RESEARCH PAPER

Comparative Phytochemical Profiling and In Vitro Antimicrobial Activity of Leaf and Bark Extracts of Glochidion zeylanicum (Gaertn.) A.Juss.

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Received: 28th Jul, 2026 | Revised: 01st Aug, 2026 | Accepted: 05th Aug, 2026 | Available Online: 17th Aug, 2026

ABSTRACT

The emergence of antimicrobial resistance necessitates the exploration of medicinal plants as alternative sources of bioactive compounds. This study investigated the phytochemical composition and antimicrobial activity of Glochidion zeylanicum (Gaertn.) A.Juss. leaf and bark extracts collected from the Paderu Forest Range, Eastern Ghats, Andhra Pradesh, India. Sequential Soxhlet extraction using chloroform, ethyl acetate, acetone, ethanol, and water produced a polarity-dependent extraction yield, with aqueous extracts showing the highest recovery (leaf: 14.65%; bark: 9.32%) and chloroform the lowest. Phytochemical screening revealed abundant glycosides, steroids, terpenoids, quinones, and alkaloids, with bark extracts exhibiting greater chemical diversity than leaves. Antimicrobial activity was evaluated against ten bacterial and four fungal reference strains using the agar well diffusion assay. All extracts exhibited concentration-dependent antimicrobial activity, although lower than the respective standard drugs. Acetone extracts demonstrated the strongest antibacterial activity against Gram-positive bacteria, achieving 55.9% and 54.6% of standard inhibition against Streptococcus mutans and Bacillus subtilis, respectively. The highest Gram-negative activity (67.8% of standard) was observed against Salmonella enterica. Antifungal activity was confined to Candida albicans and Candida glabrata, reaching 60.8% of the standard, with no inhibition against Aspergillus spp. These findings scientifically validate the traditional medicinal use of G. zeylanicum and identify acetone and ethanol bark extracts as promising candidates for bioassay-guided isolation of antimicrobial phytoconstituents.

Keywords: Phyllanthaceae; phytochemical screening; antimicrobial activity; agar well diffusion; Eastern Ghats

How to cite this article: Sowmithri C, Sujatha B. Comparative Phytochemical Profiling and In Vitro Antimicrobial Activity of Leaf and Bark Extracts of Glochidion zeylanicum (Gaertn.) A.Juss. Int J Drug Deliv Technol. 2026;16(75s): 1100. DOI: 10.25258/ijddt.16.75s.116

1. Introduction

Medicinal plants have served as the foundation of human healthcare since antiquity, with archaeological and textual evidence tracing their use back thousands of years across India, China, Egypt, and the Arab world (Ang-Lee et al., 2001; Stojanoski, 1999). In India specifically, the

Ayurveda system dates back to approximately 6000 BC and is among the oldest codified medical traditions, relying extensively on herbal formulations transmitted through oral and later written traditions, with classical texts such as the Charaka and Sushruta Samhitas documenting extensive plant-based therapeutic practice (Petrovska, 2012). This empirical, trial-and-error tradition gradually matured into structured, documented systems of knowledge through intercultural exchange and scholarly synthesis (Qiu, 2007), a transition equally evident in the classical Greek period through the work of Hippocrates, Aristotle, and later Dioscorides (Madsen & Bertelsen, 1995; Rios & Recio, 2005; Zargari, 1992).

India remains one of the most botanically diverse nations for medicinal flora, harboring over 2,500 species with therapeutic potential and supporting indigenous systems such as Unani, Siddha, and Ayurveda, yet only a small fraction of this diversity has undergone rigorous phytochemical and pharmacological validation (Gupta et al., 2005; Sandhu & Heinrich, 2005). This gap is particularly significant given that, globally, over 80–85% of the world's population depends on traditional plant-based remedies for primary healthcare (Pesic, 2015), and a substantial share of modern pharmaceutical agents trace their origin, directly or indirectly, to plant natural products (Bauer & Brönstrup, 2014).

