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Anticancer Potential of *Calotropisprocera*: An Overview

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ABSTRACT

Medicinal plants have incredible importance because of the presence of many types of components present in it, and they become the source for the synthesis of active pharmaceutical ingredients (API). *Calotropis procera* is a xerophytic shrub, with soft, tall; numerous shrubs with small flowering branches or small trees belonging to the family of Asclepiadaceae, distributed globally over the tropical and subtropical region of Asia and Africa. It is well known in traditional medicine to have cleansing and anti-ageing properties as well as used in the mitigation of various ailments as antiulcer, anti-leprotic, cytotoxic, anti-emetic, anti-coagulant and anticancer activities. It contains various chemical constituents like cardiac glycoside, triterpens, flavanoids, steroids and other phenolic components in various parts of plant was well known for its diverse pharmacological and biological activities. Different extracts of plant and parts of plants possess significant *in-vitro* and *in-vivo* cytotoxic, anti-tumour and anti-proliferative activity in various cell lines and tumor cells which indicate the considerable potential of *Calotropis procera* for anticancer adjutants. The objective of the presentr eview is to provide systematic information about the phytochemical and various extracts of *Calotropis procera* as potential candidate for cancer treatment.

Keywords: *Calotropis procera,* anti-proliferative, cytotoxic, anti-tumour, flavanoids, terpens, cardinolides, steroids, phytochemicals.

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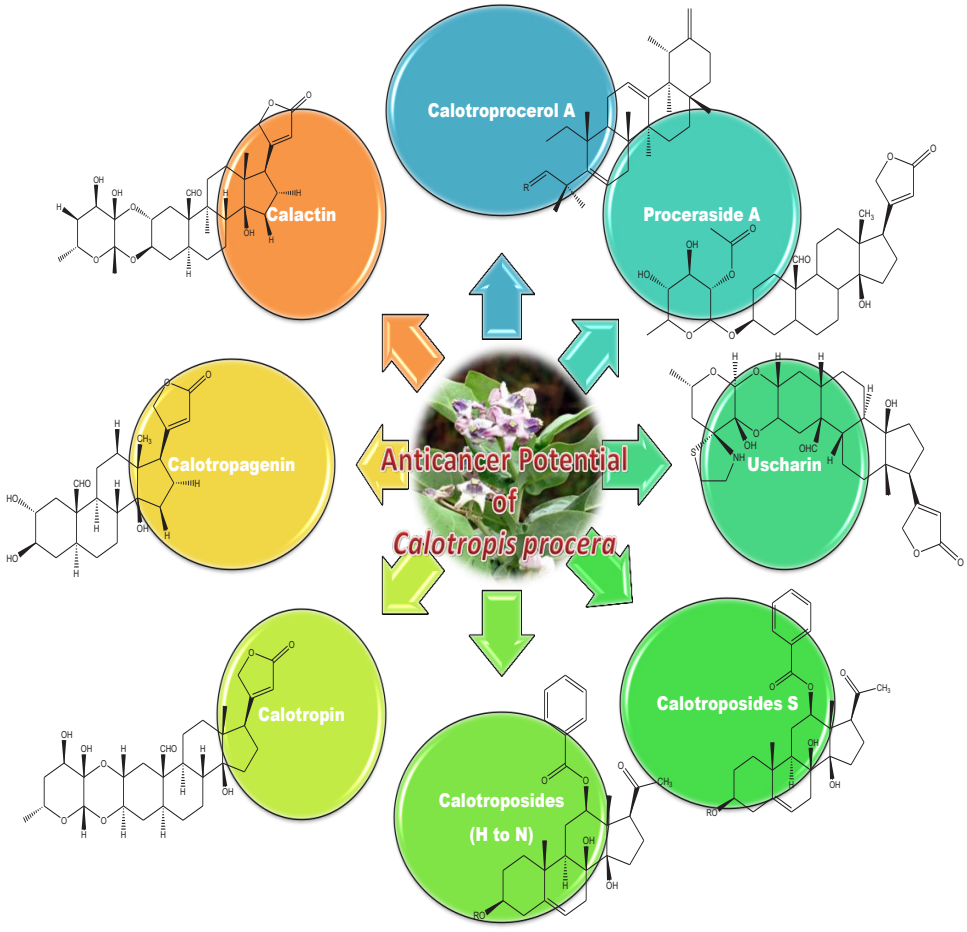
GRAPHICAL ABSTRACT



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INTRODUCTION

According to Indian traditional system, plants are the primary source of ailments for the treatments of various diseases in human and animals. The use of medicinal plants is widespread during the periods of ancient and prehistoric times for the treatment and cure of different diseases.[1] Our nature provided sufficient resources for the living world in which the medicinal plants are one of wealth to take care of the health of living creature.[2] Among the various medicinal plants, *Calotropis procera* is one of the medicinal plant having different therapeutic activities belong to the family of Asclepiadaceae. Genus *calotropis* globally distributed over the tropical and subtropical region of Asia and Africa.[3] It is found abundantly in Rajasthan and distributed in all areas of India. *Calotropis procera* is an evergreen shrub growing wildly in warm climates up to 1050 meter altitudes and Commonly Known as "Akra" or "Milkweed". Taxonomic classification is given in Table 1.The whole plant of *Calotropis procera* including leaves; roots, stem, latex and flower were used in indigenous system of medicine.[4]

Geographic Illustration: The seeds of *Calotropis procera* are dispersed through air and animals. It well tolerates the dry condition. It begins to be introduced as weeds on damaged roads, debris and overgrown traditional pastures.[5-6] It is favored and is most common in abandoned areas, especially sandy soils in areas with low rainfall, thought to be an indication of over-cultivation. *Calotropis procera* is native to India, Pakistan, Nepal, Afghanistan, Algeria, Iran, Iraq, Israel, Kuwait, Nigeria, Oman, Saudi Arabia, United Arab Emirates, Vietnam, Yemen, Zimbabwe, Africa, Mexico, Australia, Egypt, Central and South America and the Caribbean islands.[7-8]

Botanical Illustration: *Calotropis procera* is a xerophytic shrub, with tender, tall, numerous shrubs with small flowering branches or small trees that grow up to 5.4 m tall.[9-10] The bark is tender and corky with strong branches, terete with beautiful cottony pubescence (especially for young ones). Its leaves are small, rectangular, curved, wide ovate-oblong, elliptic or obovate, large, large, shiny, green lined with a hole in the most delicate cottony hair.[11] Flowers are umbellate-cymes and slightly tomentose, calyx glabrous, ovate and acute. Corolla glabrous, straight lobes, ovate, acute, coronal scales 5 - 6, later compressed and even skipped over the staminal column. Fliclicles are sub-globose or ellipsoid or ovoid. The seeds are vast ovate, acute, flattened, tomentose, brown and silky 3.2 cm long.[12] Details of different plant parts are given in Table 2.

