

Antimicrobial Activity of *Calotropis gigantea* against *Escherichia coli*: A Review

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Abstract: *Background:* Multidrug-resistant *Escherichia coli* is a leading cause of urinary tract, bloodstream and enteric infections, and new antibacterial leads are urgently needed. *Calotropis gigantea* (L.) W.T. Aiton (Apocynaceae), the crown flower, is a latex-bearing shrub widely used in traditional medicine for wounds, skin disease and diarrhoea. *Objective:* To review the published evidence on the activity of *C. gigantea* extracts, latex and plant-mediated nanoparticles against *E. coli*. *Findings:* Studies using latex, leaf, flower and root-bark preparations report measurable in vitro inhibition of *E. coli*, with reported inhibition zones ranging from about 11 mm for nanoparticle-based formulations to over 30 mm for ethanol leaf extract in agar-diffusion assays. Silver nanoparticles synthesised with leaf or flower extracts also inhibit *E. coli*. Plausible mechanisms include membrane damage by terpenoids and phenolics, protein and enzyme inhibition, and silver-ion and oxidative effects for nanoparticles, but mechanistic data specific to this plant are scarce. *Conclusion:* Evidence is largely preliminary: methods are heterogeneous, MIC data and compound isolation are limited, and in vivo efficacy and safety data are absent. *C. gigantea* is best regarded as a source of leads and a candidate for topical or adjunct applications pending standardised, mechanistic and toxicological studies.

Keywords: *Calotropis gigantea*; *Escherichia coli*; antibacterial activity; plant extracts; latex; green-synthesised silver nanoparticles; antimicrobial resistance

I. INTRODUCTION

Escherichia coli is a Gram-negative bacillus that is both a normal intestinal commensal and a versatile pathogen. Pathogenic lineages cause urinary tract infection, sepsis, neonatal meningitis and diarrhoeal disease [2]. Antimicrobial resistance (AMR) has sharply reduced therapeutic options, and *E. coli* is among the pathogens contributing most to the global burden of bacterial AMR-associated deaths [1]. This has renewed interest in natural products, including plant secondary metabolites, as sources of new antibacterial scaffolds and adjuvants [3,17].

Calotropis gigantea is a well-known medicinal plant of tropical Asia whose latex, leaves, flowers and root bark feature in Ayurvedic, Unani and folk practice [14]. Many laboratory studies have tested its preparations against bacterial panels that include *E. coli*, but the findings are dispersed and rarely compared critically. This review consolidates the evidence specific to *E. coli*, discusses likely mechanisms, and identifies the methodological and translational gaps that must be closed before the plant can be considered for pharmaceutical development.

Literature approach. Studies were identified through searches of open web and bibliographic sources using combinations of the terms *Calotropis gigantea*, *Escherichia coli*, antibacterial, extract, latex and silver nanoparticles. Primary studies reporting data on *E. coli* are summarised in Table I. This is a narrative, not a systematic, review, and additional studies may exist.



I. BOTANICAL AND ETHNOPHARMACOLOGICAL BACKGROUND

C. gigantea (Apocynaceae, subfamily Asclepiadoideae), known as crown flower, giant milkweed, akanda or madar, is an erect shrub of up to about 4 m with thick, waxy, opposite leaves, lavender-white flowers and inflated follicles that release silky-haired seeds. All parts exude a white, sticky latex. It is distributed across dry and waste lands of South and Southeast Asia and has been introduced elsewhere. Traditional uses include treatment of wounds, boils, skin disorders, ulcers, dysentery, fever, cough and asthma [14]. These uses motivated antibacterial screening, although traditional use does not by itself establish efficacy or safety. The congener *C. procera* is chemically similar and often studied alongside it; results for one species should not be assumed to apply to the other.

II. PHYTOCHEMISTRY

Qualitative screening of a methanol extract of *C. gigantea* flowers detected alkaloids, steroids, terpenoids, flavonoids, saponins, tannins and coumarins [6]. The genus is additionally characterised by cardenolide (cardiac glycoside) constituents such as calotropin, calactin, uscharin and calotoxin, pentacyclic triterpenoids (alpha- and beta-amyrin and their esters, lupeol), phytosterols, flavonoid glycosides and latex proteins including cysteine proteases. Composition varies with plant part, geography, season and extraction solvent, which is a principal source of inconsistency among antibacterial studies.

The antibacterial relevance of these classes is broadly documented: phenolics and flavonoids can bind proteins and disrupt enzymes and membranes, tannins complex with proteins and metal ions, and terpenoids can perturb membrane integrity [3]. However, which specific compounds account for the anti-*E. coli* activity of *C. gigantea* has not been established by bioassay-guided isolation.

