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Review Article

Organ-Specific Chemobiology and Translational Readiness of *Calotropis gigantea*: A Systematic Review of Preclinical Evidence

Running title: Organ-specific chemobiology of *C. gigantea*

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Abstract

Background. *Calotropis gigantea* (Apocynaceae) has been investigated for anticancer, antioxidant, anti-inflammatory, wound-healing, antidiabetic, antimicrobial, anti-angiogenic, neuropharmacological and toxicological effects. Interpretation is complicated by differences in plant organ, extraction solvent, chemical composition, experimental model, dose expression and outcome measurement. A biologically meaningful synthesis therefore requires integration of plant organ, extraction polarity, dominant chemotype, mechanism and therapeutic activity.

Objective. To systematically evaluate preclinical pharmacological and toxicological evidence for *C. gigantea* and determine how plant organ and extraction strategy relate to three major chemobiological pathways: stem-bark cardenolides, leaf/flower flavonoid-rich preparations and latex cysteine proteases.

Methods. The review was conducted in accordance with PRISMA 2020. PubMed/MEDLINE, Scopus, Web of Science and Embase were searched, with supplementary searching of Google Scholar and reference lists. English-language original studies published between 1 January 2005 and 31 December 2025 were eligible. In vitro experimental and biochemical assays, disease-relevant cell systems and animal studies were considered. Animal studies were assessed using the SYRCLE risk-of-bias tool, while in vitro and in silico reports were assessed for experimental identity,

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concentration-response testing, replication, controls, selectivity and chemical characterization. Certainty was considered using a preclinical adaptation of GRADE. Because of substantial heterogeneity in preparations, models, dose units and outcome definitions, quantitative meta-analysis was not appropriate and synthesis followed SWiM principles. A bibliographic completeness check completed on 14 March 2026 identified one additional eligible root-bark anticancer study.

Results. The search identified 450 records. After removal of 123 duplicates, 327 records underwent title and abstract screening; 241 were excluded. Eighty-six full-text reports were assessed and 65 were excluded, leaving 21 studies for qualitative synthesis. Mid-polar stem-bark fractions provided the most chemically resolved anticancer evidence. CGDCM contained 6.2 mg calotropin per 10 g extract and produced IC50 values of 5.9 microg/mL in HCT116 cells and 44.0 microg/mL in HT-29 cells. Root-bark calotroposides provided an additional chemically resolved anticancer signal, with calotroposides 2, 4 and 5 showing micromolar cytotoxicity against MDA-MB-231 cells. Leaf and flower preparations predominantly supported antioxidant, anti-inflammatory, metabolic and wound-repair effects but were generally less chemically standardized. Latex showed a distinct protein-based profile associated with wound repair and tissue-remodelling biology. Certainty was Low for the stem-bark cytotoxicity/apoptosis cluster and Very low for the other major outcome groups.

Conclusions. *C. gigantea* is best interpreted as an organ-dependent chemobiological system rather than as a chemically uniform herbal preparation. Stem-bark cardenolides represent the most developed pharmacological axis, although absent pharmacokinetic characterization and inadequate mammalian cardiac-safety assessment currently limit translation. Leaf/flower flavonoids and latex proteases require substantially improved chemical and functional standardization. Future work should prioritize validated analytical markers, normalized dosing, exposure-response relationships, disease-relevant models, pharmacokinetics and safety rather than additional nonspecific screening experiments.

Keywords: *Calotropis gigantea*; cardenolides; flavonoids; cysteine proteases; anticancer activity; wound healing; systematic review; preclinical pharmacology.

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1. Introduction

1.1 Chemobiological basis of Calotropis gigantea

Calotropis gigantea (L.) W.T.Aiton (Apocynaceae) is a perennial milkweed widely distributed across South and Southeast Asia. Different parts of the plant have long been used in Ayurveda, Siddha, Unani and regional traditional medicine. Contemporary experimental pharmacology has demonstrated a broad range of biological activities, but the available evidence does not support treating the entire plant as a single pharmacologically uniform entity.

Its biological profile can be understood through three principal chemical and functional groups. The first comprises steroidal cardenolides, particularly calotropin, calactin and related compounds. These molecules possess a steroidal core bearing an alpha,beta-unsaturated gamma-butenolide moiety and may contain characteristic sugar substitutions. Cardenolides interact with Na+/K+-ATPase and can influence intracellular ion homeostasis, mitochondrial function, cellular energetics, reactive oxygen species generation and apoptotic signalling. Their cytotoxic properties have therefore attracted considerable attention in cancer pharmacology [1-5].

The second group consists predominantly of phenolic compounds and flavonoids associated with leaves and flowers. Quercetin, kaempferol, isorhamnetin and related flavonoid structures can participate in radical scavenging, modulation of oxidative enzymes, lipid-peroxidation control and inflammatory signalling. These compounds provide a plausible chemical basis for a proportion of the antioxidant and anti-inflammatory observations reported for polar extracts, although their presence

does not by itself establish compound-specific causation because many are widely distributed across plant species.

The third group is fundamentally different from the small-molecule fractions. *C. gigantea* latex contains proteolytic enzymes, including papain-like cysteine proteases. These proteins have been associated with fibrin and extracellular-matrix degradation, clotting-related activity, debridement and tissue remodelling. Latex-based wound and hemostatic effects should therefore not be mechanistically grouped with cardenolide or flavonoid pharmacology [6].

1.2 Importance of plant organ and extraction solvent

A major source of variability in the *C. gigantea* literature is the use of the botanical name alone to describe an intervention without adequate consideration of the plant organ and extraction procedure. These variables materially influence chemical composition. Mid-polar solvents such as dichloromethane, chloroform and ethyl acetate are particularly relevant to stem-bark cardenolide fractions. Polar solvents including methanol, ethanol, hydroalcoholic mixtures and water generally recover higher proportions of phenolics and flavonoid-associated constituents from leaves and flowers. Fresh or aqueous processing of latex, by contrast, preserves proteinaceous constituents including cysteine proteases.

Plant organ and solvent polarity therefore function together as chemical selectors. Two preparations labelled simply as *C. gigantea* extract may differ profoundly in molecular composition, pharmacological target, potency, toxicity and therapeutic relevance. This organ-solvent-chemotype relationship provides the organizing framework for the present review.

1.3 Rationale for the review

The preclinical literature has expanded considerably, but several recurring limitations impede interpretation. Many antioxidant experiments rely mainly on DPPH, ABTS or FRAP assays without linking biological activity to quantitatively measured compounds. Extract doses are frequently expressed only as milligrams of crude extract rather than milligrams of a defined active constituent. Chemical profiling is inconsistently performed, and normal-cell controls are uncommon in older cytotoxicity studies.

Another difficulty is the considerable heterogeneity of experimental endpoints. Anticancer experiments report IC50 values, apoptosis markers, cell-cycle changes, tumour-associated biomarkers or survival. Wound-healing experiments use wound contraction, epithelization time, hydroxyproline content, tensile or breaking strength and histological outcomes. Antioxidant and anti-inflammatory studies report biochemical markers that cannot be directly compared across experimental models. A structured synthesis organized around organ, solvent, chemotype, mechanism and therapeutic activity is therefore more informative than a simple catalogue of positive effects.

1.4 Objectives

The review addressed four principal questions: (1) which pharmacological, therapeutic and toxicological activities of *C. gigantea* have been investigated in eligible preclinical studies; (2) how these effects are distributed across plant organs and extraction approaches; (3) which chemical classes and mechanisms provide the strongest biological explanation for the reported activities; and (4) which methodological and translational limitations should be addressed before standardized preclinical development can proceed.

The PECO framework comprised preclinical animal models, in vitro experimental and biochemical assays, and disease-relevant cell systems as the population/problem; *C. gigantea* extracts, fractions, isolated compounds or latex proteins as the exposure/intervention; vehicle, untreated controls or active comparators where available; and quantitative pharmacological, therapeutic, mechanistic or toxicological measurements as outcomes.

2. Methods

2.1 Review design and reporting

A systematic review of preclinical evidence was performed and reported in accordance with

PRISMA 2020 guidance [7,8]. Review questions, eligibility criteria, search terms, data fields and the planned organ-solvent-chemotype classification were established before synthesis. The protocol was not registered in PROSPERO; this is acknowledged because prospective registration improves methodological transparency.

2.2 Eligibility criteria

Original studies were eligible when they evaluated a preparation obtained from *C. gigantea* and reported interpretable pharmacological, therapeutic, mechanistic or toxicological findings. Eligible interventions included crude extracts, solvent fractions, isolated compounds and latex-derived preparations. The plant organ and extraction or isolation method had to be described sufficiently to allow biological interpretation.

Both in vivo studies and in vitro experimental or biochemical assays were considered. Eligible in vitro evidence included disease-relevant cell systems as well as screening assays such as DPPH and FRAP when these were used to characterize antioxidant activity. Eligible quantitative outcomes included IC50 or EC50 values, experimentally tested dose-response data, tumour or survival outcomes, wound-contraction measurements, biochemical markers, enzyme activity, inhibition zones and other quantitative endpoints. Reviews, conference abstracts, publications without original experimental data, studies exclusively concerning other Calotropis species without separable *C. gigantea* data, and reports lacking sufficient information for interpretation were excluded. Computational findings were treated as hypothesis-generating evidence rather than as equivalent to experimentally demonstrated efficacy.