Table 1 Taxonomic Classification

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Order	Gentianales
Family	Asclepiadaceae
Genus	*Calotropis*
Species	*Procera*

Table 2 Characteristics of plant parts of *Calotropis procera*

S.No.	Plant Parts	Description
1	Bark and branches	Thick, rough, corky and yellow-brown Twigs are fleshy with green in color
2	Leaves	About 30 cm×25 cm in size with ovate to obovate, Oppositedecussate,
3	Flowers	White at the base and purple at the tips and five purple-tipped stamens, five thick ovate petals
4	Fruits	Green, spongy ovoid, up to 15 cm long by 10 cm wide
5	Inflorescence	Arise from the base of the leaves in pedunculate cymes of 3-20
6	Roots	Grayish white in color and exhibit sap exudations at the places where the bark has been cut
7	Root bark	Cracked, yellowish-grey from outside and yellowish-white inside. The dried bark is bitter
8	Corolla	Regular, gamopetalous, pale rose-purple or lilac, with a short tube and five broad ovate spreading lobes

IMPORTANCE OF PLANT

Significant benefits can be derived from *Calotropis procera* in many ways, as it is traditionally known in traditional medicine to have cleansing and ageing properties as well as used in the treatment of leprosy, ulcers, tumors and plaques and vomiting, anticoagulant and anticancer. In addition, plant latex was still known for its content like cardiac glycoside that act as insecticides.[13] Different therapeutic activities are mentioned in Figure 1. Its biomass has shown a tendency to be a good source of renewable energy and hydrocarbon. Finally, the *Calotropis procera* play a significant ecological role as a habitat for several species, reflecting the phyto remedy properties of the polluted soil and their ability to regenerate abandoned land and restore health.[14-15]

According to various study performed on *Calotropis procera*, it have anti-inflammatory,[16] anti-diabetic,[17] anti-pyretic,[18-19] cytotoxic, anti-cancer,

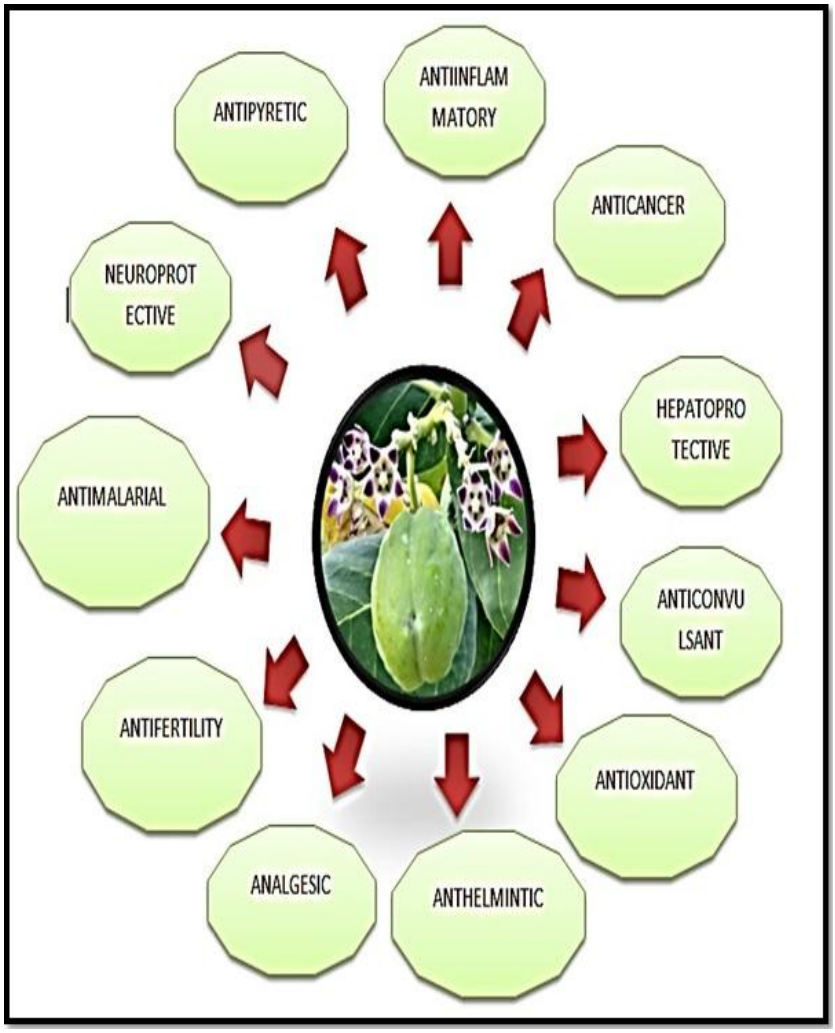


Figure 1 IMPORTANCE OF PLANT

anti-tumor,[20-22] and antioxidant[23] activities. In addition to these activities it also possess anti-arthritic,[24] analgesic activity,[25] anti-convulsant effect,[26] anti-microbial,[27] anti-fertility activity,[28] anthelmintic activity,[29] hepatoprotective activity,[30] anti-diarrhoeal activity,[31] anti-malarial,[32] neuroprotective[33] and spasmolytic activity.[34]

