REVIEW
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Calotropis procera: a review of molecular mechanisms, bioavailability, and potential anticancer property

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Competing interests

The authors declare no conflicts of interest.

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Abbreviations

Cp, calotropis procera; CAE, crude aqueous; CME, crude methanolic; CP, crude powder; DL, dried latex; PBZ, phenylbutazone; ROS, reactive oxygen species; MMP, matrix metalloproteinase.

Citation

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Abstract

Calotropis procera (Cp) is a traditional medicinal plant that has attracted significant attention for its potential anticancer properties. This review consolidates current research on the Cp bioactive compounds found in Cp, including cardenolides, flavonoids, and terpenoids, which exhibit cytotoxic effects against various cancer cells. These compounds function through multiple mechanisms, such as inducing apoptosis, inhibiting cell proliferation, suppressing angiogenesis, and modulating oxidative stress. Preclinical studies demonstrate that Cp extracts effectively reduce tumor size and improve survival rates in animal models. Furthermore, Cp influences key signaling pathways like PI3K/Akt and NF-κB, which contribute to its anticancer potential. Its therapeutic effects extend beyond oncology, encompassing, antinociceptive, anticonvulsant, antimalarial, anthelmintic, antioxidant, antidiabetic, myocardial infarction prevention, schizontocidal, antimicrobial, anti-inflammatory, larvicidal, immunomodulatory, antiulcer, Antifertility, antidiarrheal, estrogenic, and Dermatophytic properties. Despite the promising preclinical data, further investigation is necessary to address challenges such as bioavailability, toxicity, and the standardization of Cp-based treatments. This review highlights the therapeutic promise of Cp as a complementary anticancer agent while emphasizing the need for rigorous clinical trials to confirm its safety and efficacy.

Keywords: *Calotropis procera*; anticancer; PI3K/Akt; NF-κB

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Introduction

Approximately 80% of individuals in developing nations rely on herbal remedies for their primary healthcare needs [1]. A variety of bioactive compounds are derived from plants. *Calotropis procera* (Cp) is one of the notable plants extensively utilized for its medicinal properties [2]. The name is derived from Greek, meaning while it comes from Latin, referring to cuticular wax found on its leaves and stems [3]. It is commonly known as Aak, king's crown, rubber bush, Sodom apple, and rubber tree [4]. This perennial belongs to the Apocynaceae family and is classified as an evergreen, softwood xerophytic plant that thrives in various regions, particularly in the dry and semi-arid regions of tropical and subtropical Asia and Africa [5, 6]. In Ayurveda, the dried foliage of Cp is used to alleviate rheumatic discomfort and paralysis, as well as serve as an expectorant [6, 7]. The tender leaves are specifically employed for the treatment of migraines [7].

Additionally, the powdered form of these leaves is used to enhance wound healing, function as a laxative, and address issues related to indigestion. In traditional Saudi Arabian medicine, a decoction made from the aerial parts of Cp is employed to alleviate constipation, joint pain, fever, and muscular discomfort [8]. Furthermore, Cp has demonstrated efficacy in managing various skin ailments, diarrhea, and sinus fistula [9]. Previous studies have indicated that the solvent can influence the solubility of bioactive compounds. Aqueous extracts are typically cost-effective and environmentally friendly [10]. Water is particularly advantageous as a natural solvent, as it is free from usage limitations. Phytotherapy involves the use of plants to produce traditional medicines and represents a potential option for treating various cancers [11, 12]. In recent years, herbal medicine has gained attention as an alternative approach to cancer treatment [13]. Currently, there is a growing interest in the application and evaluation of the anticancer properties of plant and their compounds. However, the mechanisms by which these plant-based drugs exert their anticancer effects remain largely unclear. Nonetheless, it is evident that Cp, being a rich source of antioxidants, plays a significant role in cancer prevention and treatment by inducing antioxidant effects [14]. Over the past few decades, numerous natural and synthetic compounds have been clinically applied due to their strong pharmacological activities. For example, plant-derived saponins demonstrate physiological activities by binding to nuclear receptors, making them promising candidates for selective receptor modulators [15]. This review consolidates current research on Cp anticancer properties, providing insights into its mechanisms of action and therapeutic potential [16].

Main active ingredients and chemical composition of Cp

The primary bioactive components in the crude extract of Cp leaf include flavonoids, tannins, alkaloids, phenols, steroids, terpenoids, and saponins [17]. Neophytadiene serves various therapeutic purposes, including analgesic, antipyretic, anti-inflammatory, antimicrobial, and antioxidant functions [18]. Hexahydrofarnesyl acetone exhibits significant anti-bacterial, anti-nociceptive, and anti-inflammatory properties [19]. Lanosterol, a specific type of tetracyclic triterpenoid, is recognized as the precursor from which all steroids are derived [20, 21]. The compound 2,4-dimethyl-benzo [H] quinoline, has been extracted from the foliage of *Lasiocarpa americana* and functions as an antibacterial agent by inhibiting protein synthesis and demonstrates antiprotozoal effects [22, 23]. Squalene is utilized for its antimicrobial, antitumor, antioxidant, anti-cancer, and repellent capabilities [24].

