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Calotropis Procera: A Phytochemical and Pharmacological Review with Special Focus on Cancer

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ABSTRACT

Mankind for centuries has been using medicinal plants for different ailments. Most of the available herbal medicine could be procured as extracts or in synthetic forms. Plants naturally produce these secondary metabolites termed as phytoconstituents that are potentially lethal to microbes. These metabolites are known to have numerous biological functions beneficial to humans. This partly explains the success behind new drug discovery from plant sources. *Calotropis procera*, a medicinal plant, has been used for centuries as a traditional medicinal plant in the Middle East, India and Africa. Various phytochemicals have been reported to be extracted from different parts of *C. procera*, namely, calotropin, calotropagenin, calotoxin, calotropagenin and voruscharine, steroids, di and triterpenes, flavonoids, polyphenolic compounds. A small erect shrub, *C. procera* has been reported to possess a wide range of pharmacological activities. This review highlights pharmacological properties and the potential of *C. procera* in curbing cancer.

KEYWORDS: Calotropis, Anticancer, Antimicrobial, Pharmacological, Medicinal Plant, Herbal Drugs.

INTRODUCTION

Calotropis procera, a small shrub is of immense pharmacological importance as a traditional medicine in the Middle East, Africa and South East Asia (Figure 1). Two common species of *Calotropis* have been mentioned in literature by ancient writers, *C. procera* and *C. gigantean*. Both of these species contain similar phytochemical constituents and could possibly be used as substitutes for one another. It is widely used in Indian, Arabic and Sudanese traditional medicinal systems for curing wide range of diseases. Various tribes of the world are also known to use *C. procera* as a curative for skin disease, elephantiasis, toothache, asthma, leprosy, and rheumatism [1].

Figure 1. *C. procera*

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Various phytochemicals that have pharmacological implications are found spread across different parts of the plant, such as leaves, roots and bark, flower, fruits, stem, and latex. The plant is reported to possess acaricidal, schizonticidal, antimicrobial, anthelmintic, insecticidal, anti-inflammatory, antidiarrheal, anticancerous, and larvicidal activities [2]. This is possibly due to the presence of numerous phytochemicals, namely, calotropin, calotropagenin, calotoxin, calactin, uscharin, amyrin, amyrin esters, uscharidin, coroglaucigenin, frugoside, corotoxigenin, calotropagenin, and voruscharine, see [2].

Presence of norditerpenic esters, organic carbonates, cysteine protease, alkaloids, flavonoids, sterols, and various cardenolides makes *C. procera* a significant candidate plant for new drug discoveries. Hence, it is imperative to highlight the pharmacological benefits that could be reaped from this small erect shrub. In this review, we focus on different pharmacological benefits of numerous constituents found in *C. procera* and shed light on its potential as an anti-cancer agent.

Plant Overview

***Habitat*:** *C. procera* is mostly found in habitats with little competition from other plants. These plants tend to grow in dry and rugged habitats with little rainfall (150 to 1000 mm) annually and areas with well-drained soil having 2000 mm of annual precipitation. The plant is commonly found on roadsides, beachfront dunes, and urban areas. It is also found in areas 1000 m above sea level. Due to its xerophytic nature it can grow and propagate under harsh desert conditions. It is also grown as an ornamental plant in dry or coastal regions in the world [2,3].

Geographical Distribution

C. procera is naturally present in Southern Asia, Africa and Arabian Peninsula. The plant has been naturalized in Australia, Americas and West Indies [2,4].

Scientific Classification

Taxonomy *Calotropis procera* (Ait.) Ait.f. Kingdom: Plantae – Plants; Subkingdom: Tracheobionta – Vascular plants; Superdivision: Spermatophyta – Seed plants; Division: Magnoliophyta – Flowering plants; Class: Magnoliopsida – Dicotyledons; Subclass: Asteridae; Order: Gentianales; Family: Asclepiadaceae; Genus: *Calotropis* R.Br. – *Calotropis*; Species: *C. procera* (Ait.) Ait.f. [5].