Calotropis procera is also used by various nations of land as an agent for the treatment of diseases such as skin disease, elephantiasis, teeth, asthma, leprosy, and rheumatism.[35] Various parts such as leaves, roots and bark, flower, fruit, stem, it is reported that there is plant latex with a variety of plants phyto chemicals can have a variety of medicinal properties. This plant is described as a gift to mankind due to presence of cardiotonic agents used for medical treatment.[36] Various other compounds such as norditerpenic esters, organic carbonates, cysteine protease procerain, alkaloids, flavonoids, sterols, and many cardenolides make this plant a science attractive for centuries. It is a potent source of *in vivo* antitumor activity in the form of paclitaxel and oxaliplatin-resistant subcutaneous human NCIH727 and orthotopic A549 xenografts in naked mice.[37]

ANTICANCER POTENTIAL OF *CALOTROPIS PROCERA*

Malignant growth has been perceived for a large number of years as a human infection; however, just in the only remaining century did clinical science comprehend the simple idea of disease and how it advanced. Malignancy starts when the cell is switched here and there to recover control. A tumor is a mass shaped by a bunch of such strange cells. Most malignant growths structure tumors, however, not all tumors have a disease. Generous, or broken, tumors don't spread to different pieces of the body, nor do they grow new tumors. Threatening, or destructive, tumors emit sound cells, disturb body works, and drain the body's invulnerable framework.[38] The disease keeps on developing and spreads through an immediate expansion or a cycle called metastasis, in which the threatening cells travel through the veins or arteries, in the end, shaping new blood vessels in different pieces of the body.

According to studies performed earlier, it was seen that *Calotropis procera* possess cytotoxic and antitumor activity. The various chemical constituents of plant extracts have been indicating their inhibitory effects on tumor and cancer cell lines. Majorly the roots and latex part of *Calotropis procera* shows anticancer potential due to the presence of calotropinol, proceragenin, proceragenin, calotoxin, hydroxyketone, procesterol, multiflorenol, cyclosadol, βsitostenone, uzarigenine, βanhydroepidigitoxigenin,pentacyclictriterpinoids.[39] The growing number of published articles covering the properties of this plant, we have felt the need to prepare a review article for it. The present review highlights over the anticancer potential reported in previously occurred studies on various extracts including aqueous, methanolic, ethanolic extracts obtained from different parts of *Calotropis procera,* phytomolecules present in various part of *Calotropis procera* and semi synthetic derivatives derived upon it.

ANTICANCER POTENTIAL OF PLANT EXTRACTS

Different investigations are accounted for in literature discussing the partition of concentrate including roots, flowers, leaf and latex part of *Calotropis procera* in water, alcohol and other solvents. Subtleties of the anticancer capability of the various concentrates are summed up beneath (Table 3).

Fabiano *et al.,* in 2011 extracted root and callus proteins (CP) form plant tissue culture method and

Table 3 **Various Extracts of *Calotropisprocera* having Anticancer Potential**

S.No.	Plant Part	Type of Extract	Methods	Cell Lines Used	Ref.
1.	Callus and Root	Protein	*In-vitro*&*in-vivo* (Anti-tumor activity by mice transplanted with Sarcoma 180 cells)	HL-60 MDA-MB-43 SF-295 HCT-8	Fabiano*et al.*2011
2	Stem	Ethanol	MTT Assay Anti-mitotic assay *In-vivo* (Sarcoma 180 ascites tumor cells)	HL-60 HCT-8 B-16/F10	Hemerson*et al.*2010
3	Leaves	Aqueous	Cytosensor	Gr/Nt-G/Ln-18	Seetharamaiah*et al.* 2016
4	Latex	Aqueous	Standard Assay Method Meristem Model	-	Sehgal*et al.* 2006
5	Root	Aqueous (Silver nanoparticles)	MTT Assay	HEPK	Sagadevan*et al.* 2020
6	Root	Methanol Hexane Aqueous Ethyl acetate	MTT Assay	Hep2	Mathur *et al.*2009
7	Whole Plant	Alcohol	SRB Assay	A-549 Hep2 Colon-502713 HT-29 IMR-32	Verma*et al.*2010
8	Root Bark	Protein	MTT Assay LDH Assay	MCF-7 MDA-MB-231	Samy *et al.*2012
9	Leaves	Ethanol (*n*-Butanol fractions)	SRB Assay	HEPG2	Murti*et al,* 2016
9	Latex	Proteins	*In-vivo* (Sarcoma 180 Ascite Tumor)	-	Oliveira *et al.*2010
10	Latex	Proteins	MTT Assay	HL-60 HCT-8 MDA-MB-435 SF295	De Oliveira *et al.* 2007
11	Latex	Methanol	MTT Assay *In-vivo* (Transgenic mouse model)	HUH-7 COS-1 AML12 X15-myc	Choedon*et al.*2006
12	Latex	Proteins	MTT Assay	SF295 MDA-MB-435	Oliveira *et al.* 2010
13	Flowers	Methanol	MTT Assay	Hep2 Vero	Prabha*et al.*2011
14	Leaves	Methanol	MTT Assay	T47D	Hassan*et al.*2019
15	Stem- Leaves	Ethanol	MTT Assay	HCT-15	Bhagat*et al.*2010

conducted *in vitro* and *in vivo* cytotoxicity assay on HL-60 (lukemia), MDA-MB-43 (melenoma), SF-295 (brain) and HCT-8 (colon) cell lines. CP did not show cytotoxicity or anticancer activity, and it is therefore suggested that laticifer proteins that are involved in cytotoxicity and anticancer functions are not expressed in callus. However, this may not deliver on the root protein (RP) because the root tissue was shown to form laticifer tubes. Lack of cytotoxicity and anticancer activity in RP may be caused by a series of concerns including low accumulation of cytotoxic proteins and anticancer; laticifers in selected roots can be synthesized physically but have been implanted with biochemical and physical terms and thus anti-inflammatory proteins and ant-noniceptive are available while cytotoxic

and anticancer proteins are not available. In addition, CP and RP were non-cytotoxic at 25µg/mL, whereas laticifer proteins (LP) were active at doses less than 2µg/mL.[40]