2.3 Information sources and search strategy

Structured searches were performed in PubMed/MEDLINE, Scopus, Web of Science and Embase, with supplementary searching of Google Scholar and reference lists. Search concepts combined botanical terminology with pharmacological, chemical, organ-specific and therapeutic terms. The publication window was 1 January 2005 to 31 December 2025. Full database-specific syntax corresponding to these concepts is provided in Appendix 1 to permit reproducibility. A bibliographic completeness check was completed on 14 March 2026 using PubMed, Google Scholar and publisher records and identified the eligible root-bark anticancer study by Mahar et al. [34]. The exact execution dates of the original Scopus, Web of Science and Embase searches were not retained in the available review records and are therefore not reconstructed retrospectively.

2.4 Study selection

Records retrieved from the databases were combined and duplicates removed. Titles/abstracts and full texts were independently screened by two reviewers against the same eligibility criteria. Disagreements were resolved by discussion and consensus, with additional adjudication when required. Potentially eligible reports proceeded to full-text evaluation. Reasons for full-text exclusion included inappropriate publication type, non-target species, absence of adequate experimental data, insufficient specification of plant material or extraction, and lack of an interpretable quantitative endpoint. Reviewer identities are omitted from this blinded manuscript. The selection process is shown in Figure 1.

2.5 Data extraction and chemobiological classification

Data were extracted using a structured form. Recorded variables included author and year, country, plant organ, extraction procedure, solvent, experimental model, dose or concentration, comparator, outcome, major quantitative finding, chemical characterization and analytical technique. Each study was additionally characterized according to plant organ, extraction polarity, dominant chemical class, degree of chemical characterization and translational relevance.

Solvents were grouped broadly as polar, mid-polar, non-polar or protein-compatible aqueous systems. Dominant chemical profiles were classified as cardenolide-associated, flavonoid/phenolic-associated, protease-associated, mixed or chemically unresolved. Chemical evidence was interpreted using a four-level hierarchy:

authenticated isolated compounds evaluated in relevant biological models with pathway evidence; chemically profiled fractions with quantified markers and mechanism-linked outcomes; solvent-defined crude extracts with biological activity but incomplete chemical definition; and screening assays or computational predictions without experimental confirmation.

2.6 Risk-of-bias assessment

Animal intervention studies were assessed with the SYRCLE risk-of-bias tool [9]. Domains included sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers or investigators, random outcome assessment, blinding of outcome assessors, incomplete outcome data, selective reporting and other potential sources of bias. Domains were classified as low, high or unclear risk. When methodological information was not reported, the relevant domain was classified as unclear rather than automatically assuming high risk.

Because SYRCLE is designed for animal experiments, in vitro and computational studies were considered separately for experimental identity, concentration-response assessment, independent replication, controls, normal-cell selectivity and quantitative chemical characterization. ARRIVE 2.0 principles supported interpretation of animal-study reporting quality [12].

2.7 Effect measures and evidence synthesis

Effect measures were retained in the form reported by individual investigators, including IC50/EC50 values, experimentally tested doses, survival, wound contraction, biochemical or enzymatic changes and antimicrobial inhibition zones. Meta-analysis was not undertaken because studies differed substantially in plant organ, extraction solvent, chemical composition, species or experimental system, dosing units, duration and outcome definition. Combining these results into a single quantitative estimate would not produce a biologically meaningful effect measure. Narrative synthesis therefore followed SWiM principles [11], with evidence organized primarily according to therapeutic axis and secondarily according to plant organ and solvent system.

2.8 Certainty of evidence

Certainty was evaluated using principles adapted from GRADE for preclinical research [10]. Five major evidence groups were considered: stem-bark fraction cytotoxicity and apoptosis; in vivo antitumour survival; wound healing; redox-inflammatory effects; and systemic or cardiac safety. Potential downgrading factors included risk of bias, inconsistency, indirectness and imprecision. Certainty ratings describe confidence in the preclinical evidence and should not be interpreted as clinical treatment recommendations.

2.9 Reporting and chemical-characterization considerations

Formal funnel-plot assessment was not appropriate because individual outcome groups contained too few studies and used incompatible outcome measurements. Publication bias nevertheless remains plausible because positive pharmacological results are more likely to be published than negative screening studies. For botanical preparations, interpretation also depends on transparent reporting of plant identity, extraction conditions and quantitative chemistry; contemporary extract-characterization guidance was therefore considered when discussing translational limitations [13].

3. Results

3.1 Study selection

The search identified 450 records. After removal of 123 duplicates, 327 unique records underwent title and abstract screening. Of these, 241 records were excluded, leaving 86 full-text reports for eligibility assessment. Sixty-five full-text articles were excluded according to the eligibility criteria, and 21 studies were included in the qualitative synthesis. The bibliographic completeness check identified one additional eligible root-bark anticancer study [34]. No sufficiently homogeneous group of studies was identified for a defensible quantitative meta-analysis. The identification, screening, eligibility and inclusion process is shown in Figure 1.

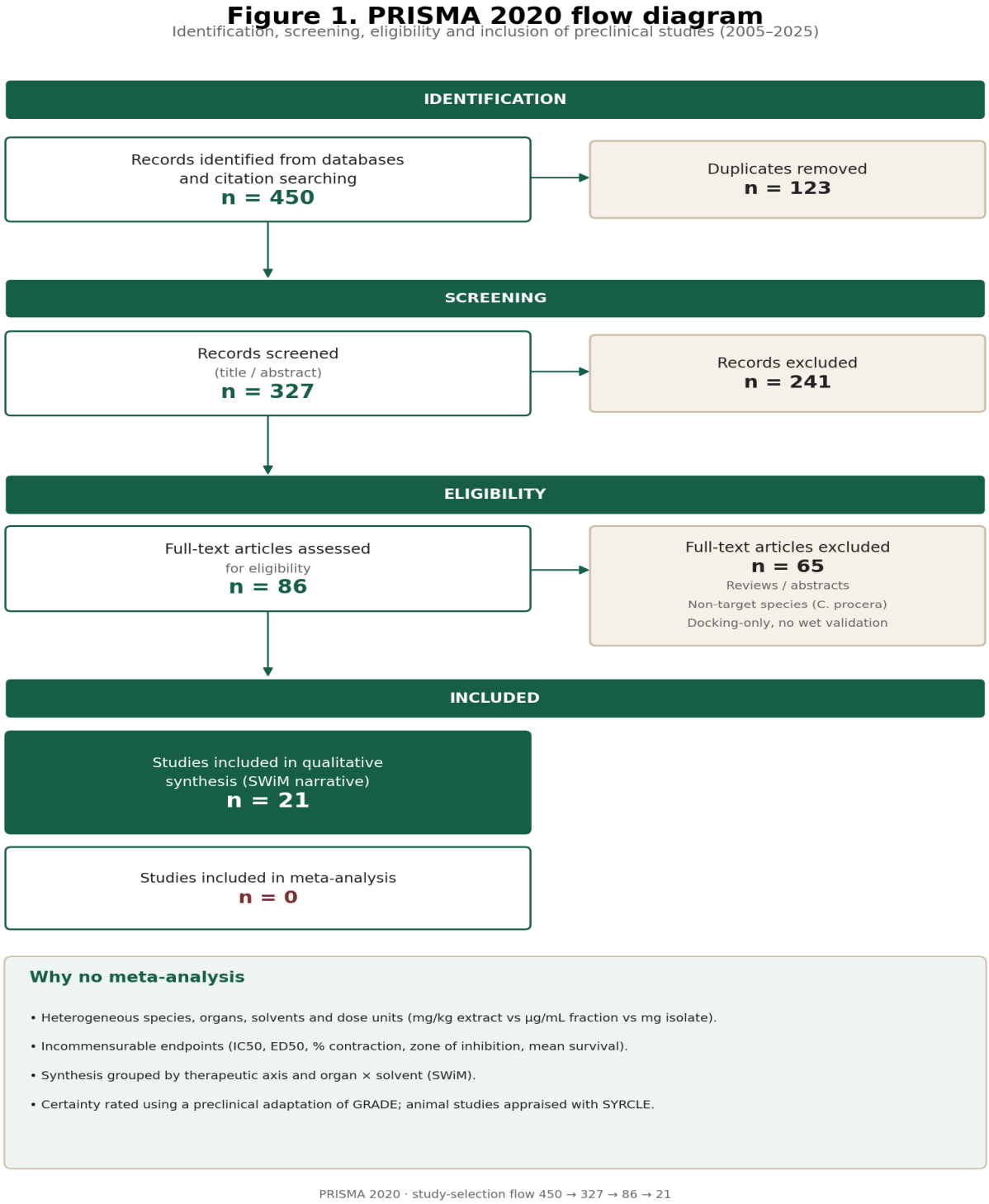

Figure 1. PRISMA 2020 study-selection flow. The search yielded 450 records, 327 remained after duplicate removal, 86 full texts were assessed and 21 studies were included in qualitative synthesis.