Botanical Description

C. procera is a soft-wooded, evergreen perennial shrub with an average height of 2.5 m. A gash on any part of the plant allows white sap to profuse out. It has a corky, furrowed bark, gray in color. It has branched roots that are woody at the base. Leaves are opposite-decussate, subsessile, and exstipulate in morphology that are leathery in touch with fine hair. It has bell-shaped flowers that are shallow and bisexual, actinomorphic, pentamerous, hypogynous, pedicellate, multiflowered, umbellate, peduncled cymes, either axillary or terminal inflorescence. A total of five sepals, 4-5 mm long that are lobe shaped and conjoined at the bottom. Five petals that are also lobe shaped gamopetalous in nature and twisted aestivation. Androecium has five stamens, gynandrous, anther dithecous, coherent. Gynoecium is bicarpellary, apocarpus with styles united at their apex, peltate stigma that has five lateral stigmatic surfaces. Anthers are adnate to the stigma forming a gynostegium. The fruit of *C. procera* is inflated and fleshy, with subglobose to obliquely ovoid follicle. It produces a large amount of seeds (3 cm) that are small, flat, obovate, compressed with silky white pappusat at one end [2,6,7].

Traditional Medicinal Uses

Its root bark secretions have been used by the physicians for treating skin diseases, intestinal worms, cough, extreme generalized edema whereas the root bark was used to treat elephantiasis. The milky sap form this plant was considered to be a strong purgative and caustic. Flowers were used for effective digestion catarrh, and to increase appetite. The latex has also been used to comfort toothache. Flowers have been used to treat asthma. Numerous other uses of the plant in treating leprosy, hepatic, and splenic enlargements have also been reported. Products obtained from boiling leaves have been used to treat paralysis. Whereas powder prepared from dry leaves was used in wound healing. Even tanners used the milky sap to remove hair from hides [2,8].

Phytochemical Reports

There are numerous research and review article on phytochemical constituents present in *C. procera*. The presence of cardenolides (cardiac glycosides) especially in the latex makes it potentially toxic. The leaf contains approximately 162 mg/g cardenolides in dry weight. Some of the important cardenolides present are voruscharin, uscharidin, uzarigenin, calotroposide, calactin, calotoxin, uscharin, ascleposide, calotropagenin, coroglaucigenin,

calotropin, proceroside, proceragenin, and syriogenin. Various plant parts contain a number of cardenolides such as uscharin and calotropagenin in the latex, calotropin and calotropagenin in the leaves, calotoxin and calactin in the root bark; uscharidin, calotropin, proceroside, and calactin in the stem, coroglaucigenin and uzarigenin in the fruit pericarp. Around 0.23-0.47 % cardenolides are present in seeds that mostly consists of coroglaucigenin or frugoside [9].

Table 1. Chemical structures of phytoconstituents present in *C. procera*.

S.no.	Chemical Composition	Class of Compound	Source	Reference
1	Calotropin	Cardenolides	Latex	[14]
2	Calotoxin			[15]
3	Calactin			[16]
4	Uscharin			[17]
5	Voruscharin			[17]
6	Uzarigenin			[17]
7	Syriogenin			[17]
8	Proceroside			[17]
9	Calotropagenin			[17]
10	Calotropain	Enzymes		[12]
11	Calotropin DI and DII			[16]
12	Calotropain FI and FII			[16]
13	β-amyrin	Terpenes		[16]
14	Lupeol			[16]
15	Taraxast-20(30)-en-3(4-methyl 3-pentenoate)			[16]
16	Quercetin-3- rutinoside	Flavonoid		[18]
17	α-Amyrin	Others	Leaves	[19]
18	α-Amyrin acetate			[19]
19	β-sitosterol			[20, 21]
20	Urosolic acid			[22, 23]
21	Calotropin			[24]
22	Calotropagenin			[25]
23	Procesterol			[26]
24	Multiflorenol			[27]
25	16-α-Hydroxy calotropagenin			[27]
26	O-Pyrocatechuic acid			[27]
27	Quercetin-3-rutinoside with D-arabinose, glucose, glucosamine and L-rhamnose	Flavonoid Polysaccharides	Flowers	[28, 29]
28	3-Proteinase	Enzymes		[28, 29]
29	Calotropain (protease)			[28, 29]
30	Lupeol	Other constituents		[28, 29]
31	Uscharin			[28, 29]
32	Proceroside			[28, 29]
33	Proceragenin(cardenolide)			[28, 29]
34	Syriogenin			[28, 29]
35	Taraxast-20(30)-en-3-(4-methyl-3 pentenoate)3 thiazoline			[28, 29]
36	Cardenolide			[28, 29]
37	Gigantin			[28, 29]
38	Giganteol			[28, 29]
39	Isogiganteol			[28, 29]
40	Uscharidin			[28, 29]
41	Uzarigenin			[28, 29]
42	Voruscharin			[28, 29]
43	α-calotropeol			[28, 29]
44	3-epimoretenol			[28, 29]
45	α- lactuceryl acetate			[28, 29]
46	α-lactuceryl isovalerate			[28, 29]
47	5-hydroxy-3, 7- dimethoxyflavone-4'-O-β-glucopyranoside		Roots	[30]
48	2β, 19-epoxy- 3β, 14β-dihydroxy-19-methoxy-5α- card-20(22)-enolide			[30]
49	β-ahydroepidigitoxigenin3β-O-glucopyranoside			[30]
50	Caroglaucigenin		Seeds	[31]
51	Frugoside			[31]
52	Carotoxigenine			[31]
53	Calotropin			[31]

