PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITY OF CALOTROPIS GIGANTEA AS A POTENTIAL MEDICINAL PLANT: AN OVERVIEW

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Summary

Calotropis gigantea R. Br. (Asclepiadaceae) a wildly growing plant has been reported to possess number of medicinal properties (1) and other purposes (2) commonly known as the (Crown flower) is a large shrub growing to 4 m tall. It has clusters of waxy flowers that are either white or lavender in colour. Each flower consists of five pointed petals and a small, elegant "crown" rising from the centre, which holds the stamens. The plant has oval, light green leaves and milky stem. Traditionally calotropis is used alone or with other medicinals (3) to treat common disease such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea (4). According to Ayurveda, dried whole plant is a good tonic, expectorant, depurative, and anthelmintic. The dried root bark is a substitute for ipecacuanha. The root bark is febrifuge, anthelmintic, depurative, expectorant, and laxative. The powdered root used in asthma, bronchitis, and dyspepsia. The leaves are useful in the treatment of paralysis, arthralgia, swellings, and intermittent fevers. The flowers are bitter, digestive, astringent, stomachic, anthelmintic, and tonic (5-6). Calotropis is also a reputed homoeopathic drug (7-8). Various phytochemical and biological evaluations have been reported in this literature for the importance of the Calotropis gigantea.

Keywords: Calotropis gigantea R. Br. | Phytochemical evaluation | Pharmacological evaluation

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Introduction

*Calotropis gigantea* R. Br. (*Asclepiadaceae*) a wildly growing plant has been reported to possess number of medicinal properties (1) and other purposes (2) commonly known as the crown flower. It has clusters of waxy flowers that are either white or lavender in colour. Each flower consists of five pointed petals and a small, elegant "crown" rising from the centre, which holds the stamens. The plant has oval, light green leaves and milky stem. It is a large shrub growing to 4 m tall, with opposite, oval leaves that are wooly beneath and has clusters of waxy flowers that are lavender in colour. The scentless flowers are about 1 cm across and long lasting and the seeds are dispersed by wind. Its stems are erect, up to 20 cm in diameter. The leaves are broadly elliptical to oblong-obviate in shape, with the size of 9-20 cm x 6-12.5 cm but sub sessile. The cymes are 5-12.5 cm in diameter. The inflorescence stalk is between 5-12 cm long, the stalk of an individual flower is 2.5-4 cm long. Sepal lobes are broadly egg-shaped with a size of 4-6 mm x 2-3 mm. Petal is 2.5-4 cm in diameter. The petal lobes are broadly triangular measuring 10-15 mm x 5-8 mm; they are pale lilac and cream coloured towards the tips. The outgrown like structure from the petal (corona) has 5 narrow fleshy scales, connected to and shorter than the stamina column, forming an upturned horn with 2 obtuse auricles on either side, cream coloured or lilac to purple, with a dense longitudinal dorsal row of short white hairs. The egg-shaped or boat-shaped fruits are mostly in pairs, inflated, 6.5-10 cm x 3-5 cm.

Phytochemical Evaluation

M. Rowshanul Habib *et al* reported as Isolation of stigmasterol from methanolic extract of Root Bark of *Calotropis gigantea*. Aim of this study was to identify and characterize the bioactive principles from the root bark of *Calotropis gigantea*. It has wide folk medicinal use. For isolation of the compounds, the dried root bark powder of *calotropis gigantea* were subjected to hot extraction and then the crude methanol (MeOH) extract was fractionated with petroleum ether, chloroform and ethyl acetate. Two compounds were isolated and purified from petroleum ether fraction of crude methanol extract and the structures were determined as stigmasterol and sitosterol by analysis of physical, chemical and spectral characteristics (9).