3.2 Characteristics of included studies

The 21 included studies encompassed crude extracts, solvent fractions, isolated phytochemicals and latex preparations [14-34]. Experimental designs included cell-culture systems, biochemical assays, microbial testing, chick chorioallantoic membrane experiments, rodent studies, zebrafish toxicology and computational modelling. The studies showed marked variation in analytical sophistication. Several earlier investigations primarily reported phenotypic outcomes from crude extracts, whereas more recent oncology studies combined chromatographic characterization with concentration-response testing and molecular or compound-level analysis. Animal studies generally used relatively small experimental groups, commonly approximately five to ten animals per group.

The clearest chemically characterized cluster originated from stem-bark oncology studies. These investigations incorporated solvent fractionation, quantitative measurements of cardenolide-associated compounds, cancer-cell models and mechanistic endpoints. Mahar et al. added a distinct root-bark anticancer study in which bioactivity-guided ethyl-acetate fractionation yielded oxypregnane-oligoglycosides (calotroposides) with micromolar cytotoxicity in breast-cancer cells [34]. By comparison, much of the leaf, flower and root literature relied on crude preparations with less extensive chemical characterization. Detailed study characteristics are presented in Table 1, and the distribution of activities across plant organs is illustrated in Figure 2.

Table 1. Characteristics of the 21 included studies

Study	Country	Plant part	Model	Extract	Chemistry	Endpoint
Deshmukh et al. 2009	India	Root bark	Wistar rats, n=6/group	EtOH 5% topical ointment; 100–400 mg/kg orally	Extract-level; hydroxyproline/collagen endpoints	Improved contraction, breaking strength and hydroxyproline
Nalwaya et al. 2009	India	Latex	Albino rats, n=6/group	Fresh latex topical, 200 mg/kg/day	Latex constituents not analytically standardized	83.42% wound-area reduction day 16; breaking strength ~485 ± 34.64 g
Rathod et al. 2009	India	Leaves & flowers	Wistar rats, n=6/group	$CHCl_3$, 10–50 mg/kg orally	Redox enzyme/lipid-peroxidation endpoints	LPO ↓; SOD/CAT ↑ across tested doses
Mahatma et al. 2010	India	Leaves	Rat n=6	Multi-solvent	Anti-inflammatory mediators	Significant anti-inflammatory/antipyretic effects (extract-dependent)
Rathod et al. 2011	India	Leaves & flowers	Wistar rats, n=6/group	$CHCl_3$, 10–50 mg/kg orally	Extract-level; no marker normalization	Serum glucose reduction in normal/STZ rats
Elakkiya & Prasanna 2012	India	Roots	In vitro DPPH/FRAP	MeOH	Phenolics	Strong DPPH activity
Suresh Babu & Karki 2012	India	Leaves	Wistar rat n=6	Multi-solvent	Phenolics, flavonoids	MeOH most effective
Habib & Karim 2013	Bangladesh	Flowers	Swiss mice n=10	A3A 10 and 20 mg/kg	Anhydrosophoradiol-3-acetate (triterpene)	MST 25.6 and 31.0 vs 19.8 d
Khan et al. 2014	Bangladesh	Leaves	Male Swiss mice, n=5/group	EtOH, 400 mg/kg	Crude extract; active constituents unresolved	Sedative/anxiolytic behavioral effects
Mahar et al. 2016	India	Root bark	MDA-MB-231, MCF-7, LNCaP, HeLa, K562, Caco-2 + HEK-293	EtOH extract -> EtOAc bioactivity-guided fraction	One new + six known oxypregnane-oligoglycosides (calotroposides); LC-MS/NMR	Calotroposides 2, 4 and 5: IC50 8.49 +/- 0.41, 6.49 +/- 0.15 and 9.65 +/- 0.44 microM in MDA-MB-231

Study	Country	Plant part	Model	Extract	Chemistry	Endpoint
Rajamohan et al. 2014	India	Flowers	In vitro antioxidant and microbial assays	Flower extract; GC-MS	Phenolics/flavonoids and GC-MS-profiled constituents	Antioxidant and antimicrobial activity
Sampath Kumar et al. 2015	India	Flowers	In vitro	MeOH/hydroalcoholic	Phenolics, flavonoids	High antioxidant capacity
Kharat & Kharat 2019	India	Leaves + stems	MCF-7; triplicate experimental sets	MeOH; 0–60 µg/mL in key assays	HPLC-detected cardenolides	IC_{50} 40.16 ± 0.54 µg/mL (24 h)
Akshatha & Kamble 2021	India	Leaves	Chick CAM n=10	EtOH 25–400 µg/mL	Angiogenesis inhibitors	Neovascularization ↓
Alafnan et al. 2021	Saudi Arabia	Leaves	Wistar rats, n=6/group	EtOH ointment 0.5% and 2% (excision)	TPC/TFC + UHPLC-MS profile	Day-16 contraction 94.33% (0.5%); 81.00% (2%)
Winitchaikul et al. 2021	Thailand	Stem bark	HCT116 / HT-29 / HFF-1	CGDCM and related fractions	Calotropin 6.2 mg/10 g (CGDCM)	IC_{50} 5.9 (HCT116), 44.0 (HT-29) µg/mL
Sawong et al. 2022	Thailand	Stem bark	HepG2 + DEN-induced Wistar model	CGDCM and related fractions	Calactin 9.6 ± 0.41 mg/10 g CGDCM; triterpenoids 689.3 ± 25.59 mg UAE/g	CGDCM IC_{50} 222.87 ± 20.91 µg/mL (HepG2)
Chaisupasakul et al. 2024	Thailand	Stem bark	HepG2 + IMR-90 fibroblasts	CGEtOAc + sorafenib	Chemically profiled CGEtOAc; mechanistic combination study	CGEtOAc IC_{50} 707.87 ± 49.05 µg/mL; 400 µg/mL + 4 µM sorafenib combo
Vinu 2025	India	Leaves	In silico only	GC-MS + docking	Azulene and other proposed ligands	Hypothesis-generating docking only
Amrutha & Kuppusamy 2025	India	Stem	Zebrafish n=30	Crude 0.01–1000 µg/mL	Oxidative-stress markers	LOEC 100 µg/mL
Khan et al. 2025	Pakistan	Leaves	In vitro microbial/antioxidant panels	EtOH, MeOH and n-hexane	LC-MS-profiled phytochemicals	Antibacterial and antioxidant endpoints

Note. A3A is a pentacyclic triterpene rather than a cardenolide. Dose and concentration values are reported as presented in the individual studies.

