

Biopesticidal Potential of *Calotropis procera*: A Green Chemistry Approach to Sustainable Pest Management

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ABSTRACT

Background: *Calotropis procera* (Aiton) W.T. Aiton (family Apocynaceae, syn. Asclepiadaceae), commonly known as Sodom apple, Ushar, or Aak, is a xerophytic shrub widely distributed across arid and semi-arid regions of Africa, the Middle East, and South Asia that contains a rich reservoir of bioactive secondary metabolites with broad pest-control properties. **Objectives:** This chapter aimed to compile and critically synthesise the phytochemical, chemical-evaluation, and bioassay literature on *C. procera* as a green-chemistry-compliant biopesticide. **Materials and Methods:** Published literature was critically synthesised to evaluate phytochemical constituents, tabulate statistical bioactivity data (LC₅₀, LD₅₀, percentage mortality, and inhibition zones) across multiple pest models, illustrate the Na⁺/K⁺Na⁺/K⁺-ATPase inhibition mechanism of toxicity, and review green-synthesised nanoparticle formulations (Ag, CuO, Fe₃O₄) capped with *C. procera* phytochemicals. **Results:** Secondary metabolites-including cardenolides (calotropin, calotoxin, calactin, uscharin, uscharidin), flavonoids, alkaloids, triterpenoids, saponins, tannins, and proteolytic enzymes-confer potent insecticidal, larvicidal, ovicidal, nematocidal, molluscicidal, and antifungal properties mediated by Na⁺/K⁺Na⁺/K⁺-ATPase inhibition. Furthermore, green-synthesised Ag, CuO, and Fe₃O₄ nanoparticle formulations capped with plant phytochemicals serve as effective, next-generation eco-friendly pest-control agents. **Conclusion:** The compiled findings position *C. procera* as a scientifically validated candidate for application in sustainable Integrated Pest Management (IPM) programmes.

Keywords: Biopesticide, *Calotropis procera*, Cardenolides, Green chemistry, LC₅₀, Nanoparticles, Phytochemical screening, Sustainable pest management.

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INTRODUCTION

The indiscriminate and continuous use of synthetic pesticides has resulted in the development of insect resistance, environmental contamination, non-target toxicity, and residual accumulation in the food chain (Benelli *et al.*, 2017; Boller *et al.*, 2005). Consequently, there is a global impetus toward botanical or “green” biopesticides derived from plants that are biodegradable, cost-effective, and ecologically compatible (Kumar and Arya, 2006; Nenaah, 2013). *Calotropis procera* is one such promising candidate. It is a perennial, latex-bearing shrub native to northern and tropical Africa, western and southern Asia, and the Middle East, thriving on wastelands, roadsides, and degraded soils (Hassan *et al.*, 2015). Every part of the plant - root, stem,

leaf, flower, and particularly the milky latex - has been shown to harbour a diverse arsenal of secondary metabolites that act as natural chemical defences against herbivores, phytopathogenic fungi, and disease vectors (Ramos *et al.*, 2007; Priyanka *et al.*, 2017).

Green chemistry, as applied to pest management, emphasises the design of biodegradable, low-toxicity, renewable-resource-based agrochemicals that minimise environmental footprint while maintaining efficacy (Boller *et al.*, 2005; Dara, 2019). This chapter presents an integrated account of the botanical background, phytochemical composition, chemical evaluation parameters, and statistically validated biopesticidal activities of *C. procera*, framed explicitly within a green chemistry and sustainable pest management context.

Botanical Profile and Distribution

C. procera belongs to the family Apocynaceae (subfamily Asclepiadoideae). It is a fast-growing, drought-tolerant shrub reaching 2-6 m in height, with large, opposite, ovate-oblong leaves and a thick, corky bark. Incision of any plant part exudes a copious



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white latex containing proteolytic enzymes, cardenolides, and resinous material that is central to the plant's defensive chemistry. Table 1 summarises its taxonomic and ecological profile (Hassan *et al.*, 2015; Dhar and Singh, 1973).