Alireza Ashori *et al* - reported the evaluation of *Calotropis gigantea* as a Promising Raw Material for Fiber-reinforced. The chemical analysis of the bark and seed fibers indicates that their main components are holocellulose 76 and 69%, cellulose 57 and 49%, lignin 18 and 23%, and alkali soluble substances 17 and 15%, respectively. There are statistically significant differences in the bark and seed fiber dimensions. The bark fibers are long, with a thin wall relative to their diameter, and are therefore lightweight. The seed and bark fibers are very similar to hard- and soft-woods, in terms of chemical compositions and fiber dimensions, respectively. The mechanical properties of the mudar bark fibers are: tensile strength 381 MPa, strain at break 2.1% and Young's modulus 9.7 GPa (10).

N. Ramamurthy, S. Kannan, sem-eds analysis of soil and plant (calotropis gigantea linn) collected from an industrial Village, cuddalore dt, tamilnadu, India. The scope of this study is to investigate the effects of the atmospheric emissions of heavy metals in soil and plants collected from an industrial area. In this connection the environmental pollution of the bioindicators (soil and plant) have been analysed by SEM-EDS method by estimating heavy metals like Na, Mg, Al, Si, Cl, K, Ca, Mn, Fe, Cr, Co, Ni, Cu, Zn, As, Se, Pb and Cd. From this analysis, a perceptible variation in the trace element concentration of samples in different seasons is found (11).
Ashis Taru Roy and DE D. N., Tissue culture and plant regeneration from immature embryo explants of Calotropis gigantea (Linn.) R. Br. Callus cultures were established from immature embryos of Calotropis gigantea (Linn.) R. Br. on a modified basal medium of Murashige & Skoog supplemented with 1 mg l\(^{-1}\) 2,4-D. In addition to 0.1 mg l\(^{-1}\) of NAA the optimal BAP concentration for promoting shoot bud formation and growth was 2 mg l\(^{-1}\). Rooting was induced when shoots were transferred to auxin-supplemented Bonner's solution or half-strength MS basal salt solutions (12).

Thitimalhinhata kool et al reported the 19 nor 18 –20-epoxy cardenolide from the leaves of calotropis gigantea, cardenolides (1 and 2) along with known compounds were isolated from the dichloromethane extract of the leaves of *Calotropis Gigantea* (13).

J. Gupta et al - studies on rare chemical constituents from calotropis gigantea roots. Four new chemical constituents including one naphthalene derivative, named calotropnaphthalene, two terpene derivatives, namely calotropisesquiterpenol and calotropisesterterpenol and an aromatic product designated as calotropbenzofuranone along with a known compound, sucrose, have been isolated from the roots of the Calotropis gigantea. The structures of these chemical constituents have been established as 1-methoxy-4-ethyl naphthalene, 6-(2-methyl-2, 3-dihydroxypentyl)-11, 11-dimethyl cyclohex-8-ene-10-one-7-oic isopentenyl ester, 14-(15, 15-dimethyl cyclohexanyl-14, 19,25-tricyclo)-3,7,11-trihydroxymethylene-tridecane and 8,15-dihydro benzofuran-18-hepta-7, 15-dione-16-oic acid, respectively, on the basis of the spectral data analyses and chemical reactions (14).

P. A. Wahid et al reported the effect of rare earth elements on growth and nutrition of coconut palm and root competition for these elements between the palm and *calotropis gigantean*. Absorption of rare earth elements (REEs) namely lanthanum (La), cerium (Ce), praseodymium (Pr), and neodymium (Nd) by coconut, competition between coconut and *Calotropis gigantea* L. for these elements in mixed culture and the effects of the REEs on growth and nutrition of the palm were studied in a pot culture. At a low rate of application, REEs promoted root growth in coconut, but at a higher level, absorption of phosphorus (P) and zinc (Zn) by the palm was reduced significantly. Absorption of REEs by the palm tended to increase with increasing application rates, but the increases were not statistically significant (15).

K. Pari et al - reported a novel insect antifeedant nonprotein Amino Acid from *Calotropis gigantea* novel nonprotein amino acid, has been isolated from a methanol extract of the root bark of *Calotropis gigantea* and its structure established by spectroscopic methods. It exhibited a significant antifeedant activity against nymphs of the desert locust *Schistocerca gregaria* (16).