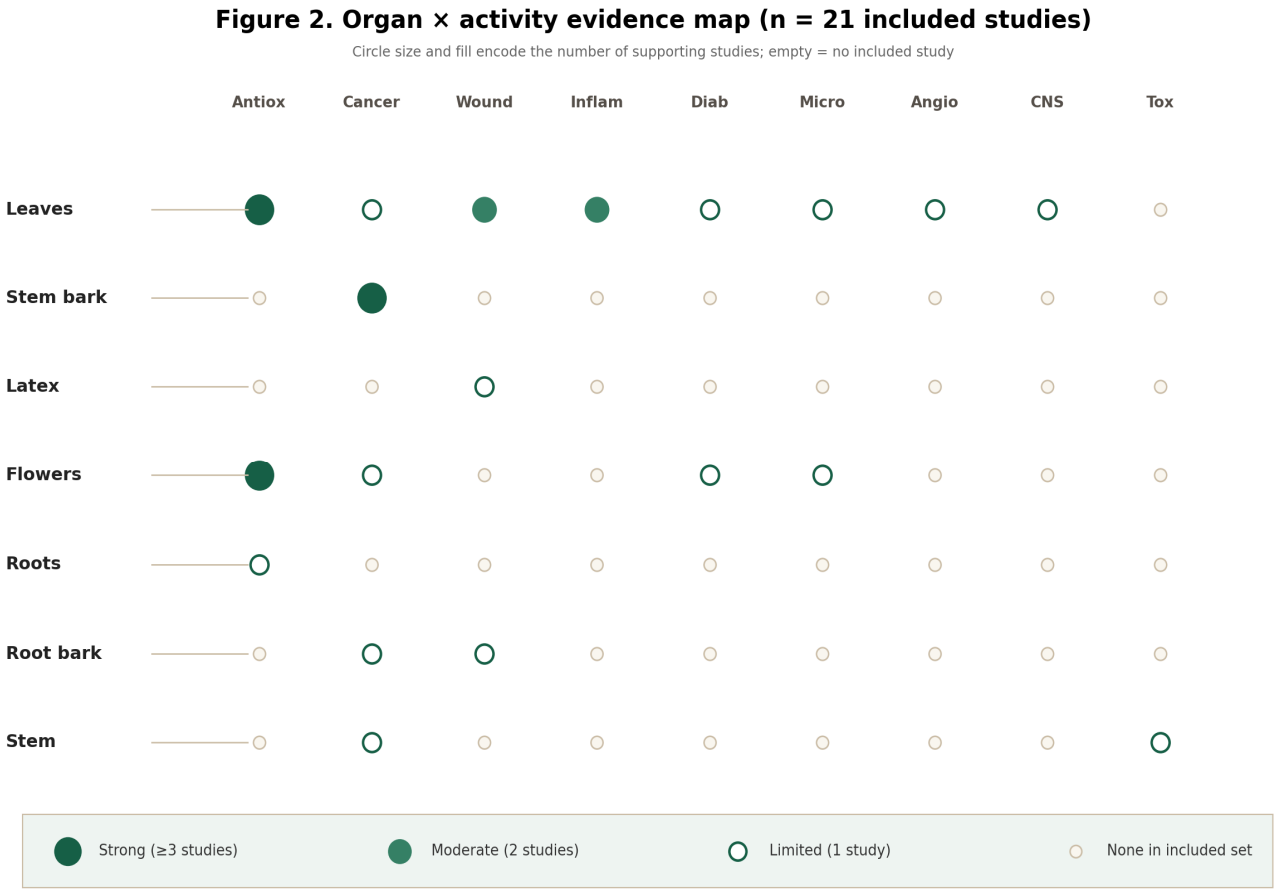

Figure 2. Organ x activity evidence map for the 21 included studies. Circle size and fill indicate the number of studies supporting each organ-activity combination. Counts are cell-specific; a study involving more than one plant organ can contribute to more than one organ row, so Figure 2 cell counts should not be summed to derive the unique-study totals in Table 4.

3.3 Risk of bias and experimental quality

Risk-of-bias assessment demonstrated a recurrent absence of sufficient methodological detail in animal experiments. Sequence generation, allocation concealment, random housing, caregiver blinding, random outcome assessment and assessor blinding were frequently not reported and were therefore classified as unclear. Baseline comparability was judged more favourably when investigators provided appropriate baseline characteristics, including body-weight ranges. Reporting of incomplete data and selective outcomes varied among studies.

The quality of in vitro reporting was strongest in studies that used chemically characterized stem-bark fractions, concentration-response experiments, appropriate controls and normal-cell comparators. Older antioxidant and antimicrobial screening studies generally provided less information about preparation identity, replicate structure, selectivity and quantitative chemical composition. Computational studies offered mechanistic hypotheses but could not substitute for experimental confirmation.

Table 2. SYRCLE risk of bias (L = low, U = unclear, H = high, NA = not applicable)

Study	Design	1	2	3	4	5	6	7	8	9	10
Deshmukh 2009	in vivo	U	L	U	U	U	U	U	U	U	U
Nalwaya 2009	in vivo	U	U	U	U	U	U	U	U	U	U
Suresh Babu 2012	in vivo	U	L	U	U	U	U	U	U	U	U
Mahatma 2010	in vivo	U	L	U	U	U	U	U	U	U	U
Rathod 2011	in vivo	U	L	U	U	U	U	U	U	U	U
Rathod 2009	in vivo	U	L	U	U	U	U	U	U	U	U
Habib 2013	in vivo	U	L	U	U	U	U	U	L	L	U
Kharat 2019	in vitro	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Winitchaikul 2021	in vitro	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sawong 2022	mixed	U	L	U	U	U	U	U	U	L	U
Chaisupasakul 2024	in vitro	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mahar 2016	in vitro	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Alafnan 2021	in vivo	U	L	U	U	U	U	U	U	L	U
Sampath Kumar 2015	in vitro	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Vinu 2025	in silico	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Khan 2014	in vivo	U	L	U	U	U	U	U	U	U	U
Rajamohan 2014	in vitro	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Elakkiya 2012	in vitro	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Khan 2025	in vitro	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Akshatha 2021	in vivo	U	U	U	U	U	U	U	U	U	U
Amrutha 2025	in vivo	U	L	U	U	U	U	U	L	L	U

Domains: 1 sequence generation; 2 baseline characteristics; 3 allocation concealment; 4 random housing; 5 caregiver/investigator blinding; 6 random outcome assessment; 7 outcome-assessor blinding; 8 incomplete outcome data; 9 selective reporting; 10 other bias.

Table 3. In vitro and in silico reporting quality (Y = yes, P = partial, N = no)

Study	Identity	Conc.–response	Replicates	Controls	Selectivity	Chemistry
Kharat 2019	Y	Y	Y	Y	N	Y
Winitchaikul 2021	Y	Y	Y	Y	Y	Y
Sawong 2022	Y	Y	Y	Y	P	Y
Mahar 2016	Y	Y	P	Y	Y	Y
Chaisupasakul 2024	Y	Y	Y	Y	Y	Y
Sampath Kumar 2015	P	P	Y	P	N	P
Elakkiya 2012	P	P	P	P	N	P
Rajamohan 2014	P	P	P	P	N	P
Khan 2025	P	P	P	P	N	Y
Vinu 2025	P	N	N	N	N	P

3.4 Organ- and solvent-dependent evidence patterns

A consistent pattern emerged when studies were classified by plant organ and solvent polarity. Mid-polar stem-bark fractions were preferentially associated with cardenolides and anticancer experiments. Polar leaf and flower extracts were predominantly associated with phenolic and flavonoid-rich preparations and with antioxidant, anti-inflammatory, metabolic and wound-related effects. Latex represented a separate protein-based axis characterized by proteolytic rather than conventional small-molecule activity. A distinct root-bark study identified cytotoxic oxypregnane-oligoglycosides (calotroposides), providing an additional chemically resolved oncology signal that is currently supported by a single study [34].

This relationship is illustrated in Figure 3, which links plant organ, solvent class, dominant chemical profile, major mechanism and therapeutic direction. The pattern is biologically important because extraction solvent should not be regarded merely as a technical laboratory choice; extraction polarity substantially determines the molecules carried forward into pharmacological testing.

Figure 3. Organ -> solvent polarity -> chemotype -> mechanism -> therapeutic-axis model. Extraction polarity functions as a major determinant of the chemical and pharmacological profile.

3.5 Findings grouped by therapeutic axis

3.5.1 Chemically resolved oncology evidence: stem-bark cardenolides and root-bark calotroposides

The strongest chemically resolved evidence was observed for stem-bark fractions. Winitchaikul et al. evaluated fractions prepared from a 95% ethanolic stem-bark extract. The dichloromethane fraction, CGDCM, showed particularly strong cytotoxicity against colorectal cancer cells. CGDCM contained approximately 6.2 mg calotropin per 10 g extract and produced IC50 values of 5.9 microg/mL in HCT116 cells and 44.0 microg/mL in HT-29 cells [28]. Mechanistic findings included increased intracellular reactive oxygen species, altered ATP production, mitochondrial dysfunction and apoptosis-related signalling. Experiments incorporating 5-fluorouracil also suggested that fraction composition may influence combination effects.

Sawong et al. extended stem-bark fraction research to HepG2 liver-cancer cells and a diethylnitrosamine-induced rat model. CGDCM contained 9.6 +/- 0.41 mg calactin per 10 g fraction and showed an IC50 of approximately 222.87 +/- 20.91 microg/mL in HepG2 cells. The study also reported changes in tumour-associated and apoptotic markers in the animal model [29].

Chaisupasakul et al. examined an ethyl-acetate stem-bark fraction in combination with sorafenib. The reported IC50 values were approximately 707.87 +/- 49.05 microg/mL for the plant fraction and 8.65 +/- 0.33 microM for sorafenib. A combination containing 400 microg/mL extract and 4 microM sorafenib produced enhanced anticancer effects and was associated with mitochondrial injury, increased ROS, apoptosis and suppression of PI3K/Akt/mTOR-related signalling [30].

Kharat and Kharat evaluated a methanolic preparation from leaves and stems in MCF-7 breast-cancer cells. The reported IC50 was approximately 40.16 +/- 0.54 microg/mL, accompanied by apoptosis and cell-cycle alterations [25]. This supports the wider anticancer potential of *C.*

gigantea but differs chemically from the more clearly defined stem-bark fractions. Taken together, these studies support an anticancer signal but do not justify direct pooling of potency estimates because the preparations, cell lines, concentrations and chemical profiles differ substantially.

Mahar et al. performed bioactivity-guided isolation from the ethanolic root-bark extract and identified one new and six known oxypregnane-oligoglycosides (calotroposides) in the active ethyl-acetate fraction. Calotroposides 2, 4 and 5 showed the greatest activity against MDA-MB-231 breast-cancer cells, with IC50 values of 8.49 +/- 0.41, 6.49 +/- 0.15 and 9.65 +/- 0.44 microM, respectively; a non-cancer HEK-293 line was also evaluated [34]. This study extends the chemically resolved oncology evidence beyond stem-bark cardenolides, but the calotroposide signal currently rests on a single publication and should be considered a distinct oxypregnane-glycoside pathway.