Botanical overview of Cp

Cp, commonly known as the apple of Sodom or milkweed, is a perennial shrub belonging to the Apocynaceae family. It thrives in tropical and subtropical regions and is recognized for its latex, which contains various bioactive compounds [25]. The plant's parts, including the leaves, roots, bark, and latex, have been utilized in traditional medicine for centuries. Phytochemical studies of Cp have revealed a diverse array of secondary metabolites, including alkaloids, flavonoids, cardenolides, and terpenoids, that are believed to contribute to its medicinal properties [26]. Among these compounds, cardenolides have shown the most promise in cancer research. The anticancer potential of Cp is attributed to various bioactive compounds, particularly cardenolides, flavonoids, and terpenoids, as illustrated in Figure 1 [27, 28].

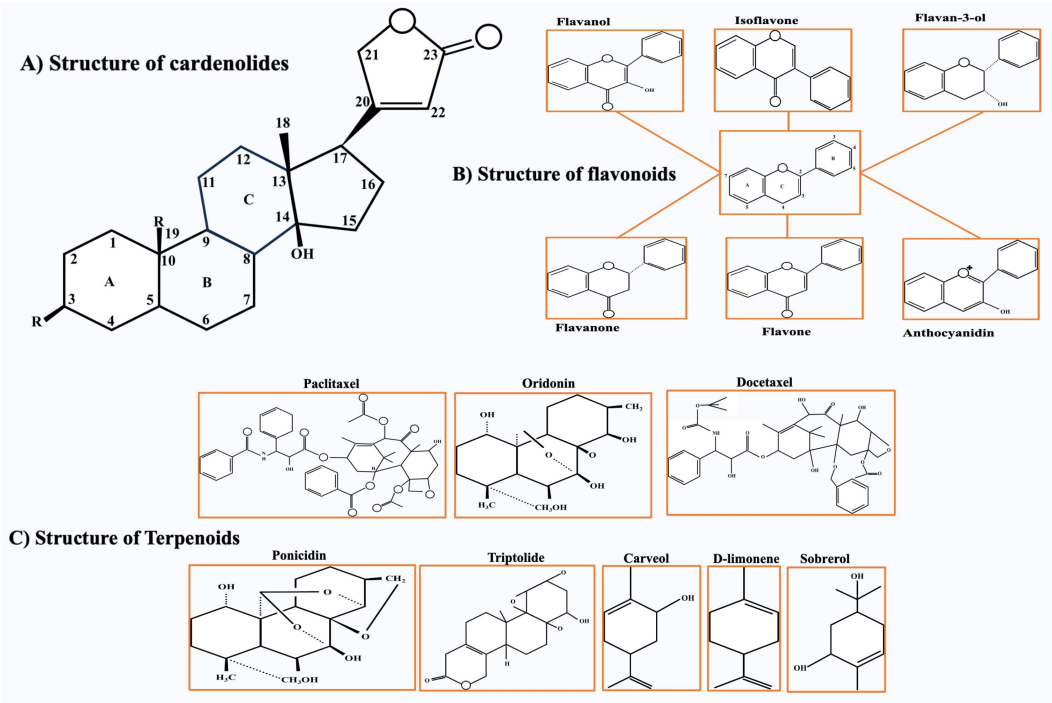

Figure 1 Chemical structure of cardenolides, flavonoids, and terpenoids [28]

Cardenolides

Cardenolides are naturally occurring cardiac glycosides found in plants and plant-eating insects worldwide. Ingestion of cardenolide can lead to severe dysrhythmias, including second or third-degree heart block and cardiac arrest, with digitalis cardenolide poisoning being the most prevalent form. Cardenolides derived from common plants have been used as insecticides and rodenticides for centuries [29]. Poisoning from cardenolide, particularly those found in yellow, pink, or white oleander and fruits from the Cerbera manghas family (e.g., sea mango, pink-eyed Cerbera, and odollam tree), is a significant cause of food poisoning in South Asia, with thousands of cases reported annually and a mortality rate of 5 to 10%. All plant components, whether fresh, dried, or boiled, are hazardous. Children can die after ingesting just one leaf, while adults may succumb after consuming 8/10 seeds, 15–20 g of the root, or 5/15 leaves [30]. Toxic effects primarily impact the cardiovascular and autonomic nervous systems. The most common molecular mechanism involved is the blockage of the Na^+/K^+-ATPase channel [31]. Initial symptoms may appear within minutes of consumption; therefore, extended hospitalization and monitoring are recommended following cardenolide administration, as the onset of serious dysrhythmias can be delayed by up to 72 h [29].