Phytochemical Profile

Qualitative and quantitative phytochemical investigations across leaf, latex, root, and flower extracts consistently reveal cardenolides (calotropin, calotoxin, calactin, uscharin, uscharidin, proceragenin), flavonoids and flavonoid glycosides, alkaloids, triterpenoids (α - and β -amyrin, lupeol, β -sitosterol), saponins, tannins, and proteolytic enzymes (Bader *et al.*, 2021; Alqahtani *et al.*, 2019). Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis of different organs identified 37 distinct constituents predominantly belonging to the cardenolide and flavonoid classes, with the highest cardenolide density recorded in the latex and root fractions (Bader *et al.*, 2021). Figure 1 depicts the representative distribution of major phytochemical classes reported across studies.

Qualitative Phytochemical Screening

Standard qualitative tests are used to detect the presence of each metabolite class prior to quantitative or instrumental analysis. Table 2 lists the principal screening tests and the corresponding positive-result indicators as reported in the literature.

Chemical Evaluation Parameters

Beyond qualitative screening, quantitative chemical evaluation is essential to standardise extracts for biopesticide formulation and to satisfy green-chemistry reproducibility criteria extracts (Bader *et al.*, 2021; Moursy, 1997; Morsy *et al.*, 2001; Doshi *et al.*, 2011; Rani *et al.*, 2019). Commonly reported parameters include Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Antioxidant Capacity (TAC), DPPH free-radical scavenging (IC₅₀), and advanced spectral/chromatographic fingerprinting (UV-vis, FTIR, GC-MS, LC-ESI-Orbitrap-MS/MS, NMR) (Bader *et al.*, 2021; Kumar *et al.*, 2013; Hanna, 2002). Table 3 consolidates

the principal chemical evaluation parameters and their analytical significance.

Green Chemistry Approach: Nanoparticle-Mediated Formulations

A defining feature of the green chemistry approach is the use of *C. procera* phytochemicals (phenolics, flavonoids, saponins) as reducing and capping agents in the eco-friendly biosynthesis of metal and metal-oxide nanoparticles, eliminating the need for hazardous chemical reductants. Silver Nanoparticles (AgNPs), Copper-Oxide Nanoparticles (CuONPs), and magnetite (Fe₃O₄) nanoparticles capped with leaf-extract phytochemicals have been reported to display enhanced antifungal and antibacterial activity against phytopathogens such as *Rhizopus oryzae*, positioning them as candidate green nano-pesticides (Table 4) (Saddiq *et al.*, 2022). Particle size in such systems is strongly influenced by the capping phytochemical class - phenolic-, flavonoid-, and saponin-rich fractions have been shown to yield nanoparticles of markedly different average diameters, underscoring the role of extract standardisation (Section 4) in reproducible nanoparticle synthesis.

Biopesticidal Activity: Statistical and Comparative Evaluation

Insecticidal and Larvicidal Activity

Ethanollic leaf extracts of *C. procera* exhibited an LC₅₀ of 282.5 mg L⁻¹ against third-instar larvae of the housefly, *Musca domestica*, under laboratory bioassay conditions, compared with 550 mg L⁻¹ for *Annona squamosa* leaf extract tested in parallel (Kumar and Arya, 2006). Against important forest defoliators, leaf extractives showed dose-dependent larvicidal action across a concentration range of 0.625-2.00% (LC₅₀ basis) on *Clostera cupreata* (poplar defoliator) and *Plecoptera reflexa* (shisham

Table 1: Botanical and ecological profile of *Calotropis procera*.