3.5.2 Flower-derived A3A antitumour evidence

A distinct antitumour experiment evaluated anhydrosophoradiol-3-acetate (A3A) isolated from *C. gigantea* flowers. A3A is a pentacyclic triterpene and should be interpreted separately from the cardenolide pathway. In Ehrlich ascites carcinoma-bearing mice, A3A administered at 10 and 20 mg/kg increased mean survival compared with tumour controls. Mean survival was approximately 25.6 days and 31.0 days, respectively, compared with approximately 19.8 days in untreated tumour-bearing controls [21]. The experiment provides isolate-level in vivo antitumour evidence, but a single animal study is insufficient to establish a general therapeutic effect.

3.5.3 Leaf and flower redox-inflammatory axis

Leaf and flower preparations showed repeated antioxidant and anti-inflammatory activity, although most studies were performed using chemically complex extracts. Antioxidant studies reported reductions in lipid peroxidation and changes in endogenous antioxidant enzymes including superoxide dismutase and catalase [16,19,24]. Root and flower preparations also demonstrated activity in DPPH, FRAP and related experimental assays [19,24]. Anti-inflammatory and antipyretic studies of leaf preparations demonstrated reductions in carrageenan-induced paw oedema and experimental pyrexia [17].

Chloroform extracts of leaves and flowers administered in streptozotocin-diabetic rats were associated with improvement in oxidative-stress variables and reduction of blood glucose across tested dose levels [16,18]. Alafnan et al. provided more detailed phytochemical characterization of an ethanolic leaf preparation and investigated wound-healing activity. At day 16, the 0.5% ointment produced approximately 94.33% wound contraction, whereas the 2% ointment produced approximately 81.00% [27]. The non-linear relationship between concentration and wound contraction illustrates why crude-extract concentration should not automatically be interpreted as equivalent to active-compound exposure. Overall, the leaf/flower evidence is broader than the stem-bark oncology evidence but considerably less chemically specific.

3.5.4 Latex, wound healing and hemostasis

Fresh latex forms a third mechanistic axis. In an experimental rat wound model, topical latex at approximately 200 mg/kg/day produced a day-16 wound-area reduction of approximately 83.42%, compared with approximately 76.22% in controls; breaking strength was also increased [15]. Root-bark ethanolic extract produced improvements in wound contraction, breaking strength and hydroxyproline-related outcomes [14], while leaf extracts likewise demonstrated wound-healing effects in excision and incision models [20].

Independent mechanistic literature describes coagulation-related and fibrinolytic activity of *C. gigantea* latex cysteine proteases [6]. Because this mechanistic study is not one of the included studies, its quantitative experimental values are not incorporated into the systematic results or Table 6 and are used only as contextual biological background. The included wound-healing evidence remains consistent with a protein-dependent pathway involving proteolysis, fibrin turnover, removal of damaged extracellular material and wound-bed remodelling. Development of a latex-derived therapeutic nevertheless requires rigorous standardization of protease activity, protein

composition, sterility, local tolerability, sensitization, formulation stability and preservation of biological activity.

3.5.5 Additional pharmacological signals

Additional studies reported antimicrobial, anti-angiogenic, neuropharmacological and toxicological effects. Flower and leaf extracts demonstrated measurable antimicrobial activity against selected organisms [23,33]. Ethanolic leaf extract produced anti-angiogenic effects in the chick chorioallantoic membrane model [26]. An ethanolic leaf preparation also demonstrated sedative and anxiolytic behavioural effects in mice [22]. A zebrafish study provided a toxicological signal for stem preparations, with a lowest-observed-effect concentration of approximately 100 microg/mL under the reported exposure conditions [32]. A computational study proposed COX-related anti-inflammatory ligand-target interactions but remains hypothesis-generating [31]. These observations expand the pharmacological profile of *C. gigantea*, but each is currently supported by too little standardized evidence to justify a separate mature therapeutic-development pathway.

3.6 Mechanistic convergence

Several recurring biological processes were apparent despite the diversity of models. The cardenolide-associated anticancer pathway involves Na+/K+-ATPase-related stress, altered cellular energetics, ROS elevation, mitochondrial membrane dysfunction, caspase activation, cell-cycle disruption and modulation of PI3K/Akt/mTOR signalling. Leaf and flower extracts more often affect oxidative and inflammatory processes through radical-scavenging activity, preservation or enhancement of antioxidant enzymes, reduction in lipid peroxidation and modulation of inflammatory mediators. Latex activity is mechanistically more distinct because cysteine protease activity affects proteins within fibrin and the extracellular matrix, creating a plausible link to debridement, coagulation-associated responses and tissue repair.

These pathways are compared in Figure 4. Apparent overlap in downstream endpoints such as oxidative stress or apoptosis should not be interpreted as evidence that the three groups share a single molecular target.

Figure 4. Mechanistic convergence across three therapeutic axes

Shared ROS / apoptosis tone is not a shared drug target — upstream chemistry differs

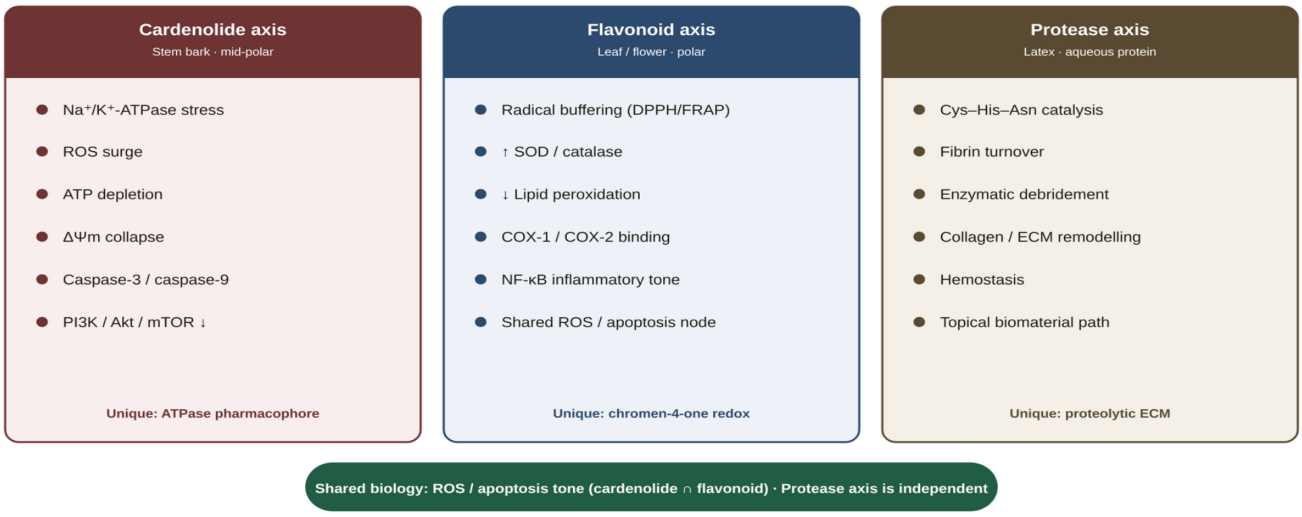

Figure 4. Mechanistic convergence across the three therapeutic axes. Shared downstream biology does not imply a shared upstream molecular target.

3.7 Distribution of pharmacological activities

The distribution of activities across the 21 studies demonstrates that pharmacological breadth does not equal evidentiary strength. Antioxidant activity was among the most frequently investigated outcomes and involved

predominantly leaves, flowers and roots. Anticancer or cytotoxic activity was represented by stem-bark fractions, a root-bark calotroposide study, a mixed leaf/stem preparation and the flower-derived A3A experiment. Wound-healing evidence arose from latex, root bark and leaf preparations. Anti-inflammatory, antidiabetic, antimicrobial, anti-angiogenic, CNS and toxicological observations occurred less frequently.

The central interpretation is that leaves display the greatest phenotypic diversity, whereas stem bark displays greater chemical specialization. Latex is narrower in the number of indications but represents a mechanistically distinctive protein-based preparation.

Table 4. Distribution of included studies by pharmacological activity

Activity	n	%	Plant parts	Key chemistry
Antioxidant	6	28.6%	Leaves, flowers, roots	Phenolics, flavonoids
Anticancer / cytotoxic	6	28.6%	Stem bark, root bark, leaves/stems, flowers	Calotropin, calactin, calotroposides; A3A (triterpene)
Wound healing	4	19.0%	Root bark, leaves, latex	Collagen-related endpoints; protease-associated biology
Anti-inflammatory	2	9.5%	Leaves	Phenolics/flavonoids; experimental and computational evidence
Antidiabetic	1	4.8%	Leaves & flowers	CHCl3-soluble fraction
Antimicrobial	2	9.5%	Leaves, flowers	Phenolics/flavonoids; GC-MS/LC-MS-profiled constituents
Anti-angiogenic	1	4.8%	Leaves	Phytochemical extract; mechanism not established
CNS activity	1	4.8%	Leaves	Active constituents unresolved
Toxicology	1	4.8%	Stem	Oxidative-stress markers

Note. Activity categories are non-mutually exclusive; percentages use n = 21 as the denominator and therefore do not sum to 100%. Alafnan 2021 is classified as an antioxidant study. Multi-organ studies contribute to each relevant organ cell in Table 5/Figure 2, but only once to the unique-study total in Table 4.