Flavonoids and phenolic compounds

Flavonoids are present in a variety of plants, as well as their derived food products. These polyphenolic chemical compounds have garnered renewed interest as possible anticarcinogens, and the molecular mechanism underlying their anticarcinogenic properties and bioavailability have been extensively studied [32]. Previous research has identified the primary dietary flavonoids-flavones, flavonols, and flavan-3-ols (catechins) and assessed their significance in cancer prevention [33]. After absorption, flavonoids are delivered to target organs, either with or without metabolic conjugation, where they exert their anticarcinogenic effect [30]. The anticarcinogenic actions of flavonoids are mediated through an antagonistic effect on the aryl hydrocarbon receptor and the regulation of phase I and II drug metabolizing enzymes and phase III transporters [34]. Experimental data indicate that flavonoids influence signal transduction pathways at all stages of carcinogenesis. To identify specific targets, it is essential to thoroughly investigate the interactions between flavonoids and biomolecules in vivo [35]. Additionally, when considering the use of flavonoid supplements for cancer prevention, it is crucial to examine potential adverse effects. Consequently, further research is needed to establish acceptable levels of flavonoid consumption as chemo-preventive agents [33].

Terpenoids

Terpenes are hydrocarbon, and their oxygenated derivatives are abundant in plants, microbes, marine creatures, fungi, and insects. Terpene compounds come in various forms, including isomers [36]. These are classified as monoterpenes (limonene), sesquiterpenes (elemene), diterpenes (camphene), and polyterpenes. Terpenoids found in Cp demonstrate considerable bioactive properties, notably their capacity to trigger apoptosis and suppress the proliferation of cancerous cells. They influence multiple signaling pathways related to cell cycle control and apoptosis, serving as effective anti-cancer agents. These compounds interfere with cellular processes, resulting in programmed cell death (apoptosis) and hindering the growth of malignant cells [37]. Terpene compounds often exhibit high lipophilicity. They dissolve in organic solvents and have asymmetric carbon atoms with optical rotation [37]. Low-molecular-weight terpenes, such as monoterpenes and sesquiterpenes, are volatile oily liquids. Their boiling point rises with increasing molecular weight and double bonds [38]. High-molecular-weight terpenoids, such as diterpenoids and triterpenoids, are primarily solid crystals as shown in Table 1 [29, 30, 37].

Extraction and purification of herbal components

The extraction and purification of herbal components is an important step in isolating bioactive compounds from plant materials for use in the pharmaceutical, nutraceutical, and other industries [39]. This process typically begins with the selection of appropriate plant parts, such as leaves, roots, or flowers, followed by the extraction of the desired components using various techniques, as illustrated in Figure 2 [40]. Extraction often involves the use of solvents, i.e., water, ethanol, or methanol, to isolate the target compounds from the plant

Table 1 Phytochemical constituents of Cp and their potential anticancer effects

Phytochemical	Class	Biological activity	Potential anticancer mechanism	References
Cardenolides	Steroidal glycosides	Cytotoxicity, apoptosis induction	Inhibition of Na^+/K^+ ATPase, apoptosis via caspase activation	[29]
Flavonoids	Polyphenols	Antioxidant, anti-inflammatory	Modulation of PI3K/Akt pathway, apoptosis induction	[30]
Terpenoids	Terpenes	Anti-inflammatory, cytotoxic	Induction of apoptosis, inhibition of proliferation	[37]
Alkaloids	Nitrogen-containing compounds	Cytotoxicity	Cell cycle arrest, apoptosis induction	[30]

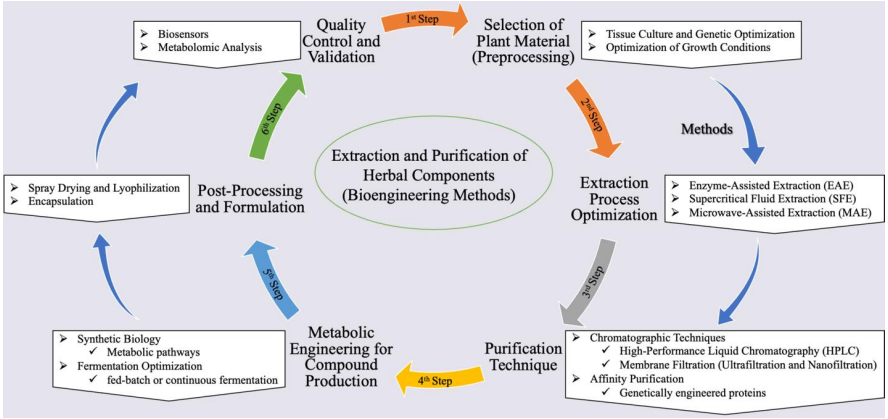

Figure 2 Step-by-step demonstration of bioengineering methods used for extraction and purification of herbal components

matrix [41]. To enhance efficiency, innovative methods such as enzyme-assisted extraction, supercritical fluid extraction, and microwave-assisted extraction are employed. These techniques can reduce extraction times, increase yields, and preserve the integrity of heat-sensitive bioactive compounds [42–45]. For example, supercritical fluid extraction using supercritical CO_2 is an environmentally friendly extraction method that eliminates the need for hazardous solvents. Microwave-assisted extraction speeds extraction by heating the plant material using microwave energy [46].