Parameter	Description
Kingdom / Family	Plantae / Apocynaceae (Asclepiadaceae)
Common names	Sodom apple, Ushar, Aak, Madar, Rubber bush
Habit	Perennial xerophytic shrub, 2-6 m
Native range	N. and tropical Africa, W. and S. Asia, Middle East
Plant parts used	Leaf, latex, root, flower, stem bark
Parameter	Description
Key defensive trait	Copious milky latex rich in cardenolides and proteases

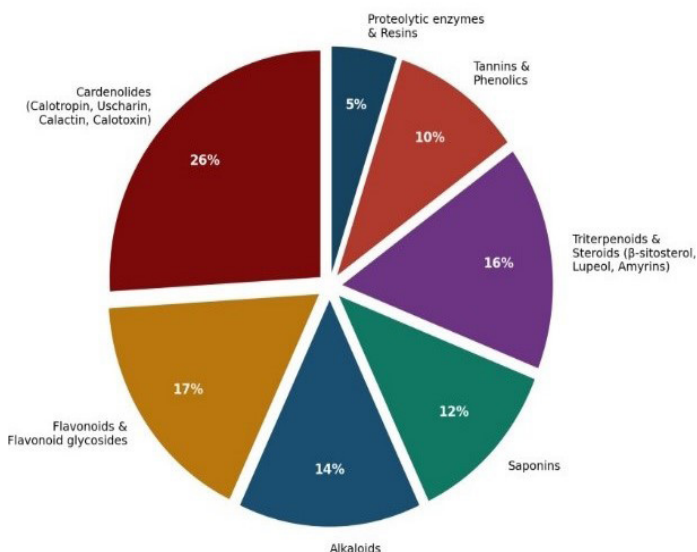


Figure 1: Representative distribution of major phytochemical classes reported in *Calotropis procera* extracts (leaf, latex and root).

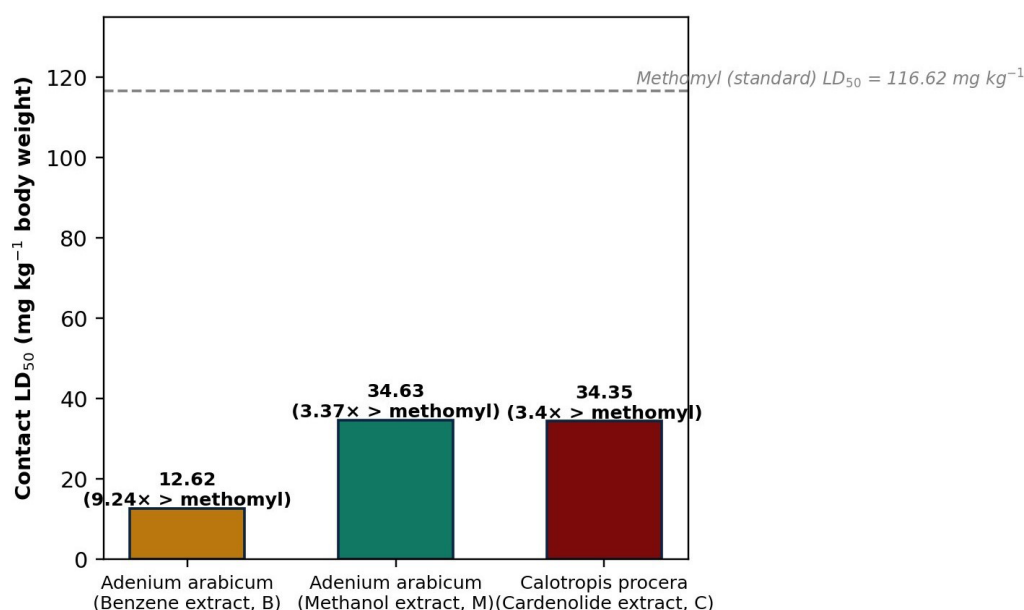


Figure 2: Molluscicidal toxicity of *C. procera* cardenolide extract vs. methomyl (standard) against *Monacha cantiana*.
Data source: (Al-Sarar et al., 2012).

Table 2: Qualitative phytochemical screening tests applied to *Calotropis procera*.