Table 5. Plant part x activity matrix (unique-study counts)

Plant part	Antiox.	Anticancer	Wound	Anti-inflam.	Antidiab.	Antimicr.	Anti-ang.	CNS	Tox.
Leaves	●●●	●	●●	●●	●	●	●	●	○
Stem bark	○	●●●	○	○	○	○	○	○	○
Latex	○	○	●	○	○	○	○	○	○
Flowers	●●●	●	○	○	●	●	○	○	○
Roots	●	○	○	○	○	○	○	○	○
Root bark	○	●	●	○	○	○	○	○	○
Stem	○	●	○	○	○	○	○	○	●

Symbols: ●●● strong (>=3 studies); ●● moderate (2); ● limited (1); ○ none in the included set. Counts are unique within each organ-activity cell; studies using more than one plant organ can appear in more than one organ row.

Table 6. Activity-specific summaries with key quantitative values

Activity	Preparation	Key value	Model
Anticancer (in vitro)	Stem bark CGDCM / calotropin 6.2 mg/10 g	IC_{50} 5.9 µg/mL HCT116; 44.0 µg/mL HT-29	HCT116 / HT-29 + 5-FU
Anticancer (in vitro)	Stem bark CGDCM / calactin 9.6 ± 0.41 mg/10 g	IC_{50} 222.87 ± 20.91 µg/mL	HepG2 + DEN rat
Anticancer (in vitro)	Root-bark EtOAc fraction / calotroposides	Calotroposides 2, 4, 5: IC50 8.49, 6.49, 9.65 microM	MDA-MB-231; additional cancer lines + HEK-293
Anticancer (combo)	Stem bark CGEtOAc + sorafenib	CGEtOAc IC_{50} 707.87 ± 49.05 µg/mL; selected combo 400 µg/mL + 4 µM sorafenib	HepG2 + fibroblasts
Anticancer (in vitro)	Leaves + stems, MeOH	IC_{50} 40.16 ± 0.54 µg/mL	MCF-7
Antitumor (in vivo)	Flower A3A isolate (triterpene)	MST 25.6 and 31.0 vs 19.8 d	EAC mice n=10
Wound healing	Root bark EtOH; 5% topical or 100–400 mg/kg oral	Improved contraction/breaking strength/hydroxyproline	Wistar excision/incision
Wound healing	Fresh latex, 200 mg/kg/day topical	83.42% day-16 wound-area reduction; breaking strength ~485 ± 34.64 g	Albino rats, n=6/group
Wound healing	Leaf EtOH ointment, 0.5% and 2%	Day 16: 94.33% (0.5%); 81.00% (2%)	Wistar + histology
Anti-inflammatory	Leaves	Significant paw-oedema/pyrexia reduction; heterogeneous extracts	Rat paw oedema
Antidiabetic	Leaves & flowers, $CHCl_3$ 10–50 mg/kg	Significant glucose lowering in STZ-diabetic rats	STZ-diabetic rat
Antibacterial	Leaves / flowers	Reported inhibition zones / antimicrobial activity (study-dependent)	Microbial panels
Toxicology	Stem crude	LOEC 100 µg/mL	Zebrafish

Note. Quantitative hemostatic values from Reference 6 are not included in this table because that mechanistic paper is not one of the included studies; it is cited only as contextual background in the Introduction and Discussion.

3.8 Certainty of evidence

Using the preclinical adaptation of GRADE, no major outcome group reached Moderate or High certainty. Stem-bark fraction cytotoxicity and apoptosis received the strongest rating, but certainty remained Low because of differences among preparations and models, limited replication across laboratories and incomplete translational validation. The A3A survival evidence was rated Very low because it depended on a single animal investigation. Wound-healing evidence was also Very low because investigators evaluated latex, leaf and root-bark preparations using incompatible doses, formulations and outcomes. Redox-inflammatory evidence was Very low owing to marked heterogeneity and frequent reliance on nonspecific antioxidant assays. Systemic and cardiac-safety evidence was Very low; zebrafish toxicology provides a useful signal but cannot replace mammalian safety studies, cardiovascular assessment, pharmacokinetic characterization and therapeutic-index determination.

Table 7. GRADE summary of findings (preclinical adaptation)

Outcome	Studies	Certainty	Finding
Stem-bark fraction cytotoxicity / apoptosis	n = 3 (Winitchaikul 2021; Sawong 2022; Chaisupasakul 2024)	Low	CGDCM IC_{50} 5.9 µg/mL (HCT116) and 44.0 µg/mL (HT-29); mechanistic apoptosis evidence. Cross-model effect sizes not poolable.
In vivo tumour survival (A3A)	n = 1 (Habib 2013)	Very low	MST 25.6 and 31.0 vs 19.8 d. A3A is a triterpene, not a cardenolide.
Wound contraction / collagen	n = 4	Very low	Four wound-healing studies; examples include 83.42% day-16 wound-area reduction with topical latex and 94.33% contraction with 0.5% leaf ointment. Heterogeneous preparations; no common potency unit.
Redox–inflammatory polar extracts	n = 6	Very low	DPPH/FRAP-positive; oedema ↓ at ~200 mg/kg. Compounds not unique.
Cardiac / systemic toxicity	n = 1 empirical (zebrafish)	Very low	LOEC 100 µg/mL. Mammalian cardiac safety untested.

3.9 Translational readiness

The three major chemobiological pathways differ substantially in translational maturity. The stem-bark cardenolide pathway has the strongest chemical characterization. Marker compounds have been identified and quantified, chromatography has been used, concentration-response experiments are available and disease-relevant models have been employed. Its major unresolved problem is safety. A compound class acting through Na+/K+-ATPase demands careful assessment of cardiotoxicity and therapeutic window.

The leaf/flower flavonoid pathway is less mature because active constituents are not unique to *C. gigantea*, quantitative standardization is inconsistent and many experiments remain at crude-extract or antioxidant-screen level. The latex protease pathway is conceptually attractive for topical applications because systemic exposure may be avoidable, but analytical characterization, enzyme-unit standardization, tolerability, sterility and formulation remain insufficiently developed. Pharmacokinetic characterization represents a particularly important deficiency across all three pathways. Without exposure data, it is difficult to relate an administered extract dose to a biologically active concentration at the relevant tissue.

Table 8. Evidence-weighted translational matrix

Axis	Organ × solvent	Chemotype	Peak evidence	Bottleneck
Cardenolide	Stem bark × CH_2Cl_2 / $CHCl_3$ / EtOAc	Calotropin · calactin · uscharin	Quantified fraction + pathway (in vitro)	Cardiac-glycoside safety window
Flavonoid	Leaves/flowers × EtOH / MeOH / H_2O	Quercetin · kaempferol · isorhamnetin	Extract-level	Non-unique compounds; weak in vivo models
Protease	Latex × aqueous protein	Papain-like Cys proteases	Enzymatic phenotype	Units, local tolerability, sterility

Table 9. Translational-readiness scores (0 = absent, 1 = emerging, 2 = partial, 3 = mature; maximum 27)

Note. The translational-readiness scores are author-derived, exploratory qualitative ratings used to compare relative evidence maturity across the three pathways. They are not a validated translational-readiness instrument and should not be interpreted as predictive clinical scores.

Criterion	Bark cardenolides	Leaf/flower flavonoids	Latex proteases
Marker-compound standardization	2	1	1
LC-MS / HPLC profiling	2	1	0
Dose normalization	2	1	1
Exposure–response modelling	1	0	0
Normal-cell selectivity	1	0	0
Pharmacokinetics	0	0	0
Cardiac / systemic toxicology	1	0	1
Formulation development	1	1	1
Disease-relevant in vivo models	2	1	2
Composite (Σ / 27)	12	5	6

Figure 5. Translational-readiness heatmap (nine development criteria)

Scores: 3 mature · 2 partial · 1 emerging · 0 absent. Composite is the sum / 27.

Figure 5. Translational-readiness heatmap across nine development criteria. Scores summarize the relative maturity of the three chemobiological pathways.

4. Discussion

4.1 Principal findings

The central finding of this review is that the pharmacology of *C. gigantea* is more coherent when interpreted according to plant organ and chemical composition than when all reported activities are attributed broadly to the species. Stem bark, particularly after mid-polar fractionation, provides the strongest cardenolide-associated anticancer evidence. Root-bark calotroposides provide an

additional chemically resolved anticancer signal but are currently supported by a single study. Leaves and flowers provide a wider but less chemically specific collection of antioxidant, inflammatory, metabolic and repair effects. Latex represents a distinct protein-based therapeutic direction dominated by proteolytic activity.

This distinction is important for future drug-development studies. Repetition of crude-extract screening across unrelated models is unlikely to resolve the most important scientific questions. Development should instead begin with a clearly defined organ, extraction process, marker compound or activity unit, disease indication and safety strategy.

4.2 Interpretation of the evidence hierarchy

The chemical-certainty hierarchy helps explain why apparently similar positive findings should not receive equal weight. A positive DPPH assay demonstrates the ability of a preparation to participate in a chemical radical-scavenging system; it does not establish oral bioavailability, tissue exposure, disease modification or clinical antioxidant benefit. Similarly, computational docking may identify a plausible ligand-target interaction but cannot establish target engagement, intracellular exposure, pharmacological selectivity or efficacy.