Purification occurs after extraction and is essential for identifying specific bioactive compounds within a mixture of plant components. Common purification techniques include high-performance liquid chromatography, ultrafiltration, and affinity chromatography [47, 48]. In high-performance liquid chromatography, compound separation is based on the interaction between the compounds and a stationary phase, along with a solvent system, which facilitates the precise isolation of desired components [49]. Membrane-based ultrafiltration further concentrates and purifies extracts by allowing smaller molecules to pass through while retaining larger undesirable ones [50]. Bioengineering techniques are employed throughout the extraction and purification process to enhance efficiency and yield [42]. Genetic engineering can be utilized to promote the synthesis of specific bioactive compounds in plants, while metabolic engineering of microbes enables the production of herbal compounds in microbial systems [42]. Following purification, spray drying, or freeze-drying is employed to stabilize the purified compounds, preparing them for long-term storage or formulation into final products. Traditional methods of extracting and purifying herbal components can be integrated with modern bioengineering and biotechnology to develop high-quality, potent, and durable bioactive compounds for various applications [51].

Therapeutic and pharmacological activity of Cp

Cp has historically been used to treat a wide range of human illnesses such as colds, fevers, Leishmaniasis, asthmatic attacks, rheumatic disorder, dermatitis, indigestion, diarrhea, elephantiasis, and skin disorders [6]. In traditional chinese medicine, Cp is esteemed for its diverse therapeutic attributes to treat various health conditions. Plant roots, leaves, and latex are utilized to exploit their medicinal benefits. These parts are traditionally recognized for their anti-inflammatory, analgesic, and antimicrobial effects, which are beneficial in managing pain, swelling, infections, and digestive ailments. Notably, latex is used topically for dermatological issues and is thought to possess purgative and detoxifying qualities. Traditional chinese medicine practitioners incorporate Cp into herbal remedies to enhance immune function, alleviate respiratory symptoms, and reduce joint discomfort (Figure 3) [52].

Analgesic and antinociceptive activity

The analgesic efficacy of the dry latex from Cp at a dosage of 415 mg/kg was significantly greater than that of an oral aspirin administration at 100 mg/kg, particularly in acetic acid-induced writhing. Additionally, a higher dose of dry latex (830 mg/kg) demonstrated a slight analgesic effect in a tail-flick assay, comparable to aspirin [53]. Furthermore, the antinociceptive properties of proteins extracted from Cp latex were assessed using three distinct nociception models: acetic acid, formalin-induced abdominal constrictions, and the hot plate test in murine subjects. The protein fraction of the latex, administered at doses of 12.5, 25, and 50 mg/kg, exhibited a dose-dependent antinociceptive effect that appeared to operate independently of the opioid system [54].

Anticonvulsant activity

The evaluation of anticonvulsant properties of aqueous and chloroform extracts from the root of Cp was conducted using various seizure models, including maximal electroshock seizures, pentylenetetrazol, lithium-pilocarpine, and electrical kindling in rat subjects [55]. The chloroform extract demonstrated a markedly significant effect in the maximal electroshock seizures and pentylenetetrazol assessments. Additionally, both extracts were effective in suppressing convulsions triggered by lithium-pilocarpine and electrical kindling [56].

Antimalarial activity

Ethanolic extracts derived from various components of Cp demonstrated IC_{50} values between 0.11 and 0.47 mg/mL against the CQ-sensitive strain of *Plasmodium falciparum* MRC 20, and between 0.52 and 1.22 mg/mL against the CQ-resistant MRC 76 strain, with extracts from flowers and buds exhibiting the highest efficacy. While these extracts are 220 to 440 times less potent than CQ, they warrant additional investigation to isolate and identify the active compounds present [57].

Anthelmintic efficacy

The anthelmintic efficacy of Cp flowers was assessed with levamisole through in vitro and in vivo experiments involving live *Haemonchus contortus*. In the in vitro phase, crude aqueous (CAE) and crude methanolic (CME) extracts were utilized, while the in vivo phase employed CAE, CME extracts, and crude powder (CP) of the flowers [58]. The percentage reduction in egg count was recorded at 88.4% and 77.8% for sheep treated with CAE and CP at a dosage of 3 g/kg, respectively; in contrast, CME demonstrated the least efficacy with a reduction of only 20.9%. All extracts displayed lower anthelmintic activity compared to levamisole, which achieved a reduction rate of 97.8–100%. Additionally, Cavalcante et al. investigated the chemical composition and in vitro activity of latex against *H. contortus* [56].