Phytoconstituent	Test Employed	Positive Indicator
Alkaloids	Mayer's / Dragendorff's test	Cream / orange-red precipitate
Flavonoids	Shinoda test	Pink-magenta colouration
Cardenolides / Glycosides	Keller-Kiliani / Kedde test	Blue-green ring / violet colour
Saponins	Froth test	Persistent honeycomb froth
Tannins and Phenolics	Ferric chloride test	Blue-black / greenish colouration
Terpenoids / Steroids	Salkowski / Liebermann-Burchard test	Reddish-brown / green-blue ring
Proteins and Enzymes	Biuret / xanthoproteic test	Violet / yellow colouration

defoliator) (Kumar and Arya, 2006). Latex-based formulations have similarly demonstrated potent activity against *Sarcophaga haemorrhoidalis* (flesh fly) and *Musca domestica* larvae under controlled toxicological assays (Moursy, 1997; Morsy et al., 2001).

Ovicidal and Mosquito-Vector Control Activity

Aqueous leaf extract at 1,000 ppm produced 100% larvicidal mortality against fourth-instar larvae of *Culex tritaeniorhynchus* and *Cx. gelidus*, together with 100% ovicidal activity against the eggs of both mosquito species, and conferred complete bite-protection for up to 240 min at the same concentration (Kumar and Arya, 2006). In a separate systematic screening across plant organs, methanolic root extracts of *C. procera* produced the strongest ovicidal effect against the stored-product pest *Cadra cautella*, preventing hatching of 50% of treated eggs at 10,000 ppm, with leaf extracts showing comparatively lower (36%) ovicidal mortality (Bader et al., 2021).

Molluscicidal Activity

In a controlled comparative bioassay, the cardenolide-rich extract of *C. procera* exhibited a contact LD₅₀ of 34.35 mg kg⁻¹ body

weight against the land snail *Monacha cantiana* - approximately 3.4-fold more toxic than the synthetic standard molluscicide methomyl (LD₅₀=116.62 mg kg⁻¹) (Al-Sarar et al., 2012), confirming the superior potency of plant-derived cardenolides over a conventional carbamate molluscicide (Figure 2).

Nematicidal, Antifungal and Grain-Protectant Activity

C. procera extracts, rich in cardiac glycosides, alkaloids, terpenes, and tannins, have demonstrated nematicidal potential against *Meloidogyne incognita* in tomato under both *in vitro* and *in vivo* conditions, attributable to the chemical groups known to disrupt nematode physiology (Alqahtani et al., 2019). Against the phytopathogenic fungus *Macrophomina phaseolina*, a causal agent of charcoal rot, *C. procera* extracts significantly inhibited mycelial growth, supporting its candidacy as a biofungicide (Waheed et al., 2016). As grain protectants, flavonoid-rich fractions, latex, and crude extracts of *C. procera* reduced infestation by two major coleopteran pests of stored rice, offering a botanical alternative to synthetic fumigants in post-harvest pest management (Nenaah, 2013; Nenaah, 2013).

Comparative Summary of Bioactivity Data

Table 5 consolidates the principal statistically reported bioactivity values of *C. procera* against different pest/target organisms, drawn from the peer-reviewed studies cited throughout this chapter.

Mechanism of Biopesticidal Action

The dominant hypothesis for cardenolide-mediated toxicity centres on potent, specific inhibition of the Na^+/K^+ -ATPase membrane pump in target insect, nematode, and mollusc cells. Cardenolides (calotropin, uscharin, calactin) bind at the extracellular ouabain-binding site of the enzyme, blocking active transport of Na^+ and K^+ ions across the cell membrane (Pandey *et al.*, 2016; Reddy *et al.*, 2020). This disrupts membrane potential and ionic homeostasis, leading to neuromuscular dysfunction, feeding cessation, impaired metamorphosis, and

ultimately mortality of the target pest (Bader *et al.*, 2021; Reddy *et al.*, 2020). Latex-borne proteolytic enzymes are thought to act synergistically, damaging the peritrophic membrane and gut epithelium of larval pests, thereby enhancing cardenolide uptake and overall toxicity (Ramos *et al.*, 2007). Figure 3 illustrates this proposed mechanism schematically.