Hemerson *et al.,* in 2010 separated the stem extracts of *Calotropis procera* in hexane, dichloromethane, ethyl acetate, acetone and methanol and tasted their cytotoxic potential against HL-60, CEM (human leukemia), HCT-8 (human colon cancer) and B-16/F10 (murine melanoma) cell lines by MTT assay and it was observed that ethyl acetate and acetone extracts showed high cytotoxic potential against tumor cells, with IC_{50} from 0.8 to 4.4µg/mL, while methanolic extract show week cytotoxic effect on cell lines. The extract produced cytotoxic effect was analysed for antimitotic activity on sea urchin egg development and *in-vivo* anti-proliferative activity in mice with Sarcoma 180 tumor. The anti-mitotic assay, exposing IC_{50} values below 5µg/mL and *in-vivo* antitumor experiments of ethyl acetate- and acetone-treated animals showed inhibition of tumor growth, 64.3 and 53.1%, respectively. The anti-proliferative activity produced by affecting, DNA and protein synthesis in Sarcoma 180 tumor cell, with making adverse effects on the kidney and liver.[41]

Seetharamaiah *et al.,* in 2016, separated aqueous leaves extract of *Calotropis procera* with soxhlet assisted extraction (SAE) and screened for anticancer effects by electrochemical methods against glioblastoma cell lines (LN-18). The extract analyzed for phytochemical screening indicates the presence of polyphenol contents by using FolinCiocalteau method. Anticancer effects of soxhlet assisted polyphenol extract (SAPE), evaluated by electrochemical characteristics using cytosenser (Gr/NT-G/LN-18) by voltammetry and differential pulse voltammetory. The DNA binding capacity of sensor studied with graplite/poly(allylamine hydrochloride)/nanotube-graphene composite/polypyrrole/deoxy ribonucleic acid (Gr/PAH/NT-G/PPy/DNA) modified electrode. The physical characteristics studied of (Gr/PAH/NT-G/PPy/DNA) modified electrode employed using EDAX and SEM reveals the ability of SAPE against glioblastoma cell lines (LN-18).[42]

Sehgal *et al.,* in 2006, performed cytotoxity and anti-mitotic activity of latex obtained from *Calotropis procera* (DL), podophyllotoxin and cyclophosphamide as standard, whereas cyproheptadine and aspirin as a control on *Allium cepa* root tip meristem model. Significant inhibition of roots growth and mitotic activity with dose dependent manner like cyclophosphamide, was observed in DL and podophyllotoxin, whereas podophyllotoxin more potent in root decay. On other side, cyproheptadine and aspirin showed mitotic activity at mach higher concentrations with marginal root growth.[43]

Sagadevan *et al.,* in 2020, separated aqueous roots extract of *Calotropis procera* with deionized water and prepared (phyto-febricted AgNPs) silver nanoparticles of extract by using typical synthesis protocol. Characterize phyto-febricted AgNPs by UV Spectroscopy, Zeta seizer, EXD and FTIR. The therapeutic efficacy phyto-febricted AgNPs against clinical pathogens was significant according to data of zone of inhibition, MIC and MBC and the cytotoxicy determined by conducting MTT assay against HEPK cell lines was not satisfactory even at high concentration of 100µg.[44]

Mathur *et al.,* in 2009,segregated methanolic extracts (CM), hexane extract (CH), aqueous extract (CW) and ethylacetate extract (CE) of C*alotropis procera* roots and investigated their cytotoxic activity on Hep2 cell lines. The morphological changes of cancer cells, observed by inverted microscope and cell parameters determined by using propidium iodide staining followed flow cytometry. Effect of different extracts at dose of 1, 5, 10 and 25µg/mL on Hep2 cell lines indicated ME, ME and CE extract possess cytotoxic effect except CW. The CE showed potent 96.3% cytotoxic activity at 10 µg/mL whereas CM and CH showed 72.7 and 60.5% cytotoxic activity on Hep2 cell lines. Treated cells exhibited typical morphological changes of apoptosis by disrupting cell cycle by arresting S phase and preventing cells from entering G2/M phase.[45]

Verma*et al.,* in 2010, separated alcoholic extract of whole plant of *Calotropis procera* and performed *in-vitro* cytotoxity assay by sulforhodamine B dye (SRB) against the human lung (A-549), liver (Hep2), colon (502613, HT-29) and neuroblastima (IMR-32) cell lines. Extract of *Calotropis procera* plant showed more than 70% growth inhibition in cell lines accept colon cell lines.[46]

Samy *et al.,* in 2012, separated protein form clear supernatant of *Calotropis procera* root bark extract by gel-filtration chromatography using Superdex G-75 column and investigated there *in-vitro* and *in-vivo* antitumor activities for *Calotropis procera* (CP-P) protein. CP-P proteins inhibit proliferation and induced apoptosis of breast cancer cells by suppressing kappa-B (NF-kB) activation. CP-P, inhibited tumor volume in 7,12-dimethyl benz(a) anthracene (DMBA)induced breast cancer, when administered individually or in combination with cyclophosphamide (CYC, 0.2 mg/kg) to rats without interfering body weight. The SOD, CAT, GST, GSH, Vitamin E and C levels were shown higher in the co-administered groups (CP-P + CYC) compared to the CYC-managed groups alone.

Also, the compound was very effective in regulating the expression of NF-kB-regulated gene products (cyclin D1 and Bcl-2) in breast tissue and found that, CP-P has significant antitumor activity compared to the widely used anticancer drug, cyclophosphamide, and may be the basis for novel cancer treatment for breast cancer.[47]

Murti *et al.,*in 2016, extracted ethanolic extractby macerating leaves of *Calotropis procera* for seven days. The dried residue partitioned with *n*-butanol and subjected to column chromatography to obtained various fraction of *n*-butanol. These fractions and ethanolic extract were tested against the HEPG2 cell lines for their anticancer activity using SRB assay. The result indicates that chloroform:methanol (9:1) elute showed maximum anticancer potential.[48]

Oliveira *et al.,* in 2010, isolated latex protein by centrifugation and performed *in-vivo* study on mice transplanted with sarcoma 180 which shows that latex protein inhibited 51.83% maturation of malignant cell and induces the endurance time of animals by 4 days and also conducted the various biochemical, hematological, histopathological and morphological studies in animal groups given oral and intra-peritonial dose of latex protein. It was found that, latex protein lost its tumor growth inhibition activity upon proteolysis, acid treatment and pretreatment with iodoacetamide. However retains activity upon heat treatment.[49]