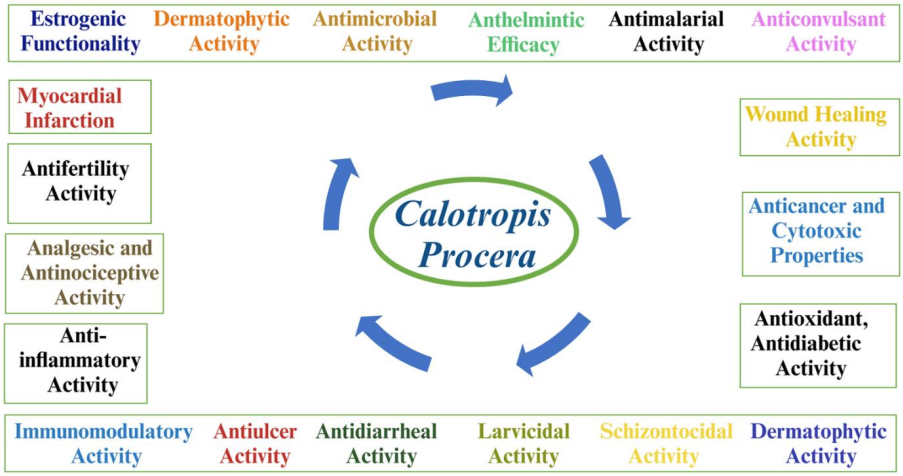

Figure 3 Therapeutic and pharmacological activity of Cp

Antioxidant and antidiabetic activity
The antioxidant properties of dried latex (DL) from Cp, as well as its antidiabetic effects in alloxan-induced diabetic rats, were investigated. Oral administration of DL at doses of 100 and 400 mg/kg was performed [59]. The findings indicated reduced blood glucose levels and increased hepatic glycogen content. Tsala et al. assessed the antioxidant potential of the ethanol extract derived from the bark of Cp in the context of surgical wound healing [60].

Myocardial infarction
The protective effects of Cp latex against isoproterenol-induced myocardial infarction (20 mg/100 g) were examined in albino rats. The administration of an ethanolic latex extract at a dosage of 300 mg/kg, given orally three times daily for 30 days, resulted in a significant decrease in the levels of elevated serum marker enzymes, including serum glutamic-pyruvic transaminase, serum glutamic oxaloacetic transaminase, and alkaline phosphatase, in both serum and heart homogenates [61].

Schizontocidal activity
The impact of crude extracts from the flower, bud, and root of Cp on two strains of *P. falciparum*. one sensitive to chloroquine (MRC 20) and the other resistant (MRC 76) was evaluated. The efficacy of these extracts was compared, revealing greater effectiveness against the chloroquine-sensitive strain in vitro than against the chloroquine-resistant strain [62].

Anticancer and cytotoxic properties
The investigation into the anticancer and cytotoxic effects of the DL of Cp in a transgenic mouse model of hepatocellular carcinoma demonstrated a complete protective effect against hepatocarcinogenesis [63]. Notable reductions in serum levels of vascular endothelial growth factor were observed, alongside significant cell death in Huh-7 and COS-1 cell lines, while AML12 cells remained viable [64]. This phenomenon was further characterized by extensive DNA fragmentation in Huh-7 and COS-1 cells. However, no alterations in Bcl2 and caspase 3 levels, which are established markers of apoptosis, were detected. Additionally, Gurung et al. identified the anticancer bioactive compound proceraside through molecular docking studies with macromolecules that play critical roles in the cell cycle and DNA replication [59].

Antimicrobial activity
The antimicrobial properties of leaf extracts from Cp were assessed, revealing a notable inhibitory effect of the latex extract against *Candida albicans* [65]. Additionally, a novel cardenolide, 7B, 14B-dihydroxy-5-card-20 (22) enolide (proceragenin), derived from CP, demonstrated antibacterial activity against *Pseudomonas pseudomallei*, the pathogen responsible for melioidosis. All fractions of the leaf extracts effectively inhibited the growth of the microorganisms tested [56]. The antimicrobial efficacy of Cp was specifically evaluated against several pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and the pathogenic fungus *C. albicans*. The study further investigated the antimicrobial effects of ethanol, aqueous, and chloroform extracts from both the leaf and latex of Cp on five bacterial strains: *Escherichia coli*, *S. aureus*, *Staphylococcus albus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*, as well as three fungal species: *Aspergillus niger*, *Aspergillus flavus*, and *Microsporum boulardii*, along with the yeast *C. albicans*, utilizing agar well diffusion and paper disk methods [66]. The findings indicated that ethanol was the most effective solvent for extracting antimicrobial compounds from the leaf and latex of Cp, followed by chloroform and aqueous extracts [67]. The ethanolic latex extracts produced the largest inhibition zone (14.1 mm) against *E. coli* in the agar well diffusion assay, while a zone of 9.0 mm was observed for the same organism in the disc plate method. The growth of six bacterial isolates was inhibited by all three extracts, except for *P. aeruginosa* and *S. pyogenes*, which were not affected by the aqueous extracts of either the leaf or latex. Similarly, the ethanol and chloroform extracts inhibited four fungal species' growth, whereas the aqueous extract exhibited the least effectiveness against the tested fungi [54].

Anti-inflammatory activity
The DL derived from the plant Cp has demonstrated significant anti-inflammatory properties when tested against carrageenan and formalin, which are known to trigger the release of inflammatory mediators. The anti-inflammatory effects of both aqueous and methanolic extracts of DL were found to be more effective than phenylbutazone (PBZ) in the context of carrageenan-induced inflammation, while showing comparable efficacy to chlorpheniramine and PBZ to histamine and prostaglandin E2, respectively [68]. These extracts achieved approximately 80%, 40%, and 30% inhibition of inflammation caused by bradykinin, compound 48/80, and serotonin. Histological evaluations indicated that the extracts surpassed PBZ's ability to inhibit cellular infiltration and subcutaneous edema. A single administration of the aqueous suspension of DL proved to be significantly effective against acute inflammatory responses [65]. Thus, the crude DL of Cp exhibits considerable anti-inflammatory activity. The methanolic dried extract was assessed in comparison to PBZ, a non-selective cyclooxygenase inhibitor, and rofecoxib, a selective cyclooxygenase-2 inhibitor. The methanolic dried extract from Cp significantly diminished cell influx, mediator release, and oxidative stress associated with arthritic conditions, suggesting its potential as an antiarthritic agent. Chaudhary et al. noted a protective effect of a high molecular weight protein sub-fraction of latex in monoarthritis models in rats [58].