Sucharita Sen et al reported the Flavonol glycosides from Calotropis gigantea. Besides isolation and characterization of isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucopyranoside and taraxasteryl acetate, a new flavonol trisaccharide was isolated from the aerial parts of Calotropis gigantea, and its structure was established as isorhamnetin-3-O-[2-O-beta-D-galactopyranosyl-6-O-alpha-L-rhamnopyranosyl]- beta-D-glucopyranoside by a combination of fast atom bombardment mass spectroscopy, 1H and 13C NMR spectra and some chemical degradations (17).

Das B.K et al performed the phytochemical studies on calotropis gigantea. Leaves were dried and powdered which was successively extracted with petroleum ether, chloroform, ethyl acetate and methanol (80%). Completion of extraction was identified by TLC analysis of siphoned liquid from the extractor (18).
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K. Sundar Rao et al performed the analysis of Calotropis gigantea, Acacia Caesia and Abelmoschus Ficulneus seeds. The percentage contents of oil and protein in the seeds of Calotropis gigantea Linn. (Asclepiadaceae), the major fatty acid was 18:1 in C. gigantea and 18:2 in the other two seeds oils. Malvalic, sterculic and dihydrosterculic acids were present in small quantities in A. ficulneus seed oil. The major essential amino acids in the seed proteins were phenylalanine, lysine and histidine in C. gigantea, threonine and arginine in A. caesia and lysine and phenylalanine in A. ficulneus (19).

V Tomar et al reported the toxic iridocyclitis caused by calotropis. The juice is acid in reaction, has specific gravity of 1021, and contains 14.8% solids. On heating or after keeping it for some time, the juice forms into a white coagulum leaving a clear serum. The coagulum yields a yellowish brown resin and a snow-white crystalline substance. The resin is slightly poisonous, about 8 gm. being necessary to kill a frog weighing about 20 gm. The crystalline substance is insoluble in water but is soluble in alcohol, acetone, ether and chloroform, and is non poisonous (20).

Kali Pada Basu et al reported the calosterol, a sterol present in the milky juice of Calotropis gigantean (21).

Shibuya Hirotaka et al reported the indonesian Medicinal Plants. V. Chemical Structures of Calotroposides C, D, E, F, and G, Five Additional New Oxypregnane-Oligoglycosides from the Root of Calotropis gigantea (Asclepiadaceae) five related oxypregnane-oligoglycosides named calotroposides C (3), D (4), E (5), F (6), and G (7) have been additionally isolated from the root of Calotropis gigantea (Asclepiadaceae), an Indonesian medicinal plant (22).

Krings, Alexander et al reported New and rediscovered milkweeds from Cuba: Calotropis gigantea and Gonolobus stephanotrichus (Apocynaceae: Asclepiadoideae). Previously only a single species of Calotropis R. Br. was reported for Cuba, While C. gigantea apparently has a native range spanning the Indian subcontinent, southern China, SE Asia and Indonesia Howard (1989) noted the presence of C. gigantea in cultivation in Barbados based on a report by Maycock (1830). The records from Cuba are apparently the first report of C. gigantea for the Greater Antilles. Based on a collection previously assigned to C. procera, C. gigantea appears to have been in Cuba for at least thirty years (23).

N. Ramamurthy et al performed the Fourier Transform infrared spectroscopic analysis of a Plant (Calotropis Gigantea Linn) from an industrial village, cuddalore dt, Tamilnadu. FT-IR spectroscopy is used to reveal some qualitative aspects regarding the organic compounds in a plant Calotropis gigantea Linn collected FT-IR spectrum is able to predict the main chemical constituents in plant materials and also to compare the quantitative differences among the similar samples (24).