Apart from the presence of cardenolides, phytochemicals such as sterols, flavonoids, coumarins, alkaloids, triterpenes, saponins, tannins, and hydrocarbons have also been isolated from *C.* procera. Rutin is a major form of flavonoids present in the plant. With highest amount found in latex at 9.7 % while roots contain 1.7 %, stem has 4.8 %, leaves comprise of 5.0 % and 7.6 % in flowers. In addition to this, the plant is also reported to contain resins, fatty acids, proteases, hydrocarbons, amino acids, and many other minerals. Polyphenol content like flavonoids also varies in different parts of plant, from 3.3 % in leaves to 4.9 % found in stems [10].

Just like other parts of the plant, flowers also contain various amounts of phytochemicals, namely, α and β-amyrins, an alkaline phosphate, cyaindin-3-rhamnoglucoside, cycloart-23-en-3 β , 25-diol, cyclosadol, multiflorenol, procestrol, quercetin-3-rutinoside, β-sitosterol, β-sitost-4en-3one, and stigmasterol, Cyanidin-3-rhamnoglucose and triterpene calotropenyl acetate [11].

Other constituents present in the leaves are ascorbic acid, calactin, calotoxin, calatropagenin, calotropin, polysaccharide containing D-arabinose, D-glucose, D-glucosamine and L-rhamnose, calotropagenin, and 3-proteinase. Calotropin, α-calotropeol, 3-epimoretenol, gigantin, giganteol, isogiganteol, α-lactuceryl acetate, α-lactuceryl isovalerate, lupeol, proceroside, proceragenin, syriogenin, taraxast-20 α -(30)-en-(4-methyl-3-pentenoate), 3'-thiazoline cardenolide uscharidin, uzarigenin, voruscharin and β-sitosterol are present in the latex [12]. Latex also contains about 11-23 % of rubber. Additionally, triterpenoids α- and β-amyrin, lupeol, taraxasteryl acetate, α- and β-calotropeol, 3-epimoretenol, multiflorenol, cyclosadol, several triterpene esters, the sterols β-sitosterol and stigmasterol, calotropin, procerain, procerain-B and the alkaloid such as choline are also found to be present in latex [12]. Enzolisoleneolone, benzollineolone, long-chain fatty acids, and C (18) isoursane are present in root-bark. Mudarine, an alkaloid which is also reported to be a principal cardioactive constituent is found in the leaves [13].

PHARMACOLOGICAL ACTIVITIES

According to the available literature all the plant parts (root-bark, stem-bark, leaves, flowers, latex) contain phytochemicals possess anticoagulant, antidiarrheal, anti-inflammatory, antioxidant, antiulcer, analgesic, hepatoprotective, smooth muscle-contracting, blocks neurotransmission and wound healing activity. It also has spermicidal activity, which explains the reason behind its usage in abortions [1].

Analgesia

Dry latex (DL) of *C. procera* is an analgesic. A dose of 415 mg/kg of dry latex is as effective an analgesic as oral administration of 100 mg/kg aspirin against acetic-acid induced writhing. The effect of DL at a dose of 415 mg/kg against acetic acid-induced writhing was more pronounced as compared to an oral dose of aspirin (100 mg/kg). DL (830 mg/kg) produced marginal analgesia in tail-flick model, which was comparable to aspirin [32, 33]. Proteins found in latex of *C. procera* have antinociceptive effect as observed in mice models where acetic acid, formalin and hot plate test were used as nociceptive stimuli. Latex proteins at doses 12.5, 25 and 50 mg/kg was shown to be antinociceptive in a dose dependent manner [34, 35].

Anticonvulsant

Maximal electroshock seizures (MES), pentylenetetrazol (PTZ), lithium-pilocarpine, and electrical kindling seizures models have been used to detect anticonvulsant effects of aqueous and chloroform extracts of *C. procera* roots [36]. Chloroform extract proved to be more effective against seizures in MES and the PTZ models. Nevertheless both aqueous and chloroform extractions from roots were comparable in subsiding convulsions in lithium-pilocarpine and electrical kindling induced models [37].