Larvicidal activity
The efficacy of Cp was assessed against the larvae of *Anopheles labranchiae*, revealing significant larvicidal properties with an LC_{50} (24 h) ranging from 28 to 325 ppm. This species of giant milkweed demonstrated effectiveness in suppressing feeding behavior and inducing mortality in the larvae. Various rubber-free fractions of the latex were tested for their impact on egg hatching and larval development of *Aedes aegypti*, showing inhibitory effects [69]. Additionally, research has been conducted on the influence of alkaloid extracts derived from the leaves of Cp at the vegetative stage, focusing on the survival rates of fifth-instar larvae and the ovarian development of *Schistocerca gregaria* [70]. The toxicological properties of crude extracts from both leaves and flowers of Cp were evaluated against two termite species, *Heterotermes indicola*, and *Coptotermes heimi*. Furthermore, Cp exhibited moderate larvicidal activity against the second and fourth instar larvae of the laboratory-reared mosquito species, *Culex quinquefasciatus*. Notably, CP demonstrated greater effectiveness compared to *Haloxylon recurvum* and *Azadirachta indica* [71].

Immunomodulatory activity
The ethanolic extract derived from the root bark of CP was assessed for its immunomodulatory effects through a series of immunological evaluations conducted on mice [72]. These evaluations included measurements of humoral antibody titers, delayed-type hypersensitivity responses, peritoneal macrophage counts, vascular permeability assessments, and a comprehensive hematological profile encompassing total red blood cell counts, total leukocyte counts, percentages of neutrophils, and percentages of lymphocyte [73]. Additionally, the study examined the extract's impact on cyclophosphamide-induced myelosuppression across three dosage levels (50, 100, and 200 mg/kg). The findings indicate that the extract enhances the immune defense mechanisms by influencing various immunological parameters. Furthermore, research by Nascimento et al. identified the immunomodulatory effects of latex protein extracts from CP which confer protection against experimental infections caused by *Listeria monocytogenes* [74].

Wound healing activity
Drawing from its historical applications, CP was chosen for an

assessment of its wound-healing capabilities in Guinea pigs. A topical application of 20 µL of a 1.0% sterile latex solution derived from the plant was administered to the subjects [75]. The results indicated a significant enhancement in the healing process, characterized by a notable increase in collagen, DNA, protein synthesis, and improved epithelialization. Additionally, Tsala et al. investigated the antioxidant properties and the healing effects of the ethanol extract from the bark of Cp on surgical wounds [6]. The methanolic extract of Cp has shown potent antimicrobial properties, with four major flavonoids, i.e., kaempferol, quercetin, isorhamnetin, and 5-hydroxy-3,7-dimethoxyflavone found to be effective, particularly quercetin against *C. albicans* and bacterial strains [76]. Quercetin is a prominent flavonoid recognized for its potent antioxidant, anti-inflammatory, and antimicrobial effects. Within the framework of your manuscript, it is essential to underscore quercetin's significance due to its ability to target multiple pathways in the fight against pathogens [77]. Distinct from other flavonoids, quercetin inhibits bacterial DNA gyrase and disrupts cellular membranes, rendering it effective against bacteria and fungi. *C. albicans*, a frequently studied pathogenic fungus, is particularly relevant due to its role in both superficial and systemic infections, especially among immunocompromised patients. Certain bacterial strains exhibit heightened sensitivity to quercetin, attributable to its interference with their growth mechanisms [78]. Quercetin can serve as a valuable indicator of broader antimicrobial efficacy [79]. Cp latex possesses antifungal properties, with Osmotin and Cysteine peptidase enzymes hydrolyzing membrane proteins and inducing cell death through reactive oxygen species (ROS) generation [80, 81]. Its antioxidant capacity has been linked to compounds like isorhamnetin and azaleatin, although quercetin remains the most effective [80].

Antiulcer activity

The investigation into the antiulcer properties of Cp was conducted utilizing various in vivo ulcer models. Findings from the study indicated that Cp markedly reduced gastric ulcerations induced by aspirin, reserpine, absolute alcohol, and serotonin in rat subjects [55]. Additionally, it demonstrated protective effects on the gastric mucosa against aspirin-induced ulceration in pyloric-ligated rats. Notably, significant protective effects were also recorded in cases of histamine-induced duodenal ulcers in guinea pigs [82].

Antifertility activity

The antifertility and hormonal effects of an ethanolic extract derived from the roots of CP (250 mg/kg) were investigated in albino rats achieving complete inhibition (100%) alongside heterotrophic activity (anti-implantation effect). However, no evidence of antiestrogenic activity was found [75].