Green Chemistry Workflow for Biopesticide Development

Figures 4 and 5 (overleaf/below) presents a consolidated flow chart integrating collection, extraction, phytochemical screening, chemical evaluation, nanoparticle-mediated green formulation, and bioassay validation stages, reflecting the complete green-chemistry pipeline discussed in Sections 2-6 (Table 6) (Benelli *et al.*, 2017; Boller *et al.*, 2005; Dara, 2019).

Table 3: Chemical evaluation parameters used in the standardisation of *Calotropis procera* biopesticidal extracts (Bader *et al.*, 2021; Kumar *et al.*, 2013; Saddiq *et al.*, 2022; Hanna, 2002).

Evaluation Parameter	Method / Instrument	Significance for Biopesticide Standardisation
Total Phenolic Content (TPC)	Folin-Ciocalteu colorimetric assay	Correlates with antioxidant and antifeedant potential
Total Flavonoid Content (TFC)	Aluminium chloride colorimetric assay	Indicates flavonoid glycoside abundance
DPPH Radical Scavenging (IC_{50})	UV-vis spectrophotometry	Quantifies free-radical scavenging efficiency
Cardenolide quantification	Kedde colorimetric method	Estimates cardiac-glycoside (active) concentration
Metabolite fingerprinting	LC-ESI-Orbitrap-MS/MS, GC-MS	Identifies and confirms bioactive constituents
Functional group analysis	FTIR spectroscopy	Confirms capping/reducing groups in nano-formulations
Structural elucidation	^1H / ^{13}C NMR	Confirms cardenolide molecular architecture
Particle characterisation (for NP formulations)	XRD, SEM, DLS	Determines size, morphology and stability of bio-NPs

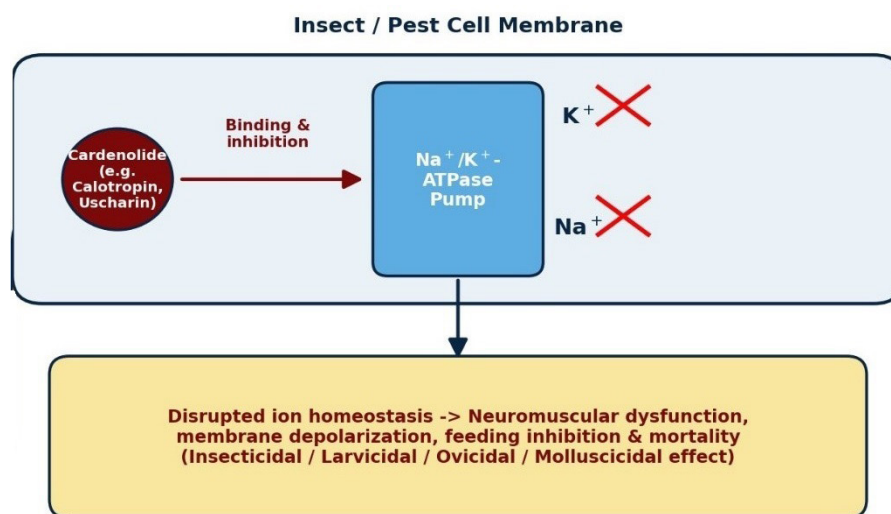


Figure 3: Proposed Mechanism of Cardenolide-Mediated Biopesticidal Action of *Calotropis procera* via Na^+/K^+ -ATPase Inhibition.

Table 4: Green-synthesised nanoparticle systems using *Calotropis procera* phytochemicals as reducing/capping agents (Saddiq et al., 2022).

Nanoparticle System	Capping Phytochemical(s)	Reported Biological Application
Ag-NPs (silver)	Alkaloids, phenols, terpenoids	Antibacterial, antifungal, electrochemical sensing
Nanoparticle System	Capping Phytochemical(s)	Reported Biological Application
CuO-NPs (Copper Oxide)	Flavonoids, glycosides, triterpenoids	Antifungal (<i>Rhizopus oryzae</i>), antibacterial
Fe ₃ O ₄ -NPs (magnetite)	Phenolics, flavonoids, saponins	Antimicrobial; carrier for targeted delivery

Table 5: Comparative statistical summary of *Calotropis procera* biopesticidal activities across pest species.