Antimalarial effect

Ethanolic extracts of different parts of *C. procera* have proven to be effective against both *Plasmodium falciparum* sensitive and resistant strains with IC_{50} value ranging from 0.11 to 0.47 mg/mL and 0.52 to 1.22 mg/mL respectively [32].

Antidiabetic and Antimyocardial Infarction Activity

Dried latex of *C. procera* when orally administered to alloxan-induced diabetic rats at doses 100 and 400 mg/kg resulted in decreased blood glucose levels and increased hepatic glycogen content [32, 35]. Additionally, isoproterenol induced myocardial infarction in albino rats was also studied. Administration of 300 mg/kg of ethanolic extract of latex before inducting myocardial infarction in albino rats considerably reduced levels of serum glutamic-pyruvic transaminase, serum glutamic oxaloacetic transaminase, and alkaline phosphatase level in serum [35].

Antimicrobial Activity

The antimicrobial activity of leaf and latex aqueous, ethanol and chloroform extracts against five species of bacteria, *Escherichia Coli*, *S. Aureus*, *Staphylococcus Albus*, *Streptococcus Pyogenes*, and *Streptococcus Pneumonia*, three fungi, *Aspergillus Niger*, *Aspergillus Flavus,* and *Microsporum Boulardii* and *C. Albicans*, a type of yeast was evaluated using paper disk method and agar well diffusion [35]. Ethanolic extract followed by chloroform showed the most promising results. Extractions by all the three solvents proved to be antimicrobial in nature. The only exception was inactivity of aqueous extract against *P. aeruginosa* and *S. pyogenes*. Likewise, aqueous extract also failed to be as effective as ethanolic and chloroform extracts against fungal and yeast species [37].

Anti-inflammatory Activity

Dry latex of *C. procera* has also been reported to exhibit anti-inflammatory activity against inflammatory mediators such as carrageenan and formalin. Both aqueous and methanolic extract of dry latex had a more profound anti-inflammatory effect against carrageenan than phenylbutazone (PBZ). Nevertheless, it was considerably comparable to chlorpheniramine and PBZ against histamine and prostaglandin E_2 induced inflammation respectively. Both methanolic and aqueous extract were also effective against bradykinin, and serotonin induced inflammation. Their increased potency compared to PBZ was also corroborated by histological analysis [38]. Interestingly crude dry latex has also proved to be potent against inflammation [39]. Additionally, methanolic dried extract was also compared with PBZ, and rofecoxib both COX-2 inhibitors. It was found that methanolic dried extract greatly reduced cell influx, release of mediators, and oxidative stress related to arthritis, and hence has the potential to be used as anti-arthritic agent. The protective role of protein sub-fraction of latex was also reported in monoarthritis rats [35, 40].

Wound Healing Activity

Topical application of latex's sterile solution greatly complemented the wound healing process in guinea pigs. The latex significantly augmented the healing process by markedly increasing collagen, DNA and protein synthesis and epithelialization. Studies of wound healing have also been carried out for surgical wounds using ethanolic extract of bark [44, 49].

Potency against Ulcer

Different ulcer models have been used to study the anti-ulcer activity of *C. procera*. The results proved to be significant as it inhibited aspirin, reserpine, absolute alcohol, and serotonin-induced gastric ulcerations in rats. Duodenal ulcers induced by histamine in guinea pigs were also greatly affected [41].

Antifertility Activity

Due to the traditional use of *C. procera* in abortions, the effect of ethanolic extract of *C. procera* root was studied in albino rats to substantiate its antifertility activity. Viable anti-implantation with inhibition rate up to 100 % at 250 mg/kg was observed though no anti-estrogenic effects were found [42].

Antidiarrheal Activity

Dried latex obtained from *C. procera* have also been tested against diarrhea. Oral administration of 500mg/kg dried latex had similar effects to atropine and PBZ in 80 % of castor oil treated rats [43].

Toxicity Studies

C. procera is a toxic plant that is avoided by grazing animals. Its latex is used by tribes to poison arrows used for hunting. If in contact with human eye, it could cause ocular toxicity, causing loss of vision and photophobia [44].