The therapeutic relevance of medicinal plants is underpinned by their capacity to biosynthesize a vast and structurally diverse pool of secondary metabolites — alkaloids, flavonoids, tannins, terpenoids, phenolics, saponins, and glycosides — many of which have served as scaffolds for clinically important drugs, from morphine and quinine (Demirkapu & Yanan, 2021; Rasoanaivo et al., 2011) to artemisinin (Liu et al., 2020) and warfarin (Hajar et al., 2017). Plants collectively produce more than 50,000 distinct secondary metabolites, many of which have been explored for their medicinal value (Teoh, 2016), and these compounds frequently act synergistically within whole-plant extracts, a phenomenon increasingly recognized as central to their efficacy in complex, multifactorial diseases such as cancer and chronic inflammation (Hassan, 2012; Phillipson, 2001).

Parallel to this, the escalating global burden of antimicrobial resistance (AMR) has renewed scientific urgency around plant-derived antimicrobials. Indiscriminate antibiotic use has driven the evolution of resistant bacterial, fungal, and viral strains through mechanisms such as enzymatic drug inactivation, efflux pump overexpression, and biofilm formation, progressively narrowing available treatment options (Chopra et al., 1996; Baquero, 1997). Medicinal plants offer a multi-target alternative: their phytoconstituents can disrupt microbial membranes, inhibit nucleic acid and protein synthesis, and interfere with quorum-sensing and biofilm pathways, a mechanistic diversity that inherently lowers the probability of resistance emergence compared with single-target synthetic antibiotics (Cowan, 1999; Khameneh et al., 2019). This has intensified interest in screening ethnomedicinally significant but pharmacologically underexplored plant species against panels of clinically relevant Gram-positive, Gram-negative, and fungal pathogens (Gupta & Birdi, 2017).

Within this framework, *Glochidion zeylanicum* (Gaertn.) A.Juss. (family Phyllanthaceae) represents a promising yet underexplored candidate. Distributed across South and Southeast Asia, including India, Sri Lanka, and Thailand, the species has a long history of ethnomedicinal use for inflammation, wounds, fever, and gastrointestinal disorders (Duangjan et al., 2019). Phytochemical investigations have confirmed that its leaves and roots are rich in phenolics and flavonoids, with reported total phenolic and flavonoid contents in the ranges of 85–145 mg GAE/g and 40–95 mg QE/g extract, respectively, depending on solvent polarity (Duangjan et al., 2019; Raunsai et al., 2022), alongside more than 20–35 characterized secondary metabolites, including catechin, resveratrol 4′-methyl ether, and the species-specific flavanol glycosides glochiflavanosides A–D (Duangjan et al., 2019; Duangjan et al., 2021; Otsuka et al., 2003). These constituents underlie the plant's demonstrated antioxidant activity, with DPPH and ABTS IC_{50} values of 18–35 µg/mL and 15–30 µg/mL respectively (Duangjan et al., 2019; Raunsai et al., 2022), corroborated *in vivo* in *Caenorhabditis elegans* models through DAF-16/FoxO and SKN-1/Nrf2-mediated stress-response activation (Duangjan et al., 2019; Kensler et al., 2007). Preliminary evidence also points to moderate antibacterial activity, with MIC values of 50–250 µg/mL against *S. aureus*, *E. coli*, and *B. subtilis* (Raunsai et al., 2022), and dose-dependent cytotoxicity against HepG2, HT-29, and PC-3 cancer cell lines, with IC_{50} values of 35–120 µg/mL (Duangjan et al., 2021; Raunsai et al., 2022).

Despite these encouraging findings, existing reports on *G. zeylanicum* remain fragmented, largely restricted to isolated phytochemical surveys or single-assay bioactivity screens, with comparatively few studies systematically correlating extract chemistry with quantitative antimicrobial and antioxidant efficacy across a broad, clinically representative panel of pathogens. Moreover, comparative data on solvent-dependent extraction efficiency and structure–activity relationships for its dominant metabolites remain limited (Jiang et al., 2024; Linh et al., 2024). Addressing this gap, the present study undertakes a systematic phytochemical and pharmacological evaluation of *G. zeylanicum*, with the objective of quantifying its phenolic and flavonoid content, profiling its bioactive constituents, and assessing its antioxidant and antimicrobial potential against a diverse panel of Gram-positive, Gram-negative, and fungal reference strains, thereby contributing toward the scientific validation of its traditional use and its prospective application as a natural antimicrobial and antioxidant agent.