Kitagawa I. et al reported as Indonesian Medicinal Plants. I. Chemical Structures of Calotroposides A and B, Two New Oxypregnane-Oligoglycosides from the Root of Calotropis gigantea (Asclepiadaceae). Two new oxypregnane-oligoglycosides named calotroposides A (1) and B (2) have been isolated from the root of Calotropis gigantea (Asclepiadaceae), an Indonesian medicinal plant, and their chemical structures have been elucidated by chemical and spectroscopic methods (25).

Pal. G., et al reported the isolation, crystallization, and properties of calotropins DI and DII from Calotropis gigantean (26). Anand et al reported the Pharmaceutical Composition Containing Uscharin. The invention provides compositions comprising uscharin and the use of uscharin to combat cell proliferation for example in the treatment of cancer.
Administration of uscharin may kill or reduce the growth rate of cancer cells and may also be of application in other medical conditions presenting symptoms of excessive or uncontrolled cell proliferation (27).

Pharmacological Evaluation

Kiuchi, F et al isolated the Cytotoxic principles of a Bangladeshi crude drug, akond mul (roots of Calotropis gigantea L.). Three cardenolide glycosides, calotropin (1), frugoside (2), and 4'-O-β-D-glucopyranosylfrugoside (3), were obtained as the cytotoxic principles of "akond mul" (roots of Calotropis gigantea L.). The cytotoxicity of these compounds against various cell lines of human and mouse origin was tested. They showed similar cell line selectivity to those of cardiac glycosides such as digoxin and ouabain. They are toxic to cell lines of human origin, but not to those from mouse at 2µg/ml (28).

S. K. Sain et al studied on A New Leaf Spot Disease of Calotropis gigantea caused by Alternaria alternata in Rajasthan. In this, they observed a leaf spot epidemic of madar growing on wasteland sites near the Sikar district of Rajasthan, India. Koch’s Postulates were completed. This is the first record of the disease from the Sikar district of the Rajasthan state of India (29).

Gaurav Lodhi et al studied on reported the hepato protective effect of calotropis gigantea extract against carbon tetra chloride liver injury in rat. Ethanolic extract (50 %) of stems of Calotropis gigantea RBr. (Asclepiadaceae) at doses of 250 and 500 mg kg–1 were studied for hepatoprotective activity in male wistar rats with liver damage induced using carbon tetrachloride, 2mL kg–1 twice a week (30).

S. Awasthi et al studied on Anti-Inflammatory Activity of Calotropis gigantea and Tridax procumbens on Carrageenin-Induced Paw Edema in Rats. The anti-inflammatory activities of extract of Calotropis gigantea R.Br. and Tridax procumbens Linn. The Ibuprofen significantly reduced paw edema gigantea R.Br gigantea and T. procumbens along with various dose regimen gigantean (31).

Sudesh Kumar et al studied the synergistic effect of calotropis plant in controlling Corrosion of Mild Steel in Basic solution. The alcoholic extracts of leaves, latex and fruit from the Calotropis procera and Calotropis gigantea are tested for corrosion inhibition in basic solution by mass loss method and thermometric method. In the present investigation the extract reduces the corrosion rate of mild steel in basic solution. The inhibition efficiency increases as the extract concentration is increased. The alcoholic extract of Calotropis is found effective corrosion inhibitor in basic media and give up to 80.89% efficiency (32).

Nanu Rathod et al reported the prevention of high-fructose diet induced Insulin resistance by Nyctanthes arbor-tristis and Calotropis gigantean in rats. The fasting serum glucose, insulin, triglyceride and cholesterol levels were measured in blood serum for 27 days of treatment. The fasting serum glucose, insulin, insulin resistance index (FIRI) levels of high-fructose diet (control) rats significantly increased, like wise, serum triglyceride, cholesterol significantly increased. The Nyctanthes arbor-tristis and Calotropis gigantea leaves and flower treatment prevent significantly increase serum glucose, insulin, levels in high fructose-diet treated rats, except in glucose Calotropis gigantea leaves 50 mg/kg, while significantly decreased in triglyceride, cholesterol, except in triglyceride Nyctanthes arbor-tristis leaves 50 mg and in cholesterol Nyctanthes arbor-tristis leaves and flowers 50 mg (33).