Anticancer and Cytotoxic Properties

Plant extracts and their antitumor potential

Root bark: Reduction in Breast Cancer Tumor Growth through NF-*κ*B Pathway Suppression

Breast cancer is one of the most common form of malignancies in women around the world. Till now, therapies in use have failed to alleviate or cure the disease. Thus, necessitating discovery of new drugs that are more potent. In this regard protein isolated from root bark of *C.* procera was used to study its *in vitro* and *in vivo* anti-malignant potential. Breast cancer cell lines, MCF-7 and MDA-MB-231 treated with *C. procera* protein restricted proliferation and induced apoptosis by suppressing nuclear factor kappa B (NF-***κ***B). The effect was also noticed *in vitro* in dimethyl benz(a)anthracene (DMBA)-induced breast cancer rats models. It was also found that the

decrease in tumor size did not affect overall body weight. The suppression of NF-kB was found to be induced by TNF-α via suppression of IkBa phosphorylation and degradation. Additionally, numerous NF-kB-dependent genes such as Bcl-2, an anti-apoptosis gene, were also down regulated [45].

Stem extracts: Anti-proliferative activity of *Calotropis procera*

C. procera is a dynamic plant in terms of its potential as a source of antitumor phytochemicals. Organic extracts from stems using ethyl acetate and acetone have also been reported to induce apoptosis and antimitotic activity in mouse models of Sarcoma 180 tumor. Additionally the toxicity against liver and kidneys was also found to be reversible.

In vitro and in vivo anti-proliferative activity of *Calotropis procera* stem extracts

Latex: Transgenic mouse model of hepatocellular carcinoma

Dried latex obtained from *C. procera* was also evaluated for its *in vitro* and *in vivo* antitumor activity in a study. In a study, HCC (hepatocellular carcinoma) mouse model, developed by integrating chimeric transgene having hepatitis B virus X and murine c-myc genes exhibiting inflammation and angiogenesis was used [46]. Mice were orally fed an aqueous solution of dried latex (400 mg/kg) for 15 weeks and their livers were examined for histological changes in ensuing weeks in addition to the serum levels of VEGF (Vascular Endothelial Growth Factor). Dried latex treatment not only provided astounding protection against hepatocellular carcinoma but also markedly reduced serum VEGF levels in treated mice. The chemoprevention observed was said to be due to its potential blockade of inflammatory mediators [47]. Interestingly, mitochondrial mediated apoptosis was not observed. However, one could not rule out the possibility of DNA fragmentation by DNAse activity irrespective of mitochondrial mediation [48, 49].

Stem-leaves: Anticancer

Total extract obtained from stem and leaves of *C. procera* show anti-proliferative activity at 10, 30 and 100 mg/mL concentration against HCT-15 cancer cell line. Further fractionation revealed chloroform soluble extract to be the most potent [50].

Laticifer proteins: Anticancer

In addition to the self-defense capabilities of latex produced by *C.* procera, it also contains laticifer proteins that have shown cytotoxic activity against SF295 and MDA-MB-435 cell lines with IC50 values ranging from 0.42 to 1.36 lg/mL respectively. Interestingly, the viability of peripheral blood mononuclear cells was not affected. Moreover, prior treatment with proteases diminished the cytotoxic effects of laticifer proteins, which further proves cytotoxicity being induced by laticifer proteins only. The mode of action was found to be the inhibitory effect of these proteins on DNA synthesis by interfering with topoisomerase I activity [51].

Cardinolides: Anticancer

In spite of the recent progress in new drug discovery, cancer still remains an elusive disease. This necessitates the discovery and development of new approaches to tackle this deadly disease. *C. procera* has been used as a source of traditional medicine against ascariasis, schizophrenia, as anti-bacterial, anthelmintic, insecticidal, anti-inflammatory, anti-diarrhoeal and larvicidal. Rich in cardinolides, recent studies led to the discovery of cardiotonic steroid, UNBS1450 01 in *C. procera* with proven anti-cancer property. A potent inhibitor of sodium pump, this cardinolide showed anti-proliferative and apoptotic properties. UNBS1450 01 interferes with the actin cytoskeleton of cells once it binds to sodium pump on cellular membrane via induction of autophagy, suppression of NF-kB and c-Myc expression in cancer cells [52].

CONCLUSION

C. procera is widely distributed geographically. The plant is of tremendous pharmaceutical importance that needs to be exploited by researchers for new drug discoveries. It is a rich source of organic compounds with vast functionalities. Recent studies also implicate its usefulness in cancer treatment. Detailed i*n vitro* and *in vivo* studies are warranted to fully utilize the anti-cancer potential of this ubiquitous plant. . The deep down knowledge about medicinal plants plays a vital role in primary health care and has great potential for the discovery of new herbal drugs.

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