By contrast, a chemically quantified fraction evaluated through concentration-response experiments, appropriate controls, normal-cell testing and mechanistic pathways provides a considerably stronger basis for pharmacological interpretation. The strongest *C. gigantea* evidence therefore comes from studies in which chemistry and biology are directly connected.

4.3 Organ-solvent-chemotype relationships

One of the most useful conclusions is the importance of extraction polarity. Mid-polar extraction of stem bark enriches lipophilic and moderately polar constituents, including cardenolide-related compounds. This provides a chemically plausible explanation for why the most detailed cancer-pharmacology studies cluster around such preparations. Polar extraction of leaves and flowers favours phenolics and flavonoids and corresponds with antioxidant and inflammatory phenotypes. These effects may be pharmacologically relevant but require careful attribution because quercetin, kaempferol and related compounds occur widely in botanical species.

Latex falls outside this solvent-polarity sequence because its principal biologically interesting components include proteins. Protein activity depends on collection conditions, processing, temperature, pH, storage and formulation, all of which can profoundly alter protease function. Consequently, pharmacological studies should report organ identity and extraction conditions with the same care given to experimental dose.

4.4 Major translational bottlenecks

Chemical standardization

Future stem-bark studies should quantify calotropin, calactin and other relevant cardenolides using validated analytical procedures. Reporting only the mass of crude extract is inadequate when cardenolide content can vary substantially among fractions. Leaf and flower preparations should similarly report defined marker concentrations rather than total extract dose alone. Total phenolic and total flavonoid measurements are useful quality-control variables but are not substitutes for compound-level characterization. Latex studies require functional standardization using validated protease-activity units supported by proteomic characterization.

Dose normalization and exposure-response

A dose expressed as 100 mg/kg of crude extract from one experiment cannot be assumed equivalent to 100 mg/kg of another extract. Future studies should normalize dosing to measured active markers whenever feasible. Pharmacodynamic effects should then be related to systemic or local exposure. This is particularly important for cardenolides, for which efficacy and toxicity may occur within relatively narrow exposure ranges.

Pharmacokinetics

Pharmacokinetic information is essentially absent from the major development pathways. Studies should determine absorption, plasma exposure, distribution, metabolism, elimination and exposure of target tissues. For botanical fractions, both the

total preparation and key chemical markers should be considered.

Selectivity

Cancer-cell cytotoxicity without comparison against relevant non-malignant cells provides limited evidence of therapeutic selectivity. Future studies should compare active fractions and isolated compounds in malignant and non-malignant cells under comparable conditions. The most informative subsequent models would include three-dimensional tumour systems, organoids and carefully selected in vivo models.

Cardiac and systemic toxicity

Cardiac safety is particularly important for the cardenolide pathway because Na+/K+-ATPase-active compounds can affect cardiac electrophysiology and contractility. A credible safety programme should incorporate concentration-response studies in cardiomyocytes, ion-channel evaluation where appropriate, electrocardiographic and telemetry endpoints, repeated-dose toxicology and histopathological assessment. General toxicity should also include hepatic, renal, neurological and hematological endpoints.

Reproducibility and reporting

Reproducibility is limited when plant authentication, collection conditions, voucher specimens, extraction yields, marker concentrations and storage conditions are incompletely reported. Future phytopharmacological studies should follow contemporary recommendations for chemical characterization of botanical extracts and rigorous reporting of animal experiments [12,13].

4.5 Development priorities for the next 3-5 years

For stem-bark cardenolides, the first priority is analytical standardization using authenticated calotropin and calactin standards. Doses should be expressed relative to cardenolide content. Subsequent experiments should establish exposure-response relationships, compare malignant and normal cells, define tumour pharmacokinetics and characterize cardiovascular toxicity. Combination approaches with established anticancer agents should be considered only after single-agent pharmacology and safety are adequately defined.

For leaf and flower flavonoid-rich preparations, research should move beyond repetitive DPPH and FRAP screening. Defined extracts should be tested in disease-relevant inflammatory or metabolic models, accompanied by quantification of major flavonoids, stability testing, oral pharmacokinetics and evaluation of potential CYP-mediated interactions.

For latex proteases, development should focus on topical applications. Protease activity should be standardized, active proteins characterized by electrophoretic and proteomic methods, and efficacy evaluated in controlled excisional, burn or chronic-wound models with histology. Local irritation, sensitization, microbial contamination, sterility and formulation stability require careful evaluation before hydrogel, film or dressing products are considered.

These development pathways are summarized in Figure 6. Representative small-molecule chemotypes and the latex protease pathway are shown in Figure 7.

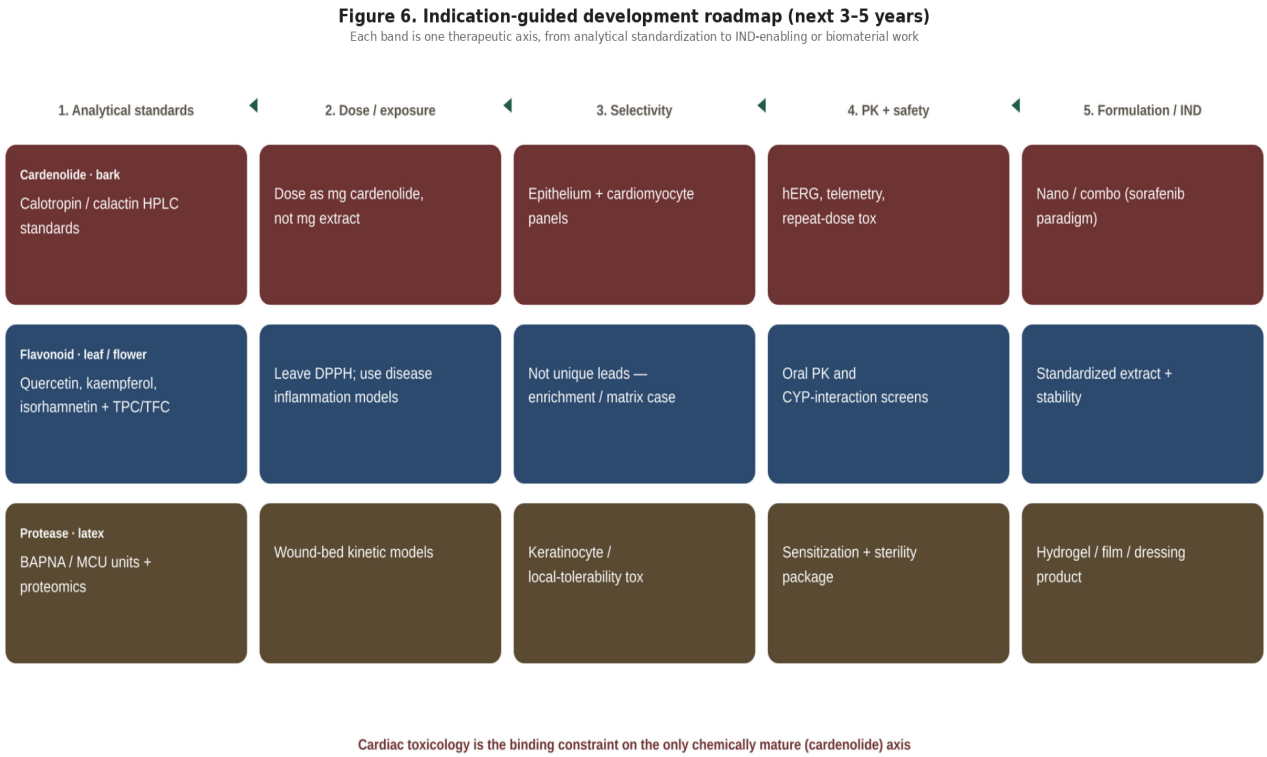

Figure 6. Indication-guided development roadmap for the next 3-5 years. The sequence emphasizes analytical standardization before exposure-response, selectivity, pharmacokinetic/safety and formulation work.

