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PHYTOCHEMICAL SCREENING AND ANTICANCER ACTIVITY OF CALOTROPIS GIGANTEA (L.) DRYAND. LEAF EXTRACT

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ABSTRACT

The preliminary phytochemical screening and cytotoxic activity of Calotropis gigantea (L.) Dryand. leaf extract on breast cancer cell lines MCF7 were investigated. The crude extract of C. gigantea leaves was prepared using ethyl acetate solvents. The plant extract was subjected to the phytochemical test; and the cytotoxic activity was tested using MTT assay. The phytochemical screening of C. gigantea leaves showed the presence of carbohydrates, proteins and secondary metabolites such as alkaloids, glycosides, tannins compounds etc. The IC_{50} value with effective anticancer activity for ethyl acetate extract was found to be 84.45 µg/ml. The findings suggest that the chemical compound present in the leaf of C. gigantea could further be exploited as effective anticancer agent.

Keywords : Calotropis gigantea, Cytotoxic activity, Phytochemical screening.

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I. INTRODUCTION

Cancer is a group of diseases and remains as a leading cause of death globally. Despite the treatment available for this disease either by surgery, chemotherapy or radiotherapy, the outcomes are limited. And finding a successful treatment strategy is still a major challenge in medical science. Although, several anticancer compounds have been discovered from various sources, they are largely ineffective due to development of resistance, lack of specificity, complex pharmacokinetics. Further, the treatment with targeted chemotherapy which is used to halt the growing tumour, also affects the normal cells (El-Naggar **et al**.)[1]. Therefore, research is being directed towards the search for new, effective chemical entities with less/no toxicity for the safe treatment of cancer.

Plants have been used for the synthesis of drugs due to their therapeutic properties. The medicinal plants contain some bioactive compounds and serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine in pharmaceuticals. Plant derived products also have a long history of usage for the treatment of cancer and are excellent sources for the discovery and development of new cancer chemotherapies (Mustafa **et al.**; Rahman **et al.**)[2] [3]. Hence, the systematic screening and regular study of various plant species with a view to identify or discover new bioactive compounds is worth praising in this regard.

Calotropis gigantea (L.) Dryand. usually known as giant milkweed or crown flower is a lactiferous weed plant which commonly occurs on wasteland. The plant species belong to the family Apocynaceae which includes latex bearing plants. It is grown widely throughout the tropical and subtropical regions of Asia and Africa. *C. gigantea* has many medicinal properties in the traditional medicinal system like Unani and Ayurveda which is used for the treatment of different kind of diseases (Mushir **et al.**) [4]. The plant is reported to have many pharmacological activities like antioxidant, analgesic, hepato-protective, anti-pyretic, pregnancy interceptive, purgative, pro-coagulant, wound healing and insecticidal activities (Sarkar **et al**.)[5]. In this study,

an effort has been made to investigate the preliminary phytochemical screening and *in vitro* anticancer activity using ethyl acetate as solvent on leaf extracts of the target plant *C. gigantea*.

II. MATERIALS AND METHODS

Plant collection, and sample preparation : The leaves of *C.gigantea* (L.) Dryand. were collected from the university campus of North Orissa University, Odisha, India. The leaves were washed under running tape water and shade-dried for ten days (Fig-1). The dried plant material was ground into powder (Fig-2) and extracted with ethyl acetate affording a crude organic extract.

Fig.-1 Shade drying of *Calotropis gigantea* (L.) Dryand. leaves

Fig.-2 Powdering of *Calotropis gigantea* (L.) Dryand. leaves

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Preliminary phytochemical analysis : The preliminary chemical tests were carried out for alkaloids, amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins, steroids and triterpenoids, The protocol was in accordance with Khandelwal [6] and Kokate [7] with modifications wherever required.