III. ESCHERICHIA COLI AS A TARGET FOR PLANT EXTRACTS

The Gram-negative outer membrane, with its lipopolysaccharide-rich outer leaflet and porin-restricted channels, limits entry of many hydrophobic or large molecules, and multidrug efflux systems expel diverse compounds [4]. Consequently, crude plant extracts frequently show lower activity against *E. coli* than against Gram-positive bacteria such as *S. aureus*. Reported activity against *E. coli* therefore deserves attention, but must be interpreted with respect to assay type, strain (laboratory versus clinical, resistant versus susceptible) and controls.

IV. EVIDENCE OF ACTIVITY AGAINST *E. COLI*

A. Latex

An aqueous latex extract collected in Rawalpindi and Islamabad (Pakistan) was tested by agar well diffusion against *S. aureus*, *Lactobacillus* and *E. coli*. The inhibition zone for *E. coli* was 24.6 ± 0.5 mm, compared with 30 ± 1 mm for *S. aureus* and 16 ± 1 mm for *Lactobacillus*; MIC values of 62.5 were reported for both *S. aureus* and *E. coli*† and 125 for *Lactobacillus* [5]. The latex may therefore contain potent antibacterial fractions, but its irritant and toxic components (Section VIII) limit direct use.

B. Leaf, Flower and Other Aerial Parts

In a comparison of ethanol and aqueous extracts of leaves, fruit, seeds, flowers and stem, the ethanol leaf extract was the most active, giving an agar-well inhibition zone of 30.08 mm against *E. coli*, larger than the zones reported for *S. aureus*, *S. typhimurium* and *P. aeruginosa* in the same study [8]. A methanol extract of flowers from Aceh, Indonesia, tested at 10, 25, 50, 75 and 100% by disc diffusion against *S. aureus* and *E. coli* (chloramphenicol as the positive control for *E. coli*), showed activity against both organisms, with inhibition increasing with extract concentration [6]. The recurring pattern is that organic solvents of intermediate to high polarity (ethanol, methanol) yield the most active extracts, in keeping with extraction of flavonoids, phenolics and glycosides.

C. Root Bark

A methanol extract of root bark and its petroleum ether, chloroform and ethyl acetate fractions were evaluated by disc diffusion at 20, 30 and 40 µg/disc against six bacteria, with kanamycin as the reference. The methanol extract and



chloroform fraction were comparatively the more active overall, while the ethyl acetate fraction inhibited *P. aeruginosa* and *E. coli*; MICs were determined by serial dilution [7]. This points to moderately polar constituents of the root bark as relevant to Gram-negative activity, though root bark is also the part richest in toxic cardenolides.

D. Green-Synthesised Silver Nanoparticles

Aqueous leaf and flower extracts of *C. gigantea* have been used as reducing and capping agents to produce silver nanoparticles (AgNPs). In one study, AgNPs prepared from plants growing in a geothermal area of Aceh showed surface plasmon resonance peaks at 410–460 nm, agglomerated spherical particles of about 88–257 nm and zeta potentials of -41.8 to -25.1 mV; the flower-derived particles were the most potent, with a disc-diffusion inhibition zone of 12.05 ± 0.58 mm against *E. coli* [9]. Optimised leaf-derived AgNPs (smallest size 56 nm) were later hybridised with patchouli oil, with reported enhancement of antibacterial and antifungal effects [10], and an AgNP-based handwash formulated from these particles produced a 10.95 ± 0.8 mm zone against *E. coli* with no irritation reported in the product evaluation [11]. Other groups report that leaf-extract AgNPs are active against both Gram-positive and Gram-negative organisms [12,13]. A key caveat is that the activity of nanoparticles may derive mainly from silver rather than from the plant; controls using silver nitrate alone, extract alone and commercial AgNPs are needed to apportion the effect.

TABLE I: SELECTED STUDIES REPORTING ACTIVITY OF *C. GIGANTEA* PREPARATIONS AGAINST *E. COLI*

Preparation / source	Method	Reported finding for <i>E. coli</i>	Ref.
Aqueous latex (Pakistan)	Agar well diffusion; MIC	Zone 24.6 ± 0.5 mm; MIC 62.5†	[5]
Methanol extract of flowers (Aceh, Indonesia)	Disc diffusion, 10–100% extract	Active; zone increased with concentration	[6]
Methanol extract and fractions of root bark (Bangladesh)	Disc diffusion, 20–40 µg/disc; MIC	Ethyl acetate fraction active against <i>E. coli</i>	[7]
Ethanol extracts of leaf, fruit, seed, flower, stem	Agar well diffusion	Leaf ethanol extract highest: zone 30.08 mm	[8]
AgNPs from aqueous flower extract (AgNPs-FCg)	Kirby–Bauer disc diffusion	Zone 12.05 ± 0.58 mm	[9]
AgNP-based handwash (leaf-derived AgNPs)	Zone of inhibition	Zone 10.95 ± 0.8 mm (best formula)	[11]
Leaf-extract AgNPs	Antimicrobial screening	Activity against Gram-positive and Gram-negative strains	[12]