De Oliveira *et al.,* in 2007 isolated laticifer proteins (LP) from latex of *Calotropis procera* by centrifugation method and performed cytotoxicity assay on various cell lines including HL-60, HCT-8, SF295 and MDA-MB-435 were treated with laticifer proteins and analyzed after 72 h by MTT assay. Result showed apparent cytotoxicity with IC_{50} values ranging from 0.42 to 1.36 µg/mL,SF-295 and MDA-MB-435 cell lines. In healthy mononuclear cells from LP (10µg/mL) 72 h, no significant effects on functional or cell morphology were observed. The separation of latex protein into ion exchange chromatography opened up a new component (PI-protein unbound) that retains almost all cytotoxicity. The cytotoxic effects of latex protein and PI were reduced when previously treated with expression, or 2-mercaptoethanol, elevating the natural state of active protein molecules; however, pre-incubation with dithiothreitol (DTT) reduces the activity of PI only.PI did not show the activity of cysteine proteinase, indicating that cysteine proteins, found in latex protein, are not affected by latex protein cytotoxicity. In this study, using the HL-60 cell as a model, LP was shown to inhibit DNA formation. This is probably due to changes in DNA topology, because it was observed that LP is able to disrupt the activity of topoisomerase-I somehow adhere to DNA. LP triggered a decrease in cell numbers but did not cause a significant increase in the number of inactive cells. These findings are combined with morphologic analysis, in which LP-treated cells exhibit morphology of apoptotic process with multiple gaps, chromatin inflammation and nuclei fragmentation. The results of this study suggested that LP is a target of DNA topoisomerase-I triggers apoptosis in cancer cell lines.[50]

Choedon *et al.,* in 2006 separated the petroleum ether and methanol fraction of dried latex in a sequential order and evaluated anticancer activity of hepatocellular carcinoma in the X15-myc transgenic mouse model. Dried latex showed complete protection against hepato-carcinogenesis without producing any adverse effect on mice. Serum VEGF levels are significantly reduced in mice compare to control group of animals. MTT assay showed that the methanolic fraction of dried latex as well as its 8^{th} fraction caused high cell death in both Huh-7 and COS-1 cells while AML12 cells were rescued. This was accompanied by a major division of DNA into Huh-7 and COS-1 cells and thereis no significant changes were seen in levels of Bcl2 and caspase 3 canonical markers of apoptosis.[51]

Oliveira *et al.,* in 2010 performed cytotoxicity of lacticifier protein obtained from latex of *Calotropis procera* by MTT assay among the SF295 and MDA-MB-435 cell lines. It produced IC_{50} of 0.42-1.36µg/mL to SF-295 and MDA-MB-435 without producing noticeable change in their morphology and cell viability of healthy mononuclear cells upto 72 h at 10 g/mL.[52]

Prabha *et al.,*in 2011 performed MTT assay on Hep2 and Vero cells to find out the cytotoxic activity and radical scavenging activity with ferric thiocyanide, hydrogen peroxide and DPPH assay from methanolic extract of *Calotropis procera* flowers. The result revealed 100% cytotoxic potential against Hep2 cell lines. The presence of hydroxyl group in phenolic compounds present in flower extract is essential for radical scavenging activity.[53]

ANTICANCER POTENTIAL OF PHYTO-CONTITUENTS

Calotropisprocera have many types of phyto-constituents as their active chemical moiety to produced cytotoxic effects on various cancer cell lines. It possesses various groupsof chemicals like Cardiacglycosides, Terpines, flavanoidsetcgiven in Table 4.The various components of the plant used to treat various ailments in traditional medicine, and their result have been scientifically based, reports of antimicrobial activity have been proven by experiments with the molecules involved in these studies.

Table 4 Various Phytochemicals of *Calotropis procera* having Anticancer Potential