Antidiarrheal activity

The antidiarrheal properties of Cp DL were assessed. Similar to the effects observed with atropine and PBZ, a single oral administration of DL (500 mg/kg) resulted in a notable reduction in both the frequency of defecation and the intensity of diarrhea, with 80% of the rats treated with castor oil showing protection against diarrhea [83].

Estrogenic functionality

Research was conducted on the impact of ethanolic and aqueous extracts derived from the roots of Cp on the estrous cycle and various aspects of estrogenic functionality in rats. It was determined that both extracts disrupted the normal estrous cycle in 60% and 80% of the treated rats, respectively [84].

Dermatophytic activity

The antifungal properties of fresh latex from Cp were evaluated against dermatophytes, specifically *Trichophyton* spp., *Microsporum* spp., and *Epidermophyton* spp. The findings indicate that *Trichophyton* spp. exhibited the highest susceptibility, while *Microsporum* spp. showed moderate inhibition, and *Epidermophyton* spp. demonstrated the least antifungal response [85].

Mechanisms of anticancer action

The anticancer activity of Cp has been demonstrated through multiple mechanisms.

Induction of apoptosis

The key phytochemicals involved were cardenolides and flavonoids. Apoptosis is a critical mechanism in cancer treatment [86]. Cardenolides from Cp induce apoptosis in cancer cells by activating the mitochondrial (intrinsic) apoptotic pathway [55]. This leads to the release of cytochrome-C, activation of caspases (particularly caspase-3 and caspase-9), and ultimately cell death. Cardenolides disrupt mitochondrial membrane potential, causing a cascade of apoptotic events. It is triggered through caspase activation, leading to DNA fragmentation and cancer cell death [87]. Cardenolides also downregulate anti-apoptotic proteins like Bcl-2 and upregulate pro-apoptotic proteins like Bax, enhancing cell death (Figure 4A) [88].

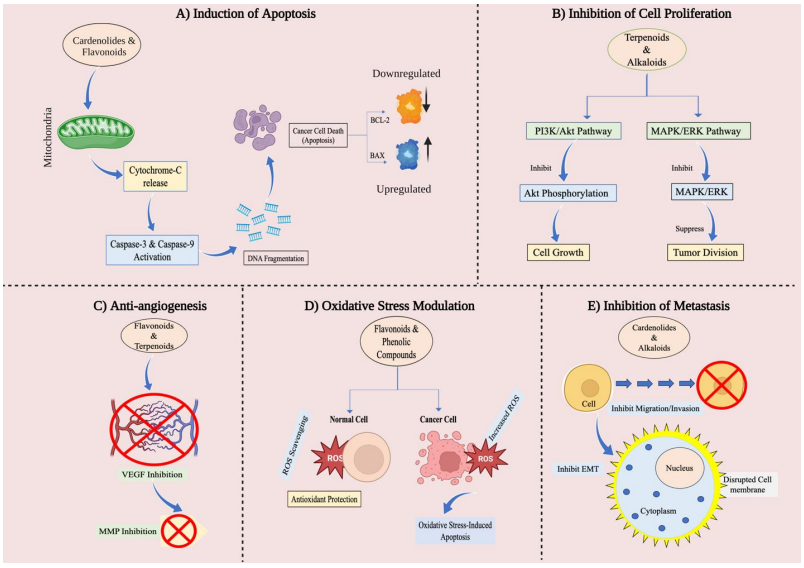

Figure 4 Pathway of anticancer actions. (A) Induction of apoptosis. (B) Inhibition of cell proliferation. (C) Anti-angiogenesis. (D) Oxidative stress modulation. (E) Inhibition of metastasis. MMP, matrix metalloproteinase.

Inhibition of cell proliferation

The key phytochemicals involved were terpenoids and alkaloids. The cancer cells proliferate uncontrollably due to dysregulation of cell cycle checkpoints and signaling pathways. Cp extracts inhibit cell proliferation by affecting key growth-regulatory pathways, such as the PI3K/Akt pathway which is critical for cancer cell survival and growth [89]. Cp extracts have been shown to inhibit Akt phosphorylation, thereby suppressing downstream signals that promote cell growth and proliferation. MAPK/ERK Pathway includes inhibiting this pathway in which Cp can suppress tumor cell division and growth [90]. Cell cycle arrest in which Cp extracts induce cell cycle arrest at the G1 or G2/M phase, preventing the progression of cells through the cell cycle and thus halting their proliferation (Figure 4B) [91].

Anti-angiogenesis

The key phytochemicals involved were flavonoids and terpenoids. The mechanism includes tumor growth and metastasis depending on angiogenesis and the formation of new blood vessels to supply oxygen and nutrients to the tumor [92]. Cp inhibits angiogenesis by VEGF Inhibition in which the extracts downregulate vascular endothelial growth factor (VEGF) which is a key pro-angiogenic factor that stimulates the growth of blood vessels in tumors [93]. Moreover, the matrix metalloproteinase (MMP) are enzymes involved in the degradation of the extracellular matrix, which facilitates tumor angiogenesis. Cp extracts inhibit MMP activity, reducing the ability of cancer cells to form new blood vessels (Figure 4C) [94].

