 hours, the supernatant was decanted and filtered through celite powder and the process was repeated four times, pooled extract was concentrated on rotavapour and freeze dried.

For the bioassay test, samples were dissolved in Dimethyl sulphoxide (DMSO) and further diluted in culture media. No concentration of the DMSO used showed cytotoxicity.

Preparation of plant fractions:

The alcoholic extract was fractionated sequentially with n-hexane, chloroform, n-butanol and water. The dried alcoholic extract was macerated with n-hexane and combined solvent portion was evaporated under reduced pressure to yield hexane fraction. The residue was further macerated with chloroform and combined organic layer was evaporated to yield chloroform fraction. The residue obtained was dissolved in distilled water and partitioned between n-butanol and water. The organic layer was dried over anhydrous sodium sulfate and concentrated to yield n-butanol fraction. The aqueous part was concentrated to give aqueous fraction.

The hydro-alcoholic extract was fractionated sequentially with chloroform, n-butanol and water. The dried hydro-alcoholic extract was macerated with chloroform. The combined organic layer was evaporated under reduced pressure to yield chloroform fraction. The residue obtained was dissolved in distilled water and partitioned between n-butanol and water. The suspension was extracted with n-butanol. The combined n-butanol extract was evaporated to dryness under reduced pressure below 50°C. Aqueous fraction was filtered and freeze dried.

The aqueous extract fraction was fractionated sequentially with n-butanol and water. The aqueous extract was extracted with n-butanol and combined n-butanol extract was evaporated to dryness under reduced pressure. Aqueous fraction was filtered and freeze dried.

Media and cell line:

The human cancer cell line was obtained from National Center for Cell Science, Pune, India and maintained with Dulbecco's minimum extract medium (pH 7.4), supplemented with 10% fetal calf serum and containing 100 units/ml penicillin, 100 µg/ml streptomycin and 2 mM glutamine. Positive controls paclitaxel was prepared in Dimethyl sulfoxide (DMSO) and then diluted in gentamycin medium to obtain desired concentrations of 1×10^{-6} M.

Cytotoxic assay:

Cellular viability in the presence and absence of experimental agents was determined using the standard sulforhodamine-B (SRB) assay as described previously by Skehan *et al.* (1990). In brief, the stock solution (20 mg/ml) of the alcoholic, hydro-alcoholic and aqueous

extracts was prepared in dimethylsulfoxide (DMSO), dimethylsulfoxide – water (1:1) and hot water, respectively and were further diluted with growth medium (RPMI-1640/ DMEM with 2mM glutamine, pH 7.4, 10% fetal calf serum, 100 µg/ml streptomycin, and 100 U/ ml penicillin) to obtain desired concentration. The cells were grown in tissue culture flasks in growth medium at 37°C in an atmosphere of 5% CO₂ and 95 % relative humidity in a CO₂ incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in the growth medium. Cells with more than 97% viability (Trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 µl of cell suspension (10^5 to 2×10^5 cells/ml depending upon mass doubling time of cells) was transferred to a well of 96-well tissue culture plate. The cells were incubated for 24 hours. The test materials (100 µl) were then added to the wells and cells were further allowed to grow for another 48h. The cell growth was stopped by gently layering 50µl of 50% trichloroacetic acid. The plates were incubated at 4°C for an hour to fix the cells attached to the bottom of the wells. Liquids of all the wells were gently pipetted out and discarded. The plates were washed five times with distilled water and air-dried. Sulforhadamine B (100 µl, 0.4% in 1% acetic acid) was added to each well and plates were incubated at room temperature for 30 min. The unbound SRB was quickly removed by washing the wells five times with 1% acetic acid. Plates were air dried, tris-HCl buffer (100 µl, 0.01 M, pH 10.4) was added to all the wells, and plates were gently stirred for 5 min on a mechanical stirrer. The optical density was recorded on ELISA reader at 540 nm. Suitable blanks and positive controls were also included. Each test was done in triplicate. The value reported here in are mean of three experiments.

RESULTS AND DISCUSSION

Percentage yield of the three extracts from aerial parts of the *C. procera* had clearly shown that alcoholic extract had yielded greater percentage of extract followed by the aqueous and hydro-alcoholic extracts (Table 1). Further the cytotoxic effect of extracts and fractions from aerial part of *Calotropis procera* was evaluated against CNS cancer cell line via, 95% alcoholic, hydro-alcoholic

Table1 : Extractive value of aerial parts of *Calotropis procera*

Sr. No	Extracts	Percentage yield
1.	Alcoholic	11.5
2.	Hydro-alcoholic	11
3.	Aqueous	11.4

and aqueous extract at three different concentration of 10, 30 and 100µg/ml. Growth inhibition was observed in a dose dependent manner in both the cell lines by all the extracts. Data expressed as mean ± S.E., unless otherwise indicated. The study indicates that among all the three extracts hydro-alcoholic extract showed high cytotoxicity followed by alcoholic and aqueous extracts. The percentage growth inhibition found to be 27, 67 and 73 of hydro-alcoholic extract, 45, 59 and 70 of alcoholic extract and 11, 32 and 56 of aqueous extract at 10, 30 and 100µg/ml (Fig. 1). Further to get some insight of the active constituents responsible for the cytotoxicity of this plant we have fractionated the extracts with different solvent of increasing polarity. Four fractions of alcoholic