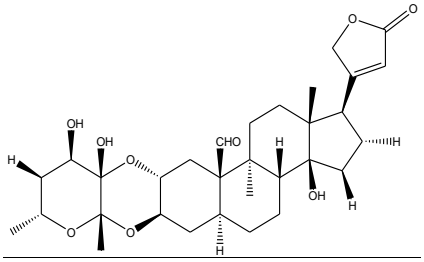
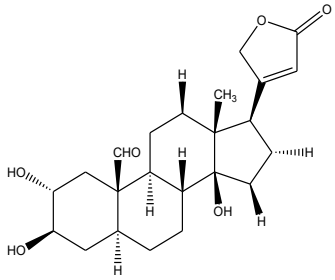
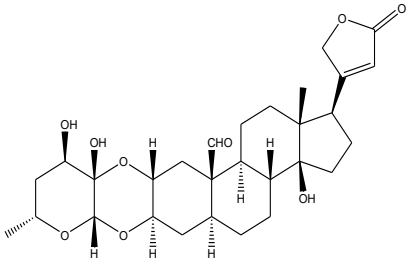
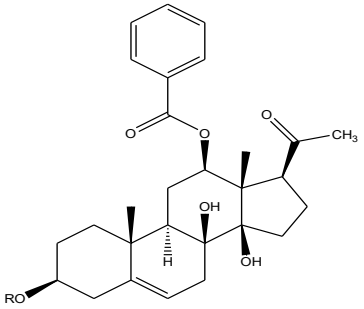
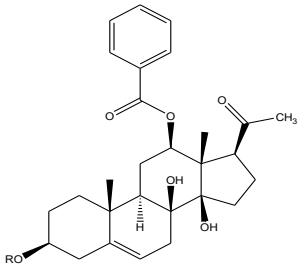
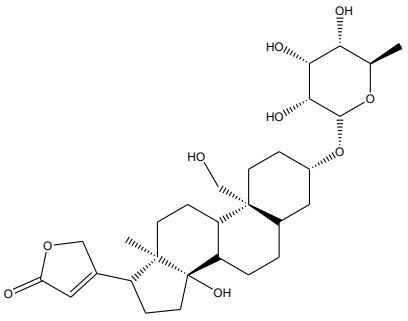
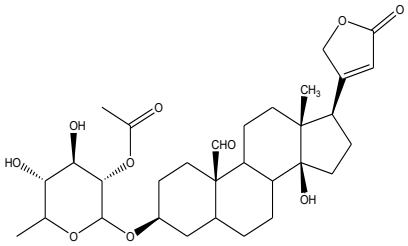
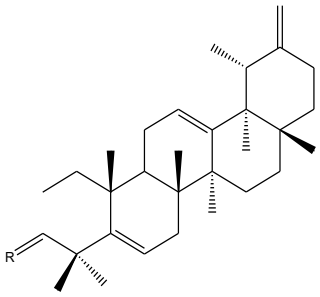
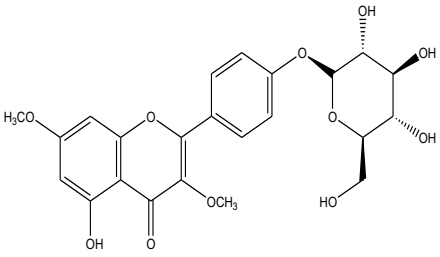
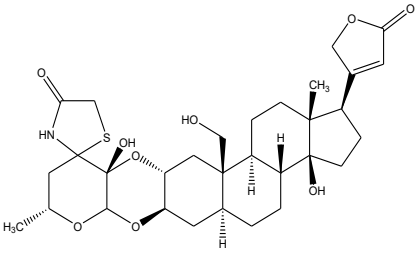
S. No.	Phytochemicals (Class)	Plant part (Extract)	Chemical Structure	Methods	Cell line	Ref.
1	Calactin (Cardinolides)	Latex (Chloroform)		MTT Assay	MCF-7	Parhira *et al.* 2016
2	Calotropagenin (Cardinolide)	Leaves (Choroform and butanol fraction)		MTT Assay	HepG-2 A-549 MCF-7 HCT-116	Kanojiya *et al.* 2012
3	Calotropin (Cardinolide)	Root Bark (Methanol)		MTT Assay	K562	Ibrahim *et al.2014*
4	Calotroposides (H to N) (Oxypregnanes)	Root Bark (*n*-Butanol)	 H: R= Cym-Ole-Ole-Cym-Cym I: R= Ole-Ole-Ole-Cym-Cym J: R= 6-Ac-Glu-Cym-Ole-Ole-Cym-Cym K: R= Glu-Cym-Ole-Ole-Cym-Cym L: R= Ole-Ole-Cym-Glu-Glu-Cym M: R= 6-Ac-Glu-Ole-Ole-Ole-Ole-Cym-Cym N: R= Glu-Glu-Cym-Ole-Ole-Ole-Cym-Cym	MTT Assay	A549 U373 PC-3	Ibrahim *et al.* 2015
5	Calotroposides- S (Oxypregnanes)	Root Bark (*n*-Butanol)	 R= Ole-Ole-Ole-Cym- Ole-Ole-Cym-Cym	MTT Assay	PC3, A549, U373	Ibrahim *et al.* 2016

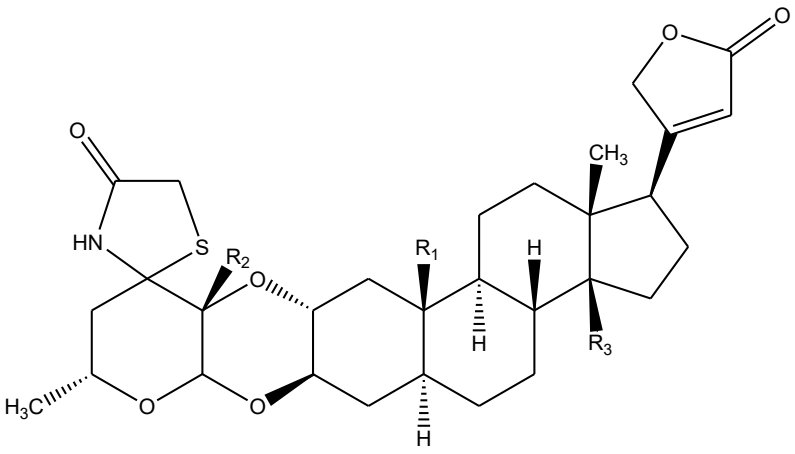
Table 4 Continue

S. No.	Phytochemicals (Class)	Plant part (Extract)	Chemical Structure	Methods	Cell line	Ref.
7	Frugoside (Cardinolide)	Root Bark (Methanol)		MTT Assay	A549, U373, PC-3	Ibrahim *et al.2014*
6	Proceraside A (Cardinolide)	Root Bark (Methanol)		MTT Assay	A549, U373, PC-3	Ibrahim *et al.2014*
7	Ursane (Triterpene) (Calotroprocerol A, Calotroproceryl acetate A, Calotroprocerone A)	Root Bark (Hexane fraction)		MTT Assay	A549, U373, PC-3	Ibrahim *et al.* 2012
8	5-Hydroxy-3,7-dimethoxyflavone-4'-*O*-***β***-glucopyranoside (Flavonoid)	Stem (Ethanol)		MTT Assay	HT29, HepG2, NIH-3T3	Shaker *et al.* 2010
9	2"-Oxovoruscharin (UNBS1244) (Cardinolide)	Root Bark (Methanol)		MTT Assay	58 human cell lines	Van Quaquebeke *et al.* 2005

Calactin: Parhira *et al.*, in 2016 investigated thatcalactin has IC_{50} estimation of 21.8 nM with intense inhibitor of HIF1. Their exploration uncovered presence of the aldehyde group in C-19 calactin expanded the HIF-1 inhibitory action. Calactin was likewise tried on the MCF-7 malignant growth cell line and was discovered to be an intense cytotoxic operator with IC_{50} an aggregate of 45.2 nM.[56]

Calotropagenin: Kanojiya *et al.*, in 2012 segregated calotropagenin from chloroform and butanolfractions of leaves of *Calotropis procera* utilising HPLC in a mix of electrospray ionisation couple mass spectrometry.[30] Calotropagenin cytotoxic