Oxidative stress modulation

The key phytochemicals involved were flavonoids and phenolic compounds. The mechanism includes ROS which play a dual role in cancer progression, contributing to both the promotion of tumor growth and cell death induction [95]. Cp modulates oxidative stress by antioxidant properties, i.e., flavonoids and phenolic compounds act as antioxidants, scavenging ROS and reducing oxidative damage in cells [96]. This helps in the selective killing of cancer cells while protecting normal cells from oxidative damage [97]. Cp reduces oxidative stress in normal cells and paradoxically increases ROS generation in cancer cells, leading to oxidative stress-induced apoptosis in the tumor environment (Figure 4D) [98].

Inhibition of metastasis

The key phytochemicals involved were cardenolides and alkaloids. The mechanism includes the spread of cancer cells from the primary site to distant organs, which is a major cause of cancer-related mortality. Cp may inhibit metastasis by downregulating metastasis-related genes [99]. The plant extracts downregulate genes associated with epithelial-mesenchymal transition; a process crucial for cancer metastasis [100]. Studies have shown that Cp inhibits the cancer cell's migration and invasion abilities by disrupting the cytoskeletal architecture and inhibiting cell adhesion molecules (Figure 4E) [101]. Potential mechanisms of action of Cp in cancer treatment are shown in Table 2 [91, 92, 94, 98–102].

Preclinical studies

Several in vitro and in vivo studies have highlighted the anticancer efficacy of Cp extracts. In vitro, cytotoxicity assays have demonstrated that Cp extracts exhibit significant activity against various cancer cell lines, including breast (MCF-7), liver (HepG2), and colon (HT-29) cancers [103]. In vivo studies using animal models have shown that Cp extracts can reduce tumor volume and increase the lifespan of treated animals, providing strong evidence for its potential as a natural anticancer agent [104]. The in vitro studies on the anticancer activity of Cp extracts are shown in Table 3 [105–108].

Toxicity and safety concerns

Cp has a promising role in anticancer therapy but shows concerns regarding its toxicity. The plant's latex contains potent compounds that can be toxic if ingested in large quantities. Preclinical studies indicate that the therapeutic window for Cp is narrow, and further research is needed to determine safe and effective dosing regimens. The development of standardized extracts and formulations will be crucial in ensuring the safe use of this plant in cancer treatment.

Table 2 Potential mechanisms of action of Cp in cancer treatment

Mechanism of action	Key phytochemicals involved	Description	References
Induction of apoptosis	Cardenolides, flavonoids	Activation of caspases, mitochondrial dysfunction, modulation of Bcl-2/Bax proteins leading to apoptosis in cancer cells.	[91, 102]
Inhibition of cell Proliferation	Terpenoids, alkaloids	Inhibition of PI3K/Akt and MAPK/ERK pathways, cell cycle arrest at G1 or G2/M phases, suppression of tumor cell growth.	[91, 99]
Anti-angiogenesis	Flavonoids, terpenoids	Downregulation of VEGF, inhibition of matrix metalloproteinases (MMPs), reduced blood supply to tumors, and suppression of tumor growth.	[92]
Modulation of oxidative stress	Phenolic compounds, flavonoids	Scavenging of ROS into normal cells (antioxidant effect), induction of ROS into cancer cells leading to apoptosis, and selective oxidative stress modulation.	[94, 98]
Inhibition of metastasis	Cardenolides, alkaloids	Downregulation of metastasis-related genes, inhibition of cancer cell migration and invasion, and disruption of epithelial-mesenchymal transition.	[100, 101]

Table 3 In vitro studies on the anticancer activity of Cp extracts

Cancer cell line	Source	Observed effects	Mechanism of action	Reference
MCF-7 (Breast)	Latex extract	Reduced cell viability, apoptosis	Mitochondrial pathway activation	[105]
HepG2 (Liver)	Leaf extract	Cell cycle arrest, apoptosis	Inhibition of PI3K/Akt	[106]
HT-29 (Colon)	Root extract	Inhibition of proliferation	Suppression of NF-κB	[107]
A549 (Lung)	Latex extract	Induction of apoptosis	Caspase activation, ROS generation	[108]

Conclusion

Cp has significant potential as a natural source of bioactive chemicals with many therapeutic uses, notably in anticancer treatments. Cardenolides, flavonoids, and terpenoids have been demonstrated to have considerable cytotoxic effects on cancer cell lines, acting via mechanisms such as apoptosis induction, cell proliferation inhibition, and angiogenesis suppression. In addition to anticancer properties, Cp has antibacterial, anti-inflammatory, antioxidant, and anti-ulcer properties, making it a multipurpose therapeutic plant. Despite the excellent preclinical evidence, there are still problems, i.e., toxicity, bioavailability, and plant extract standardization. Further research is needed to enhance the therapeutic potential of Cp and prove its efficacy in robust clinical studies. With proper development, this traditional plant could play an important role as a complementary or alternative treatment in modern cancer therapy and other health conditions.

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