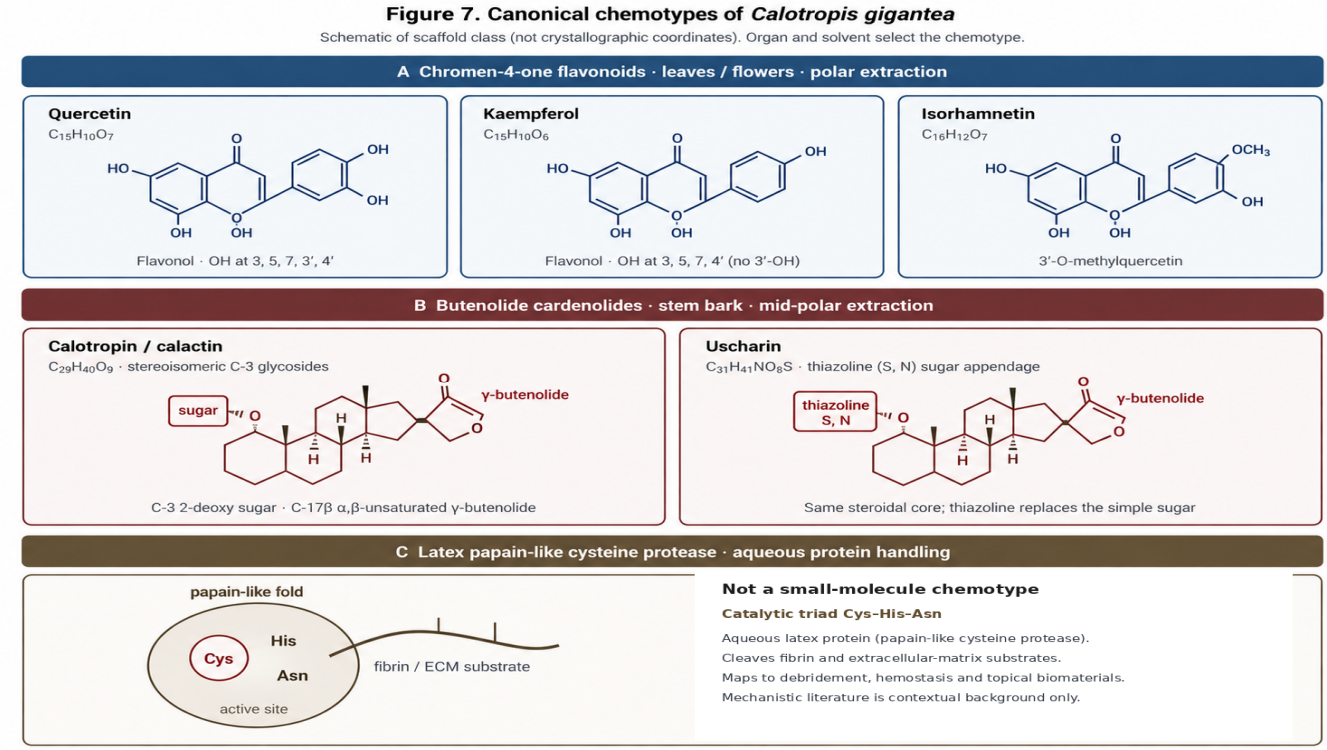

Figure 7. Canonical chemotypes of C. gigantea. The structures are schematic representations of representative flavonoid and cardenolide classes; the latex panel represents a protein-based papain-like cysteine-protease

pathway. Mechanistic latex literature is used as contextual background and is not included as quantitative systematic-review evidence.

4.6 Limitations

Several limitations should be considered. The evidence base is relatively small and predominantly preclinical. Many animal studies used modest sample sizes and incompletely described methods of randomization, allocation concealment and blinding. In vitro investigations differed markedly in extract composition, model system, exposure time and endpoint definition. Chemical standardization was inconsistent, particularly in older studies, so nominal extract doses cannot be assumed to represent equivalent pharmacologically active exposures.

Substantial heterogeneity prevented meta-analysis and necessitated structured narrative synthesis. The English-language restriction may have excluded relevant publications. Publication bias is also possible because studies demonstrating little or no pharmacological activity are less likely to appear in the literature. The review protocol was not prospectively registered. The exact execution dates of the original Scopus, Web of Science and Embase searches were not retained; reproducible database syntax is therefore provided in Appendix 1, and a bibliographic completeness check was completed on 14 March 2026. Geographic concentration of studies in a limited number of countries may also reduce generalizability because phytochemical composition can vary with genotype, climate, geography, collection season and processing. Evidence from *Calotropis procera* was not substituted for *C. gigantea* because the two species should not be treated as chemically interchangeable. These limitations mean that the findings should be interpreted as preclinical pharmacological evidence rather than as support for human therapeutic use.

5. Conclusion

The available preclinical evidence indicates that *C. gigantea* is best understood as an organ-dependent chemobiological system rather than as a single uniform phytomedicine. Mid-polar stem-bark fractions form the most chemically developed pathway and are characterized by cardenolide-associated anticancer activity. Calotropin-containing CGDCM has demonstrated substantial cytotoxic activity in colorectal cancer models, accompanied by ROS-related, mitochondrial, energetic and apoptotic changes. Root-bark calotroposides provide a separate chemically defined anticancer signal with micromolar activity in breast-cancer cells, although this evidence currently derives from one study. The overall certainty of the principal preclinical evidence remains Low or Very low, and the absence of adequate pharmacokinetic and cardiovascular-safety characterization represents a major barrier to translation.

Leaf and flower preparations produce a wider range of antioxidant, inflammatory, metabolic and wound-related effects, but the evidence is predominantly extract-level and chemically less specific. Their future development depends on quantitative marker-based standardization and transition from general antioxidant assays to disease-relevant experimental systems. Latex-derived cysteine proteases constitute a distinct protein-based therapeutic pathway with potential relevance to wound debridement, tissue remodelling, fibrin turnover and topical biomaterials. Progress requires reproducible enzyme standardization, molecular characterization, local-safety assessment, sterility and suitable formulation technology.

The most productive next phase of research should therefore emphasize chemical identity, standardized potency, exposure-response relationships, selectivity, pharmacokinetics and safety. Further nonspecific screening of poorly characterized extracts is unlikely to provide comparable translational value. No *C. gigantea* extract, fraction, isolated compound or latex preparation can currently be recommended for clinical treatment on the basis of the available preclinical evidence.

Declarations

Data availability. All data used in this systematic review are derived from published studies and are summarized in Tables 1-9 and Figures 1-7.

Funding. No external funding was received for this work.

Competing interests. The authors declare no competing interests.

Ethics statement. This systematic review synthesizes previously published experimental research. No new experiments involving humans or animals were conducted, and separate ethics approval was therefore not required.

Use of artificial intelligence

The authors declare that no generative artificial intelligence tool was used to generate the scientific findings, data, analyses, interpretations or conclusions presented in this manuscript. Any use of AI-assisted tools, if applicable, was limited to language editing, grammar correction or improvement of manuscript readability, and the authors take full responsibility for the accuracy, originality and integrity of the final manuscript.

Appendix 1. Reproducible database-specific search syntax

The following syntax corresponds to the review concepts and publication window and is provided to permit replication. The exact execution dates of the original Scopus, Web of Science and Embase searches were not retained in the available study records. A bibliographic completeness check was completed on 14 March 2026.

PubMed/MEDLINE: ("Calotropis gigantea"[Title/Abstract] OR "crown flower"[Title/Abstract] OR "giant milkweed"[Title/Abstract] OR arka[Title/Abstract]) AND (pharmacolog*[Title/Abstract] OR therapeut*[Title/Abstract] OR bioactiv*[Title/Abstract] OR anticancer[Title/Abstract] OR cytotoxic*[Title/Abstract] OR antioxidant*[Title/Abstract] OR "anti-inflammatory"[Title/Abstract] OR "wound healing"[Title/Abstract] OR antimicrobial*[Title/Abstract] OR antidiabet*[Title/Abstract] OR cardenolide*[Title/Abstract] OR calotropin[Title/Abstract] OR flavonoid*[Title/Abstract] OR "latex protease"[Title/Abstract] OR toxic*[Title/Abstract]) AND ("2005/01/01"[Date - Publication] : "2025/12/31"[Date - Publication])

Scopus: TITLE-ABS-KEY(("Calotropis gigantea" OR "crown flower" OR "giant milkweed" OR arka) AND (pharmacolog* OR therapeut* OR bioactiv* OR anticancer OR cytotoxic* OR antioxidant* OR "anti-inflammatory" OR "wound healing" OR antimicrobial* OR antidiabet* OR cardenolide* OR calotropin OR flavonoid* OR "latex protease" OR toxic*)) AND PUBYEAR > 2004 AND PUBYEAR < 2026

Web of Science Core Collection: TS=(("Calotropis gigantea" OR "crown flower" OR "giant milkweed" OR arka) AND (pharmacolog* OR therapeut* OR bioactiv* OR anticancer OR cytotoxic* OR antioxidant* OR "anti-inflammatory" OR "wound healing" OR antimicrobial* OR antidiabet* OR cardenolide* OR calotropin OR flavonoid* OR "latex protease" OR toxic*)) AND PY=(2005-2025)

Embase: ('calotropis gigantea':ti,ab,kw OR 'crown flower':ti,ab,kw OR 'giant milkweed':ti,ab,kw OR arka:ti,ab,kw) AND (pharmacolog*:ti,ab,kw OR therapeut*:ti,ab,kw OR bioactiv*:ti,ab,kw OR anticancer:ti,ab,kw OR cytotoxic*:ti,ab,kw OR antioxidant*:ti,ab,kw OR 'anti-inflammatory':ti,ab,kw OR 'wound healing':ti,ab,kw OR antimicrobial*:ti,ab,kw OR antidiabet*:ti,ab,kw OR cardenolide*:ti,ab,kw OR calotropin:ti,ab,kw OR flavonoid*:ti,ab,kw OR 'latex protease':ti,ab,kw OR toxic*:ti,ab,kw) AND [2005-2025]/py

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