V. PROPOSED MECHANISMS OF ACTION

Mechanistic studies of *C. gigantea* against *E. coli* are lacking; the following are inferred from the behaviour of the constituent classes and of silver nanoparticles.

- Membrane disruption: lipophilic terpenoids and phenolics may perturb the outer and cytoplasmic membranes, increasing permeability and causing leakage of cell contents [3].
- Enzyme and protein inhibition: flavonoids, tannins and other phenolics can bind microbial proteins and inactivate enzymes [3].
- Resistance modulation: some plant compounds inhibit efflux or act synergistically with antibiotics; this has not been systematically tested for *C. gigantea*.
- Nanoparticle effects: silver nanoparticles damage the *E. coli* envelope, alter membrane morphology and permeability, and release Ag^+ ions that interfere with respiratory and DNA-associated functions [15,16].
- Latex proteases: cysteine proteases may damage surface proteins, although their role against Gram-negative bacteria is not established.



It is noteworthy that cardenolides act principally on the eukaryotic Na^+/K^+ -ATPase, which bacteria lack; they are therefore unlikely to be the direct antibacterial principle and are better regarded as a toxicity liability.

VI. LIMITATIONS OF THE CURRENT EVIDENCE

- Heterogeneity: plant part, collection site, extraction method and solvent differ widely, so results cannot be pooled or ranked.
- Assay type: most studies use agar diffusion, which is influenced by compound diffusion and gives only qualitative comparisons; MIC and MBC values are less frequently reported [17].
- Controls and reporting: solvent, positive and negative controls, replicates and statistics are not consistently described.
- Characterisation: extracts are seldom standardised or profiled by HPLC or LC-MS, and active compounds are not isolated.
- Strains: reference strains predominate; multidrug-resistant and clinical isolates and different pathotypes are rarely tested.
- Nanoparticle studies: missing precursor and extract controls make it difficult to attribute activity to the plant.
- No in vivo data: no animal infection model or pharmacokinetic study for *E. coli* was identified.

Adoption of standard methods such as CLSI and EUCAST broth microdilution, with quality-control strains and appropriate natural-product reporting practices, would improve comparability [17,18,19].

VII. SAFETY CONSIDERATIONS

Calotropis latex is a strong irritant that can cause severe dermatitis and corneal or mucosal injury, and cardenolide-containing parts can be cardiotoxic if ingested. Antibacterial activity in vitro therefore cannot be translated to oral or systemic use without careful toxicological evaluation. Topical or device-related uses and purified, cardenolide-free fractions appear more realistic, subject to cytotoxicity testing. Nanoparticle formulations raise additional questions about cytotoxicity, environmental release and regulatory status.

VIII. FUTURE PERSPECTIVES

- Bioassay-guided fractionation to isolate and identify anti-*E. coli* constituents, with MIC and MBC determination.
- Standardised, chemically fingerprinted extracts from authenticated, vouchered plant material.
- Testing against multidrug-resistant and clinical *E. coli* isolates, including ESBL producers and distinct pathotypes.
- Time-kill kinetics, anti-biofilm and anti-virulence assays, and checkerboard synergy studies with conventional antibiotics.
- Mechanistic work: membrane integrity, electron microscopy, efflux and transcriptomic analyses.
- In vivo efficacy models (for example, urinary tract or wound infection) with toxicity and pharmacokinetic studies.
- Well-controlled nanoparticle studies that separate the contributions of silver and plant constituents and assess safety.

IX. CONCLUSION

Published studies indicate that *Calotropis gigantea* latex, leaf, flower and root-bark preparations, and nanoparticles prepared with its extracts, inhibit *Escherichia coli* in vitro, with ethanol or methanol extracts and latex giving the largest reported zones. The evidence is preliminary and heterogeneous, mechanisms are inferred rather than demonstrated, and toxicity and in vivo data are lacking. *C. gigantea* merits further rigorous investigation as a source of



antibacterial leads and as a candidate for topical or adjunct use, but should not be considered a validated antibacterial therapy.

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