Table 5 SAR of 2″-Oxovoruscharin



S.No.	R1	R2	R3	MTD* (mg/kg)	Na/K activity IC_{50} (nM)
1	CHO	OH	OH		
2	CH_2OH	OH	OH	80	75
3	CH_2OAc	OH	OH	>40	Nd
4	$CH_2OCO\ \phi$	OH	OH	>40	Nd
5	$CH_2OCO(CH_2)\ \phi$	OH	OH	>40	Nd
6	$CH_2OSO_2\phi\ CH_3$	OH	OH	nd	Nd
7	$CH_2OSO_2CH_3$	OH	OH	nd	Nd
8	$CH_2OCO(CH_2)_2COCH_3$	OH	OH	20	Nd
9	$CH_2OCO(CH_2)_2CHOHCH_3$	OH	OH	>40	Nd
10	CH_2OSiMe_3	$OSiMe_3$	$OSiMe_3$	nd	Nd

activity was resolved against four disease cell lines including MCF-7, HepG-2, A-549 and HCT-116. Calotropagenin shows critical cytotoxic potential in HepG-2 and A-549 malignant growth cell line containing IC_{50} estimations of 10.40 and 6.50g/mL, individually.[57]

Calotropin: Ibrahim *et al.,* in 2014 segregated calotropin from the *Calotropis procera* root barks and led MTT colorimetric measure for A549, U373 and PC-3 cell lines for assessing anti-proliferative capability of calotropin. They discovered calotropin is intense cytotoxic compound with IC_{50} esteem 0.005μg/mL against all the cell lines was tried.[58]

As indicated by Tian *et al.*, in 2018 calotropin instigated apoptosis in NSCLC by directing T-lymphocytes connected antigen 4-intervened TGF-β/ERK flagging pathway. Calotropin hindered the attack and development, prompts in-vitro apoptosis of H-358 cells and delivered extension and customised cell passing of NSCLC. What's more, the consequences of Western blot examination likewise uncovered that calotropin levels repressed the outflow of Fibronectin (FN), Vimentin (VIM) and E-cadherin (Eca) proteins in H358 cells. The outcomes likewise indicated that the degrees of cytotoxic T_lymphocyte-related antigen 4 (CTLA-4) levels diminished with H358 cells in calotropin treatment. The exploratory cycle beneath proposed that calotropin was likewise demonstrated to have the option to repress TGF-β (factor-factor development factor) and ERK (extracellular sign controlled kinase) articulation. In-vivo concentrates likewise indicated that calotropinorganisation altogether diminished tumor development and expanded creature endurance during a 120-day treatment period. Joined with in-vitro impacts, insusceptible histochemistry indicated a lessening in CTLA-4 articulation levels and a diminishing in TGF-β and ERK articulation in calotropin administration.[59]

Calotroposides: Ibrahim *et al.,* in 2015 isolated seven calotroposides (oxypregnaneoligo glycosides) labeled as calotroposides H-N from the *Calotropis procera* root bark. *In-vitro* development restraint work Calotroposides H-N tried with A549, U373 and PC-3 malignant growth cell lines with MTT examine. Calotroposide K and M were discovered to be conceivably dynamic compound at sub nanomolar development restraint work with IC_{50} esteem

from 0.5 to 0.7μM against the malignancy cell PC-3 and PC-3 lines. Calotroposide-M indicated an expansion in demonstrated an expansion *in-vitro* development inhibition activity because of the 6-*O*-acetylation of terminal glucose unit.[60]

Calotroposides-S: From the *n*-butanol fraction of *Calotropis procera* root bark, the oxypregnaneoligo glycoside (Calotroposide-S) a novel compound was isolated by Ibrahim *et al.,* in 2016. It has shown strong anti-proliferative properties function to PC-3, A549 and U373 cell lines with IC_{50} 0.18, 0.2, and 0.06 6μM respectively.[61]

Proceraside-A: Ibrahim *et al.,* in 2014 separated proceraside-A from methanolic concentrates of root barks of *Calotropis procera.* A colorless amorphous with sub-atomic recipe of $C_{31}H_{44}O_{10}$ was acquired. Compound assessed for A549, U373 and PC-3 malignant growth cell lines. proceraside-A demonstrated huge enemy of proliferative operator with IC_{50} esteem 0.03μg/mL, 0.05μg/mL and 0.06 μg/mL for A549, U373 and PC-3 malignancy cell lines individually.[57]

Ursane type triterpenes: Ibrahim *et al.,* in 2012 segregated diverse sort of ursanetriterpenes, labeled as calotroprocerol A, calotroproceryl acetic acid derivation A, calotroprocerone An and calotroproceryl acetic acid derivation B, among others notable terpenes comprise pseudotaraxasterol acetic acid derivation, taraxasterol, and calotropursenyl acetic acid derivation from hexane division of Calotropisprocera root bark. Its structures were set up with the assistance of 1D and 2D NMR (1H-1H COZY, HSQC, just as HMBC) and HRMS phantom information. The *in-vitro* development inhibitory action of all the above detached compound were tried against the human malignancy cell lines including A549, U373 and PC-3 cell lines. The presence of free OH bunch at the situation of C-3 in calotroproceryl acetic acid derivation A (Ursane type triterpenes) discovered vital for delivering in vitro development inhibitory action in disease cell lines. The acetylation of OH gathering (CalotroproceroneA)or oxidation (Calotroproceryl acetic acid derivation B) prompts loss of *in vitro* development inhibitory movement. Among all the Calotroprocerol A has been shown significant potential of growth inhibition activity against three cancer cells lines, magnitude of effect are in range of cisplatin and carboplatin, used as a standard drug.[62]

Uscharin: Uscharin was separated by Kushwaha *et al.,* in 2010 from *Calotropis* latex and inspected the harmful impact of uscharin on snail propagation. The outcomes proposed that uscharin had the option to diminish the generation of snails and increment the term of hatchability, so it was utilized to control destructive snails, as it decreases the endurance of youthful snails.[63]

5-Hydroxy-3,7-dimethoxyflavone-4′-O-β-glucopyranoside (flavonoid)

Shaker *et al.,*in 2010, isolated 5-hydroxy-3,7-di methoxyflavone-4'-O-β-glucopyranoside (flavonoid) from ethanolic extract of *Calotropis procera* stems and performed MTT assay to analyze the cytotoxicity of isolate flavonoid against the human cell lines HT29, HepG2 and mouse fibroblast(NIH-3T3). They found that the isolated flavanoid decreased the metabolic activities of NIH-3T3 and HepG2 cells by 18% and 10% at concentration of 50μM.[64]