Target Organism	Plant Part / Extract	Reported Activity (Value)	References
<i>Musca domestica</i> (housefly larvae)	Ethanollic leaf extract	LC50=282.5 mg L ⁻¹	(Kumar and Arya, 2006)
<i>Culex tritaeniorhynchus</i> / <i>Cx. Gelidus</i>	Aqueous leaf extract	100% larvicidal and ovicidal @ 1000 ppm	(Kumar and Arya, 2006)
<i>Cadra cautella</i> (almond moth)	Methanolic root extract	50% ovicidal mortality @ 10,000 ppm	(Bader et al., 2021)
<i>Monacha cantiana</i> (land snail)	Cardenolide extract	LD ₅₀ =34.35 mg kg ⁻¹ (3.4x > methomyl)	(Al-Sarar et al., 2012)
<i>Clostera cupreata</i> / <i>Plecoptera reflexa</i>	Leaf extractives	LC50 range 0.625-2.00%	(Kumar and Arya, 2006)
<i>Macrophomina phaseolina</i> (fungus)	Crude extract	Significant mycelial growth inhibition	(Waheed et al., 2016)
Coleopteran rice pests (stored grain)	Flavonoids / latex	Reduced grain infestation (grain protectant)	(Nenaah, 2013, Nenaah, 2013)
<i>Meloidogyne incognita</i> (root-knot nematode)	Aqueous leaf extract	Reduced root galling in tomato (<i>in vivo</i>)	(Alqahtani et al., 2019)

Comparative Advantages over Synthetic Pesticides

Table 6: Comparative advantages of *Calotropis procera*-based green biopesticides over conventional synthetic pesticides.

Attribute	Synthetic Pesticides	<i>C. procera</i> -Based Biopesticides
Biodegradability	Low - persistent residues	High - readily biodegradable
Attribute	Synthetic Pesticides	<i>C. procera</i> -Based Biopesticides
Non-target toxicity	Often high (mammals, pollinators)	Generally lower at effective doses
Resistance development	Widely reported in target pests	Multi-compound action reduces resistance risk
Raw material cost	Petrochemical-dependent, costly	Abundant, low-cost wasteland biomass
Environmental compliance	Regulatory restrictions increasing	Aligned with green chemistry principles
Mode of action	Often single-target	Multi-target (ion-pump inhibition + proteolysis)



Figure 4: *Calotropis procera* in habitat. Photograph showing the thick glaucous ovate-oblong leaves, an immature fruit (follicle), and umbellate clusters of the characteristic star-shaped corona flowers.