MTT assay : The MCF7 breast cancer cell lines were purchased from National Centre for Cell Science (NCCS), Pune and maintained in Dulbecco's modified Eagles medium (MEM) supplemented with 10 % EDTA Phosphate Buffered Saline (FBS) and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2 /95% air at 37 °C. Cell viability was evaluated by the 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay following Venkanna **et al**. [8] with three independent experiments with five concentrations of compounds in triplicates. Cells were trypsinized and the trypan blue assay was performed to know viable cells in cell suspension. Cells were counted byhaemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µl media in a 96 well plate culture medium and incubated overnight at 37° C. After incubation, the old was taken off and fresh media was added with 100 µl of different concentrations of test extract (5 µg, 10 µg, 25 µg, 50 µg and 100 µg) in their respective wells. After 48 hrs, the drug solution was discarded and the fresh media with MTT solution (0.5 mg / mL-1) was added to each well, and plates were incubated at 37°C for 3 hrs. At the end of incubation time, precipitates were formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in Dimethyl Sulfoxide (DMSO) was measured at 570nm on a microplate reader. The percentage growth inhibition was calculated using the formula, % Inhibition = 100 (Control-Treatment)/Control. The IC_{50} value was determined by using linear regression equation Y = Mx + C.Y = 50, M and C values were derived from the viability graph.

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III. RESULTS AND DISCUSSION

Natural products from plants have played an important role in the treatment of cancer and in reality, most new clinical applications of secondary metabolites and their derivatives over the century have been developed as anticancer agents (Mazumder **et al**.)[9]. Hence, the present study was undertaken to evaluate anticancer potential and to investigate the phytochemical analysis of *C. gigantea* (L.) Dryand. leaves (Fig-3).The preliminary phytochemical screening of the leaf powder was carried out by solvent extraction procedure using ethyl acetate as organic solvent.

Fig.-3 *Calotropis gigantea* (L.) Dryand.

The resultant organic extract when subjected to various qualitative phytochemical analysis using some generalized and standardized methods showed the presence of carbohydrates, proteins, certain amino acids and secondary metabolites such as alkaloids, tannins (gallotannins and ellagitannins), cardiac glycosides etc (Table-1).

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Table-1. Results of Preliminary Phytochemical Investigation of *Calotropis gigantea* leaf extracts.

S. No.	Name of the Test	RESULT
	Test for Proteins	
1.	Trichloroacetic Acid Test	POSITIVE
2.	Xanthoprotein Test	POSITIVE
3.	Biuret Test	POSITIVE
	Test for Alkaloids	
1.	Dragendroff's Test	POSITIVE
2.	Tannicacid Test	POSITIVE
	Test for Amino Acids	
1.	Millons Test	NEGATIVE
2.	Ninhydrin Test	POSITIVE
	Test for Carbohydrates	
1.	Molisch's Test	POSITIVE
2.	Barfoed's Test	NEGATIVE
3.	Seliwinoff's test	NEGATIVE
4.	Test for Pentoses	NEGATIVE
	Test for Flavonoids	
1.	Shinoda Test	NEGATIVE
2.	Alkaline reagent Test	NEGATIVE
3.	Zinc hydrochloride test	NEGATIVE
	Test for Phenolic Compounds	
1.	Ferric chloridetest	NEGATIVE
	Specific chemical test for Tannins	
1.	Test for Gallotannins	POSITIVE
2.	Test for Ellagitannins	POSITIVE
	Test for Steroids & Triterpenoids	
1.	Libermann-Burchard Test	NEGATIVE
2.	Salkowaski test	NEGATIVE
3.	Sulfur powder test	NEGATIVE
	General Test for Glycosides	
1.	Test A & B	POSITIVE
	Specific Chemical test for Glycosides	
	Cardiac Glycosides	
1.	Legal's test	POSITIVE
2.	Baljet's test	POSITIVE

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Sapon in Glycosides		
1.	Froth formation Test	NEGATIVE
Anthraquinone Glycosides		
1.	Borntrager's test	NEGATIVE
2.	Test for Saponins	NEGATIVE
3.	Test for Anthocyanins	NEGATIVE

A study carried out on the phytochemical analysis of the target plant by Singh **et al.** [10] and Kori and Alawa [11] have also reported the presence of alkaloids, glycosides and tannins in the root and latex extracts. The leaves extract was also determined for the anticancer property. Different concentration of the crude metabolites starting from 10 – 100 µg/mL was used against the human breast cancer cell line MCF7 and IC_{50}values were calculated. The results indicated that the ethyl acetate derived organic extract exhibited considerable anticancer activity against MCF7 cell line (Fig.-4) at a calculated IC_{50} of 84.45.