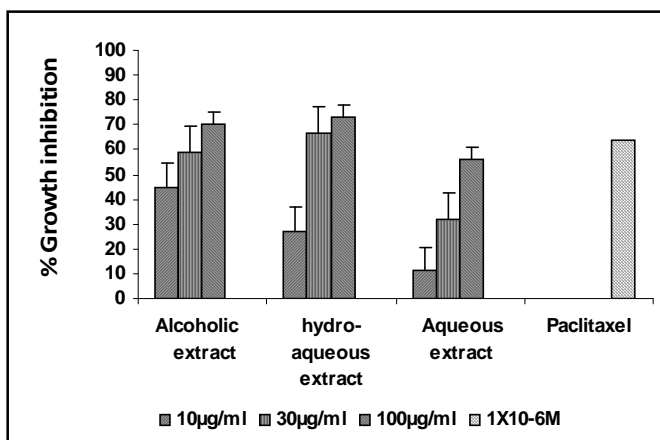


Fig.1 : *In vitro* cytotoxicity of extracts from aerial part of *Calotropis procera* against central nervous system (SNB-78) human cancer cell line. Data are mean±S.E. (n = 4-wells) and representative of three similar experiments

extract were prepared and per cent growth inhibition was found to be highest for n-butanol fraction (29, 65 and 76) followed by aqueous fraction (33, 60 and 66) at three different concentrations of 10, 30 and 100µg/ml (Fig.2). Chloroform fraction and n-hexane fraction had showed less than 35 per cent growth inhibition. Similarly three fractions of hydro-alcoholic extract were prepared. Out of these three fractions, n-butanol fraction showed 63, 70 and 71 and chloroform fraction showed 58, 66 and 69 per cent growth inhibition at 10, 30 and 100µg/ml (Fig.3) whereas aqueous fraction showed less than 26 per cent growth inhibition. Likewise two more fractions of aqueous extract were also prepared but it showed almost negligible growth inhibition and were considered inactive (data not included).

Earlier studies showed that this plant is responsible for various pharmacological activities, viz., the alcoholic extract of the plant possesses antimicrobial and

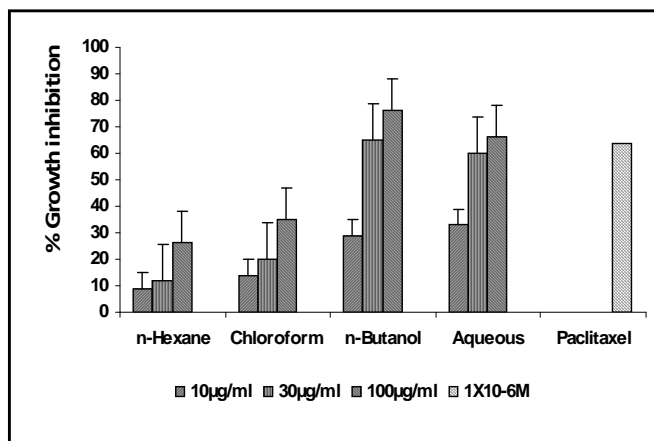


Fig. 2: *In vitro* cytotoxicity of fractions (alcoholic extract) from aerial part of *Calotropis procera* against central nervous system (SNB-78) human cancer cell line. Data are mean±S.E. (n = 4-wells) and representative of three similar experiments

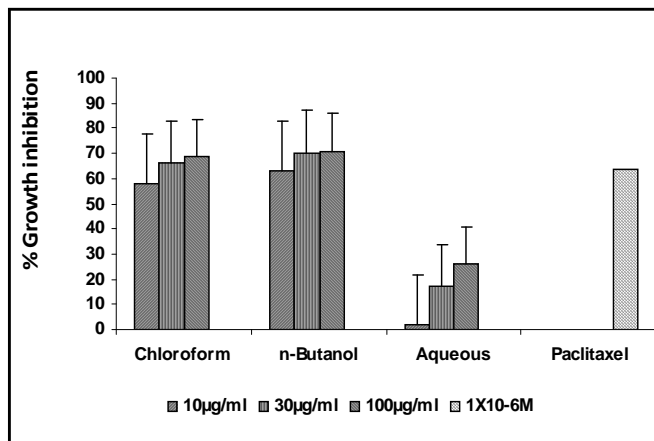


Fig. 3: *In vitro* cytotoxicity of fractions (hydro-alcoholic extract) from aerial part of *Calotropis procera* against central nervous system (SNB-78) human cancer cell line. Data are mean±S.E. (n = 4-wells) and representative of three similar experiments

spermicidal activity (Kamath and Rana, 2002), latex showed potent anti-inflammatory, antioxidant and cytotoxic properties (Choedon *et al.*, 2006). The phytochemical analysis of the plant confirmed the presence of compounds like cardenolides, cardio glycosides from latex and leaves, anthocyanins from flowers others are hydrocarbons, calotropin etc (Ahmed *et al.*, 2005, Parotta 2001), which can be responsible for the cytotoxicity of the CNS cancer cell lines. Thus, it is concluded from present studies that hydro-alcoholic extract and alcoholic extract both possess strong potential for cytotoxicity and n-butanol fraction of both the hydro-alcoholic and alcoholic extract had shown more potential for growth inhibition against CNS cancer cell line. Therefore, constituents responsible for the

cytotoxic effect has medium polarity and lies in the n-butanol fraction of the plant. However, it is important to point out the n-butanol fraction of the plant needs to be further processed to determine the active constituents having anticancer potential.

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