2. Materials and Methods

2.1 Study Area

The study was conducted in the Eastern Ghats of Andhra Pradesh, south-eastern India, a biodiversity-rich mountain system extending parallel to the Bay of Bengal. The region comprises tropical moist and dry deciduous forests, semi-evergreen forests, grasslands, and freshwater ecosystems, supporting a rich diversity of medicinal plants and endemic flora. Major river systems, including the Godavari, Krishna, and Penna, originate from or traverse these hills, sustaining regional agriculture and livelihoods. The Eastern Ghats are inhabited by indigenous tribal communities possessing extensive ethnomedicinal knowledge. Despite their

ecological significance, these forests face increasing anthropogenic pressures from deforestation, mining, agricultural expansion, and infrastructure development.

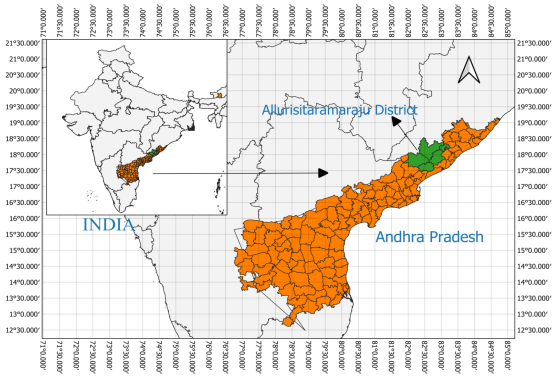

Figure 1: Schematic study area map showing the location of Andhra Pradesh within India and

2.2 Plant Material Collection and Authentication

Fresh leaves and bark of *Glochidion zeylanicum* were collected during January 2023 from the forest tract surrounding Kommula Mamidi village, located within the Paderu Forest Range of the Paderu Division, Alluri Sitarama Raju district, Andhra Pradesh, India (17°59'47.27" N 82°29'12.41" E). The collection site lies within moist deciduous forest vegetation and represents a biodiversity-rich habitat suitable for the sourcing of medicinal plant resources.

Plant material was taxonomically identified and authenticated by S. B. Padal, Department of Botany, Andhra University, Visakhapatnam, India, and voucher specimens were deposited in the Andhra University Herbarium (AUV) under accession number AUV-24825 for future reference.

Following authentication, plant material was cleaned to remove adhering soil and extraneous matter, and the leaves and bark were separated and shade-dried at ambient temperature (30-32 °C) under well-ventilated conditions until a constant weight was attained. The dried material was pulverised using a mechanical grinder and passed through a 0.5 mm mesh sieve to obtain a homogeneous coarse powder, which was stored in sterile, airtight containers under dry conditions until further use.

2.3 Soxhlet Extraction

Sequential Soxhlet extraction was performed to enable continuous solvent reflux and repeated washing of the plant matrix, allowing efficient recovery of both polar and non-polar constituents *(Sasidharan et al., 2011)*. Solvents were selected in order of increasing polarity to fractionate phytoconstituents according to their solubility characteristics *(Ajanal et al., 2012)*.

2.3.1 Reagents and chemicals

All solvents and reagents used were of analytical reagent (AR) or HPLC grade, comprising chloroform, acetone, ethanol, ethyl acetate, and double-distilled water.

2.3.2 Extraction procedure

Dried, powdered leaf and bark (100 g each) material of *G. zeylanicum* was accurately weighed into cellulose extraction thimbles and subjected to sequential Soxhlet extraction using solvents of increasing polarity-chloroform, ethyl acetate, acetone, 70% ethanol and water. Extraction with each solvent was continued for approximately 60 siphon cycles (~72 h) or until the siphoning solvent ran colourless. The resulting extracts were concentrated under reduced pressure using a rotary vacuum evaporator, and the semi-solid crude extracts were stored at 4 °C in sterile glass containers until further use in phytochemical screening and bioactivity evaluation *(Abubakar & Haque, 2020)*.