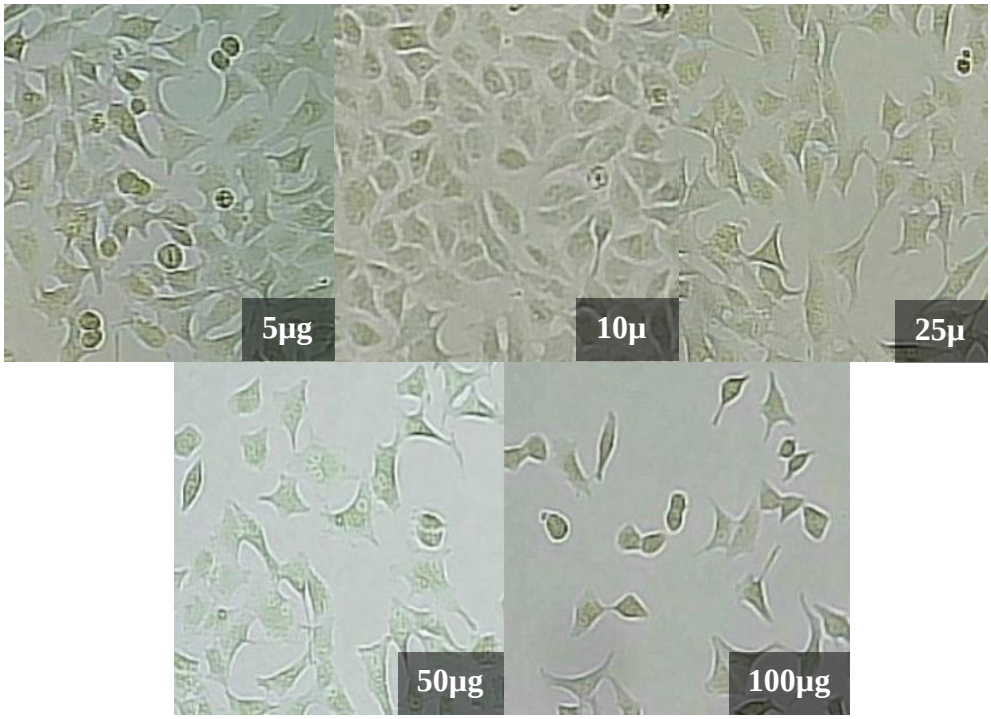

Fig.-4 Anticancer activity against MCF7 breast cancer cell line.

Alkaloids have a wide distribution in the plant kingdom especially in higher plants and are an important class of chemical compounds that serve as a rich reservoir for drug discovery. Several alkaloids isolated from natural herbs exhibit anti-proliferative and anti-metastatic effects on several cancer types both *in vitro* and *in vivo* (Lu **et al.**; Isah)[12][13] . Similarly, cardiac glycosides are also known to exhibit significant anticancer properties that influence the immune response at multiple levels (Kepp **et al.**)[14]. Besides alkaloids and cardiac glycosides, tannins are also widely distributed in plant flora and are found in root, bark, stem and outer layers of plant tissues. Several studies have proven the anticancer activities of gallotannins and ellagitannins against human breast cancer and colon cancer cells (Yildirim and Kutlu; Gonzalez-Sarria **et al.**; Catalan **et al.**)[15] [16] [17]. Therefore, it may be concluded that anticancer activity of the extract might be due to the presence of some ethyl acetate soluble chemical entities belonging to alkaloids, cardiac glycosides and tannins in *C.gigantea* (L.) Dryand. leaves. However, a more detailed study on the purification and chemical characterization of the chemical constituents is highly needed for a better understanding of biological properties and mechanistic action.

IV. CONCLUSION

The phytochemical screening and anticancer activity of *Calotropis gigantea* (L.) Dryand. leaf extract reveals that the biological activity of the extract is due to the presence of alkaloids, glycosides and tannins in the leaves of target plant. Furthermore, the leaves could better be exploited as antimicrobial and anticancer agents for pharmaceutical applications.

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