Narendra Nalwaya et al - studied on Wound Healing Activity of latex of Calotropis gigantea. The Latex of Calotropis gigantean (200 mg/kg/day) was evaluated for its wound healing
activity in albino rats using excision and incision wound models. Latex treated animals exhibit 83.42 % reduction in wound area when compared to controls which was 76.22 %. The extract treated wounds are found to epithelize faster as compared to controls. Significant (p<0.001) increase in granuloma breaking strength (485±34.64) was observed. The Framycetin sulphate cream (FSC) 1 % w/w was used as standard (34).

Deshmukh PT et al studied on Wound Healing Activity of Calotropis gigantea Root Bark in Rats. In this they investigated the effects of Calotropis gigantea root bark on wound healing activity in rats by excision, incision and dead space wound healing models in rats. Wistar albino rats of either sex weighing between 180-200g of either sex were topically treated with extract formulated in ointment by using simple ointment BP as base. 5 % (w/w) ointment once daily in excision wound model. Calotropis gigantea ethanolic extract were given orally at a dose of 100mg/kg, 200mg/kg, and 400mg/kg in incision and dead space wound healing models. Rats of standard groups were treated with 5% Povidone iodine ointment topical. The percentage wound closure, epithelization time, hydroxyproline content and scar area on complete epithelization were measured (35).

K. Usha et al studied the antifungal activity of Datura stramonium, Calotropis gigantea. Present studies clearly showed strong antifungal activity of a concoction brewed from Datura stramonium, Calotropis gigantea, Azadirachta indica (neem) and cow manure (T1) followed by methanol-water (70/30 v/v) extracts of Datura stramonium, Calotropis gigantea and Azadirachta indica (T2) against Fusarium mangiferae (36).

M. A. Rahman et al reported as A taxonomic revision of Calotropis (Asclepiadaceae)The genus Calotropis of the tribe Asclepiadeae of Asclepiadaceae subfamily Ascle-piaidoideae is revised throughout its range in tropical and subtropical Africa and Asia. The three species recognized are keyed out, illustrated and described with appropriate bibliography, synonymy, notes on ecology, distribution and uses. Distribution maps for the species are provided (37).

Zhu Nian Wang et al studied on new cytotoxic pregnancy calotropis gigantea. A new pregnanone, named calotropone (1), was isolated from the EtOH extract of the roots of Calotropis gigantea L. together with a known cardiac glycoside. The structures were elucidated by a study of their physical and spectral data. Compounds 1 and 2 displayed inhibitory effects towards chronic myelogenous leukemia K562 and human gastric cancer SGC-7901 cell lines (38).

D. Madhv Vaidya et al studies on preliminary studies on antmyotic an anticancer activity of calotropis gigantea. The Total aqueous extract (CAI) of the aerial parts was prepared by decoction method using distilled water as solvent. The fresh latex was collected and dried in vacuum oven at 60°C for 48 hours. The dried latex was suspended in water and filtered to get the water soluble fraction (CAII) (39).

Zacharia J et al reported the method and preparation of herbal extract of calotropis gigantea for cancer treatment. Method of preparation of a potential herbal anti-cancer medicine from the leaves and twigs of Calotropis gigantean for prevention and treatment of all types of cancers, using fresh extract of leaves and twigs of Calotropis gigantea fortified with unrefined sugarcane sugar (jaggery). The aqueous extract of leaves and twigs of Calotropis gigantea was desiccated. The [3 H]-Thymidine growth assay was performed against cancer cell lines, WM 902-B and WM1341 -D. The extract [28mgms] was dissolved in 2ml control at 0.5%, 0.1% DMSO. The extract is highly inhibitory at all dilutions and highly inhibitory to both the cancer cell lines (40).
M. Ashraful Alam et al studied the Antimicrobial Activity of Akanda (Calotropis gigantea L.) on some pathogenic bacteria. The antibacterial activity of methanol extract from the root bark of Akanda (Calotropis gigantea L.) and its petroleum ether, chloroform and ethyl acetate fractions were investigated. Both of methanol extract and its chloroform fraction showed activity against Sarcina lutea, Bacillus megaterium and Pseudomonas aeruginosa. Petroleum ether fraction showed activity against Bacillus subtilis and Shigella sonnei whereas ethyl acetate fraction showed activity against Pseudomonas aeruginosa and Escherichia coli at 20 µg/disc, 30 µg/disc and 40 µg/disc doses (41).