2.4 Qualitative Preliminary Phytochemical Screening

Extracts of *G. zeylanicum* were screened for carbohydrates, proteins, amino acids, fixed oils and fats, phenols, tannins, flavonoids, glycosides, steroids, alkaloids, saponins, terpenoids, phytosterols, quinones, gums and mucilage, phlobatannins, and reducing sugars, following standard qualitative protocols *(Harborne, 1998)*. Reducing sugars were detected using Benedict's and Fehling's tests; proteins and amino acids using the Ninhydrin and Biuret tests; fixed oils and fats using spot and saponification tests; phenols and tannins using ferric chloride and lead acetate tests; flavonoids using the Shinoda and sodium hydroxide tests; glycosides using the Liebermann and Keller-Killiani tests; steroids and phytosterols using Liebermann-Burchard-type colour reactions; alkaloids using Mayer's and Wagner's tests; saponins using the froth test; terpenoids using the Salkowski test; phlobatannins using the aqueous HCl heating test; and quinones using sulphuric acid and alcoholic potassium hydroxide tests. All reagents were freshly prepared from analytical-grade chemicals, and the appearance of characteristic colour changes or precipitates was recorded as indicative of the corresponding phytoconstituent class.

2.5 Antimicrobial Activity Evaluation

2.5.1 Collection and maintenance of microorganisms

Microbial strains were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh, India, and revived following standard MTCC protocols. Bacterial cultures - *Staphylococcus aureus* (MTCC 737), *Streptococcus mutans* (MTCC 497), *Bacillus subtilis* (MTCC 28), *Enterococcus faecalis* (MTCC 2729), *Bacillus cereus* (MTCC 1272), *Pseudomonas fluorescens* (MTCC 664), *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 1687), *Salmonella enterica* (MTCC 98), and *Klebsiella pneumoniae* (MTCC 109) - were maintained on Mueller-Hinton Agar at 37 °C for 24 h. Fungal strains - *Aspergillus niger* (MTCC 282), *Aspergillus brasiliensis* (MTCC 1344), *Candida albicans* (MTCC 227), and *Candida glabrata* (MTCC 3019) - were maintained on Potato Dextrose Agar at 26 °C for 72 h. Ciprofloxacin and clotrimazole served as positive controls for the antibacterial and antifungal assays, respectively.

2.5.2 Preparation of microbial inoculum

Bacterial suspensions were adjusted to the 0.5 McFarland standard (~1×10^8 CFU/mL) using sterile 0.85% saline. Fungal inocula were prepared by suspending spores/cells in sterile saline and adjusting the turbidity to a comparable standard density.

2.5.3 In vitro screening of antimicrobial activity

Antimicrobial activity was assessed using the agar well diffusion method *(Murray et al., 1995)*, performed in triplicate under aseptic conditions.

Antibacterial activity

Standardised bacterial inocula (100 µL) were spread onto Mueller-Hinton Agar plates, and 6 mm wells were punched aseptically. Extracts (70% ethanol and acetone fractions) were dissolved in 10% DMSO to obtain test concentrations of 100, 250, and 500 µg, and 20 µL of each concentration was dispensed into the wells. Ciprofloxacin (20 µg) served as the positive control and 10% DMSO as the negative control. Plates were incubated at 37 °C for 24 h, and zones of inhibition were measured in millimetres and

expressed as mean ± SD of three replicates.

Antifungal activity

Fungal suspensions were spread onto Potato Dextrose Agar plates, and 6 mm wells were punched aseptically. Extract solutions (100, 250, and 500 µg in 10% DMSO) were dispensed into the wells, with clotrimazole (20 µg) as the positive control and 10% DMSO as the negative control. Plates were incubated at 26 °C for 72 h, and inhibition zone diameters were recorded in millimetres as mean ± SD of triplicate determinations.