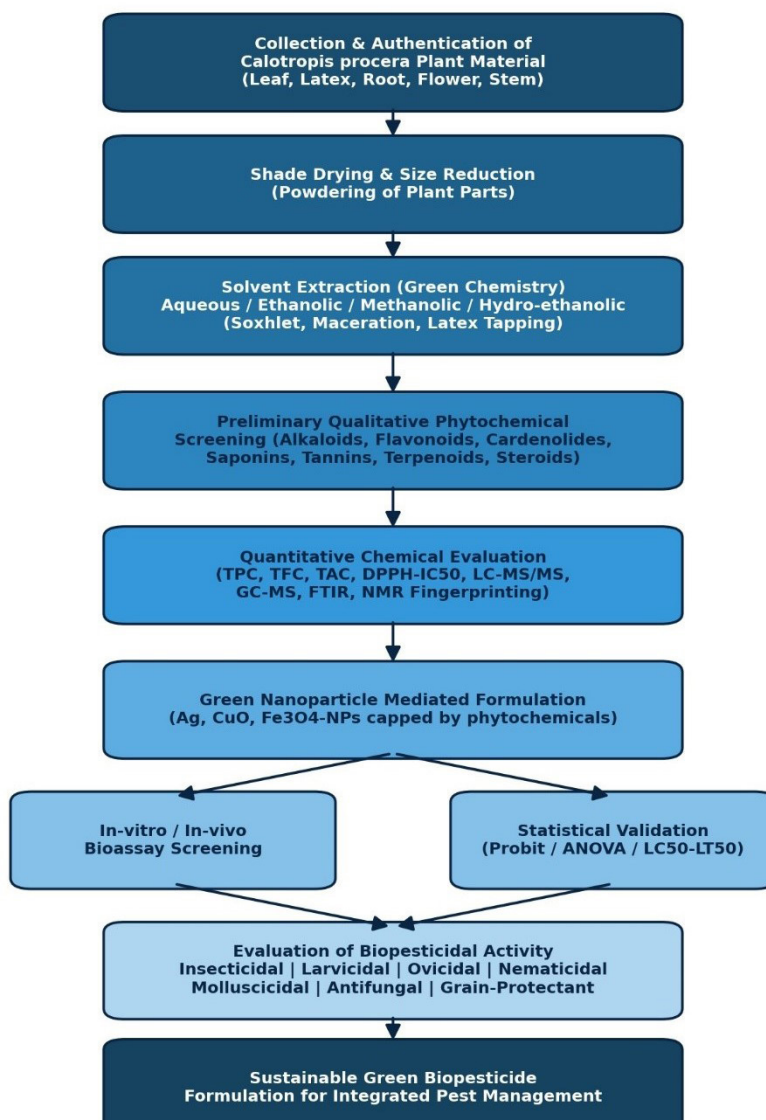


Figure 5: Workflow of the green chemistry approach for development of *Calotropis procera*-based biopesticides.

CHALLENGES AND FUTURE PROSPECTS

Standardisation

Cardenolide and flavonoid content varies with plant part, season, and geographic origin, necessitating robust chemical-evaluation protocols (Section 4) prior to commercial formulation.

Mammalian safety margins

Although generally safer than synthetic analogues, cardenolides possess cardiotoxic activity in mammals and require rigorous dose-toxicity profiling before field-scale deployment.

Formulation stability

Green-synthesised nanoparticle carriers may improve shelf-life, dispersibility, and controlled release of active cardenolides in field conditions.

Regulatory pathway

Clear registration guidelines for plant-derived biopesticides are needed to accelerate translation from laboratory bioassay to commercial product.

Field-scale validation

Most reported data derive from laboratory bioassays; multi-season, multi-location field trials remain a priority research gap.

CONCLUSION

Calotropis procera represents a scientifically well-documented, phytochemically rich, and statistically validated candidate for green-chemistry-based biopesticide development. Its cardenolide-dominant chemistry underlies broad-spectrum insecticidal, larvicidal, ovicidal, molluscicidal, nematocidal, and antifungal activity, while its compatibility with eco-friendly nanoparticle synthesis further extends its applicability to next-generation crop-protection formulations. Continued standardisation, toxicological profiling, and field validation will be essential to translate this considerable laboratory evidence base into a commercially viable, sustainable pest-management tool.

ABBREVIATIONS

LC₅₀: Median Lethal Concentration; **LD₅₀**: Median Lethal Dose; **IC₅₀**: Half-Maximal Inhibitory Concentration; **TPC**: Total Phenolic Content; **TFC**: Total Flavonoid Content; **TAC**: Total Antioxidant Capacity; **DPPH**: 2,2-Diphenyl-1-Picrylhydrazyl; **FTIR**: Fourier-Transform Infrared Spectroscopy; **GC-MS**: Gas Chromatography-Mass Spectrometry; **NMR**: Nuclear Magnetic Resonance; **XRD**: X-ray Diffraction; **SEM**: Scanning Electron Microscopy; **DLS**: Dynamic Light Scattering; **IPM**: Integrated Pest Management; **AgNPs**: Silver Nanoparticles; **CuONPs**: Copper Oxide Nanoparticles; **Fe₃O₄**: Magnetite.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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