S. goldeeperdasi et al studied the immunomodulatory Activity of Calotropis Gigantea by Cyclophosphamide Induced Myelosuppression (42).

S. Srivastava et al, studied the pregnancy interceptive activity of the roots of Calotropis gigantea Linn. in rats. They conducted this study to evaluate the pregnancy interceptive activity of the roots of Calotropis gigantea Linn. in colony-bred adult Sprague–Dawley rats when administered during the preimplantation and/or peri-implantation periods (43).

A. K. Pathak et al studied on analgesic activity of calotropis gigantea. The alcoholic extract of the flowers of Calotropis gigantea was administered orally and explored for its analgesic activity in chemical and thermal models in mice. In acetic acid induced writhing test, an inhibition of 20.97% and 43.0% in the number of writhes was observed at the doses of 250 and 500 mg/kg, respectively. In the hot plate method the paw licking time was delayed. The analgesic effect was observed after 30 min of dose administration which reached its maximum after 90 min (44).

Prateek Shilpkar et al reported an alternate use of Calotropis gigantea in biomethanation. To obtain 6% TS, 30 g buffalo dung was mixed with 90 ml water. From the 41st day onwards, 6 g buffalo dung was replaced with C. gigantea powdered leaves. Digested slurry (120 ml) was withdrawn daily from the digester. Results showed the favourable effect of co-digestion over the control. Significantly higher biogas production (109.82% increase) was recorded in test digesters than the control (45).

Ameeta Argal et al. studied the CNS activity of calotropis gigantea roots. Alcoholic extract of peeled roots of Calotropis gigantea R.Br. (Asclepiadaceae) was tested orally in albino rats at the dose level of 250 and 500 mg/kg bodyweight for CNS activity (46).

J. C. Sakthivel et al performed the Some Studies on Mudar Fibers. In this work, Mudar fibers (Calotropis procera) have been characterized for their physical, chemical, and tensile properties. Mudar fibers have good length, strength, uniformity, fineness, and excellent moisture absorption. The study highlights the difficulties in spinning 100% Mudar yarn. Subsequently, a 75/25 Mudar/cotton blend is successfully spun in a cotton spinning system and the results are analyzed. Smooth Mudar fibers develop convolutions when treated with 5% NaOH, which can render spinning possible with fibers treated in such a way. The yarns have enough potential in natural fiber-reinforced composites and other industrial textiles application (47).

R. Rajesh et al studies on Procoagulant activity of Calotropis gigantea latex associated with fibrin (ogen)olytic activity. Pharmacologically the crude extract is hemorrhagic and induces skin hemorrhage at >75 µg and reduces the coagulation time of citrated plasma from 150 to 47 s and promotes blood coagulation. Procoagulation and blood clot hydrolysis are important in wound healing process. This is due to unique cysteine proteases of plant latex and is responsible for the pharmacological actions observed in folk medicine (48).
Chitme,-H-R, et al performed the studies on Evaluation of antipyretic activity of Calotropis gigantea (Asclepiadaceae) in experimental animals. The present communication evaluated its antipyretic activity by using yeast-induced and TAB (Typhoid) vaccine-induced pyrexia in rats and rabbits. In both yeast-induced and TAB vaccine-induced fever, the fever was significantly reduced and the body temperature was normalized by administration of 200 and 400 mg/kg dose intraperitoneally. Based on the results of the present study it can be concluded that the extract of C. gigantea has potential antipyretic activity against both yeast-induced and TAB vaccine-induced fever, indicating the possibility of developing C. gigantea as a cheaper and potent antipyretic agent (49).