SEMI SYNTHETIC MOLECULE

UNBS1450 is totally different from ouabain, digitoxin, and digoxin in structure.[65] The substance change of 2"- oxovoruscharin dependent on understanding the basic action connections inside the arrangement, prompted the advancement of UNBS1450 with improved execution and *in vivo* resilience in mice with helpful markers expanded by 10 folds. The structure of the UNBS1450 varies from that of the old cardenolides given by the disclosure of a novel association connected to the steroid spine (dissimilar to a solitary connect to digitalis-like cardenolides) and the inclusion of rings A and B of the trans-spinal line, steroid (not at all like the situation of cis on cardenolides, for example, digitalis). This determination of the structure might be answerable for the entirely amazing enemy of malignant growth capacity and explicit system showed by UNBS1450.[66-67]

It is conceivable that these distinctions have given the UNBS1450 a high restricting fondness for α1 and therefore extraordinary potential to battle *in vitro* multiplication against examined malignant growth lines. The UNBS1450 subsequently speaks to a novel restorative treatment that has likewise indicated solid enemy of development and hostile to vascular properties in an assortment of plants *in vivo.* Fundamental security information likewise demonstrate the separation of PC-3 cell lines helped hostile to tumor action from its possible cardiotoxicity and in this manner a higher clinical evaluation contrasted with differentcardiotonic steroids(CSs) in disease treatment.[68-79]

Autophagy-related cell passing that prompts the thrashing of significant systems answerable for the disappointment of disease chemotherapy bolsters its development as an anti of malignant growth

specialist that centers around the sodium siphon α1 subunit.[70-71]

The auxiliary uniqueness of UNBS1450 improves its capacity to disorganize the actin cytoskeleton, disorganize nucleolar structure and capacities and deactivate constitutively enacted cytoprotective flagging pathways. UNBS1450 (hemi-synthetic cardenolide) provides evidence that identifying an α1 subunit pump subunit containing cardenolides may be helpful in the treatment of serious diseases characterized by sodium α1 pumps (Figure 2).[72] 2"- Oxovoruscharin is one of the significant gatherings of proteins that go about as specific ligands for cardenolides is Na^+/K^+ ATPase (additionally called sodium siphon), which is under the name "Na^+/K^+- ATPase signalosom". The butenolide part and R3 gathering of cardenolide structure are important for authoritative to Na^+/K^+-ATPase. Information given in table speaks to the presence of 14α-hydroxy gathering at R3 position are basic for cytotoxic, endless supply of R3 bunch by trimethylsilane bunch prompted a significantly decreases the degree of antitumor action found in compound 10 than on account of compound 2. While regulation at R4 position didn't appear to any effect on their cytotoxic movement, it was a lot of impact on their in-vivo resilience.[73-75] For example, R4, adjustment in R2 position likewise not impacted any major changes in both of *in-vitro* antitumor action and inhibitory movement toward the Na^+/K^+-ATPase (Table 5).

Then again regulation at R1 position by the formyl in an essential liquor (in compound 2) made it conceivable to keep up the *in vitro* antitumor action saw with compound 2"- oxovoruscharin, it drastically diminished (by around one log) the *in vivo* poisonousness. Compound 2 has all the earmarks of being the most intriguing one among the all mixes gritty here as far as *in vitro* antitumor movement, *in vitro* enemy of Na^+/K^+-ATPase action, *in vivo* resistance, and concoction accessibility from regular compound 2"- oxovoruscharin.[76-79]

FUTURE PROSPECTIVE

The future will lies in the plants and plant derived products for everlasting source of phytomolecules. The community of anticancer drug lacking behind for their adverse effects, drug targeting and decreased in quality of patients life, to overcome these effect the medicinal herbs getting the attention of the researchers'. *Calotropis procera* is the herb with full of opportunities and encourages the researchers for exploration of active lead molecules for anticancer drug development. Various molecules were isolated from the *Calotropis procera* and evaluated for their anticancer potential and the result encourages the scientists to do more research on these molecules. For future work more studies are required for identification of exact mechanism behind these molecules as anticancer agent.

CONCLUSION

Conventional arrangement of medication keeps on being broadly rehearsed for different reasons. Quick population growth, insufficient gracefully of marked medications, alarmingly restrictive expense of treatment, unfriendly symptoms of a few allopathic medications and ever increasing protection from current medications for irresistible infections have prompted developing accentuation on the utilization of plant-materials as a wellspring of medications for a wide assortment of human afflictions. Plants are always be the indispensible sources of natural phytoconstituents and still the beneficial exploration zone and offers the scientist for incredible trust in finding the phytomolecule forestall incessant human body issues. According to studies, phenol contents present in *Calotropis procera,* maximum in naturally growing as compared to *in-vitro* growing.[80]

The present paper is an endeavor to explore the ongoing researches on *Calotropis procera* and its subordinates for anticancer potential. *Calotropis procera* is considered as an important medicinal herb that have previously mentioned for their pharmacological activities in various ailments of human being. It is foreseen that various investigations on *Calotropis procera* and its subsidiaries will open new roads for additional bio-prospection and will absolutely lead to new genera for the treatment of various ailments. At last, we can infer that to approve future applications of *Calotropis procera* and its lead molecules will be investigated further point by point.

LIST OF ABBREVIATIONS

CP	-	callus protein
RP	-	root protein
LP	-	laticifer proteins
SAE	-	soxhlet assisted extraction
m	-	meter
cm	-	centimeter
ml	-	mililiter
µg	-	micro gram
µM	-	micro molar

nM	-	nano molar
IC_{50}	-	half maximum inhibitory concentration
EDAX	-	Energy Dispersive X-Ray Analysis
SEM	-	Scanning electron microscopy
FTIR	-	fourior transform infra-red spectroscopy
MIC	-	minimum inhibitory concentration
MTT assay	-	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NSCLC	-	non small celllung cancer
DNA	-	DeoxyRibo Nucleic Acid

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest concerning this article.

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