Chitme et al studies on antidiarrhoeal activity of calotropis gigantean. The anti-diarrhoeal effect of hydroalcoholic (50:50) extract of aerial part of Calotropis gigantea was studied against castor oil-induced diarrhoea model in rats. The gastrointestinal transit rate was expressed as the percentage of the longest distance traversed by the charcoal divided by the total length of the small intestine. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method (50).

P Oudhia et al studied on Allelopathic effects of Ageratum conyzoides and Calotropis gigantea on germination and seedling vigour of rice. Allelopathic effects of Ageratum conyzoides and Calotropis gigantea on germination and seedling vigour of rice were investigated. The aqueous extracts of root, stem, leaf and stem+leaf of these weeds were used for study. The extracts of different parts of these weeds produced significant effects on germination and seedling vigour of rice. Ageratum leaf extract identified as a promising extract and produced maximum root and shoot elongation without affecting the germination of rice seeds (51).

Fukao Yumi et al studied the Cytotoxic Principles of a Bangladeshi Crude Drug, Akond Mul (Roots of Calotropis gigantea L.) Three cardenolide glycosides, calotropin (1), frugoside (2), and 4'-O-β-D-glucopyranosylfrugoside (3), were obtained as the cytotoxic principles of "akond mul" (roots of Calotropis gigantea L.). The cytotoxicity of these compounds against various cell lines of human and mouse origin was tested. They showed similar cell line selectivity to those of cardiac glycosides such as digoxin and ouabain: they are toxic to cell lines of human origin, but not to those from mouse at 2µg/ml (52).

Ashis Taru Roy et al Studies on Differentiation of Laticifers through Light and Electron Microscopy in Calotropis gigantea. The distribution, cytological organization and differentiation of non-articulated laticifers in the primary and mature tissues of Calotropis gigantea (Linn.) R.Br., were studied by the use of optical and electron microscopy. Laticifers occur in the cortex, vascular bundle and pith of the plant axis. At the earliest detectable stage a laticifer is a cell which undergoes rapid elongation and nuclear division. This results in a multinucleate elongated cell which undergoes further increase in length with gradual degeneration of the cytoplasm. At the electron microscopic level the presumptive laticifer cell shows increasing vacuolation which forms a large central vacuole. Simultaneously the cytoplasmic organelles undergo degeneration by autophagic processes. Later numerous vesicles can be observed in the large central vacuole, the remaining cytoplasm being pushed to a thin layer. Mature laticifers show three types of spherical structures of which the highly electron dense globules are the latex particles (53).

Narong Chungsamarnyart et al studies on Acaricidal activity of the Combination of Plant Crude-extracts to Tropical Cattle Ticks ethanol crude extracts of plant species were studied the acaricidal activity of the two combined crude-extracts to the engorged female tropical
cattle tick (*Boophilus microplus*). The concentration of crude-extract was 2.5% in mixed solution of 9 parts of 1% Tween 88® and 1 part of 100% ethanol. The acaricidal activity of the combined crude-extracts were tested by dipping method and observed the corrected mortality of the ticks afterdipping 24 h, 48 h and 7 days (54).

S. K. Datta *et al* reported the Laticifer Differentiation of *Calotropis gigantea* R. Br. ex Ait. in Cultures. The initiation and subsequent development of laticifers were studied in callus tissues of *Calotropis gigantea* grown on MS (Murashige and Skoog) medium supplemented with 1 mg l\(^{-1}\) IAA. Laticifer development was related to the age of the culture and could be preserved by repeated subculturing on similar medium with IAA. Our previous work had established that cardenolide biosynthesis was related to rhizogenesis and here we had reported the culture system of laticifer, long term preservation and differentiation of this hydrocarbon producing energy plant (55).

Kiuchi, F., *et al* reported the Cytotoxic Principles of Bangladeshi Crude Drug, Akond Mul (Roots of *Calotropis gigantea* L.). Three cardenolide glycosides, calotropin (1), frugoside (2), and 4'-O-β-D-glucopyranosylfrugoside (3), were obtained as the cytotoxic principles of "akond mul" (roots of *Calotropis gigantea* L.). The cytotoxicity of these compounds against various cell lines of human and mouse origin was tested. They showed similar cell line selectivity to those of cardiac glycosides such as digoxin and ouabain (56).

Mohammad Ramezani *et al* studies on Evaluation of leishmanicidal effect of *Calotropis gigantea* extract by in vitro leishmanicidal assay using promastigotes of *L*. Four different concentration of extract either perculated or soxhlet (0.12, 0.25, 0.50 and 1.0 mg/ml), one positive control, one negative control and one solvent (DMSO) control were prepared and were placed in 24 well plates that contained 50,000 parasites/well. Positive control group contained Amphotericin B (0.5 mg/ml) and negative control group contained only culture media. Then they were incubated at 25°C for 3 days and amount of parasites in each well determined on days 1, 2 and 3 of experiment (57).

K.I Abraham *et al* Studies on proteinases from *Calotropis gigantea* latex. I. Purification and some properties of two proteinases containing carbohydrate. Two proteinase containing carbohydrate, called calotropain-FI and calotropain-FII, were purified from *Calotropis gigantea* latex by CM-Sephadex C-50 chromatography. Both calotropain-FI and FII were found to be homogeneous by rechromatography on CM-Sephadex C-50, gel filtration on Sephadex G-100, electrophoresis on polyacrylamide gel and by N-terminal amino acid analysis. Some properties of these enzymes are reported (58).

Habib MR *et al* reported the isolation of stigmasterol and beta-sitosterol from methanolic extract of root bark of *Calotropis gigantea* (Linn). Aim of this study is to identify and characterize the bioactive principles from the root bark of *Calotropis gigantea*. It has wide folk medicinal use. For isolation of the compounds, the dried root bark's powder of *Calotropis gigantea* were subjected to hot extraction and then the crude methanol (MeOH) extract was fractionated with petroleum ether, chloroform and ethyl acetate. Two compounds were isolated and purified from petroleum ether fraction of crude methanol extract and the structures were determined as stigmasterol and beta-sitosterol by analysis of physical, chemical and spectral characteristics (1D NMR and mass spectrometry) (59).

C. gadgoli *et al* studied on Investigation on Immunomodulatory activity of *calotropis gigantean* (60).
Srivastava SR et al, Pregnancy interceptive activity of the roots of Calotropis gigantea Linn. in rats. We conducted this study to evaluate the pregnancy interceptive activity of the roots of Calotropis gigantea Linn. in colony-bred adult Sprague-Dawley rats when administered during the preimplantation and/or peri-implantation periods (61).

M. Ashraful Alam et al, Insecticidal Activity of Root Bark of Calotropis gigantea L. Against Tribolium castaneum (Herbst). The residual film toxicity, fumigant toxicity and repellent effect of methanol extract of root bark of Calotropis gigantea (Linn) and its chloroform and petroleum ether (40-60°C) soluble fractions were evaluated against several inster of larvae and adult of Tribolium castaneum (62).

Adak M et al evaluated the effect of Calotropis G in various experimental animal models. The anti-inflammatory activity was evaluated using carrageenin-induced kaolin -induced rat paw oedema for acute and cotton-pellet granuloma, adjuvant-induced arthritis model for chronic inflammation. Antipyretic activity was carried out using yeast induced pyresis method. Phenylquinone--induced writhing method in mice was used for analgesic activity. Test compounds exhibited variable anti-inflammatory activity and peak activity of the test compounds were reached at 2 h. Alkaloid fraction possesses comparatively high initial anti-inflammatory activity. The residual anti-inflammatory activity of alkaloid fraction of Calotropis G suggest either a greater protein binding nature of the compound there by providing a slow released pool of active drug molecule in the system or non available of possible bioactive metabolites to retain the activity profile relation (63).

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