4. Results and Discussion

4.1. Extraction yield of Glochidion zeylanicum leaf and bark

Successive Extraction of leaf and bark powders (100 g) of *Glochidion zeylanicum* in 500 mL of solvents of increasing polarity (chloroform, ethyl acetate, acetone, ethanol and water) produced a clear polarity-dependent gradient in extractive yield (Fig. 2). In the leaf, the aqueous extract gave the highest recovery (14.65% w/w), followed by ethanol (10.34%), acetone (7.21%), ethyl acetate (6.94%) and chloroform (4.48%); the aqueous yield was accordingly 3.3-fold and the ethanolic yield 2.3-fold higher than the chloroform yield. Bark extracts followed the same order but with consistently lower absolute values – aqueous 9.32%, ethanol 7.62%, ethyl acetate 5.89%, acetone 5.64% and chloroform 1.28%, a 7.3-fold aqueous-to-chloroform difference.

The superior performance of water and ethanol is consistent with a predominance of hydrophilic secondary metabolites – phenolics, flavonoids, tannins, glycosides and sugars – that hydrogen-bond readily with polar solvents, whereas chloroform recovers only the smaller lipophilic fraction (sterols, terpenoids, fatty acids). This trend mirrors reports for *Glochidion ellipticum*, where methanol (8.49%) outperformed chloroform (5.18%) and petroleum ether (2.66%) (Ravi et al., 2022). Leaves consistently out-yielded bark in both species, suggesting a higher density of soluble primary and secondary metabolites in photosynthetically active tissue relative to the more lignified, structural bark.

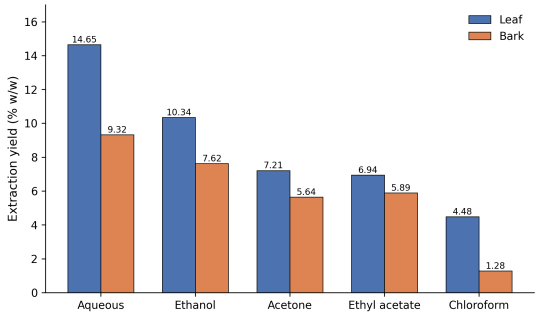

Fig. 2: Percentage extractive yield of *Glochidion zeylanicum* leaf and bark powders in solvents of increasing polarity (aqueous > ethanol > acetone > ethyl acetate > chloroform; 100 g dried powder per 500 mL solvent).

4.2. Qualitative phytochemical screening

Preliminary screening of the five leaf extracts (Table 1) revealed a chemically restricted, strongly solvent-selective profile. Proteins, amino acids, lipids, phenols, tannins, mucilage, saponins and phlobatannins were undetected in every solvent, whereas glycosides, steroids and reducing sugars were concentrated almost exclusively in the ethanol extract (Liebermann and steroid tests, both ++), flavonoids appeared only as a weak positive in ethanol, and alkaloids/terpenoids were confined to the acetone and ethanol fractions. Phytosterols, in contrast, partitioned into the more lipophilic acetone, ethyl-acetate and chloroform extracts (strongest in chloroform, ++), and quinones were

uniquely and strongly (+++) detected in acetone.

Table 1: Qualitative phytochemical screening of *G. zeylanicum* leaf extracts.

Phytoconstituent	Aq	Et	Ac	EA	Chl
Carbohydrates (Benedict's/Fehling's)	+	+	–	–	++
Proteins & amino acids	–	–	–	–	–
Fixed oils & fats	–	–	–	–	–
Phenols	–	–	–	–	–
Tannins	–	–	–	–	–
Mucilage	–	–	–	–	–
Flavonoids (Shinoda)	–	+	–	–	–
Glycosides (Liebermann)	–	++	–	–	–
Steroids	–	++	–	–	–
Alkaloids (Mayer's)	–	+	+	–	–
Terpenoids	–	+	+	–	–
Saponins	–	–	–	–	–
Phlobatannins	–	–	–	–	–
Reducing sugars	–	++	–	–	–
Phytosterols	–	–	+	+	++
Quinones	–	–	+++	–	–

Aq, aqueous; Et, ethanol; Ac, acetone; EA, ethyl acetate; Chl, chloroform. +++, *abundant;* ++, *positive;* +, *slightly positive;* –, *not detected.*

Bark extracts (Table 2) were markedly more chemically diverse than leaf extracts. Glycosides, steroids and terpenoids were detected across nearly all organic solvents (++), phenolics/tannins and flavonoids were concentrated in acetone and ethanol (++), alkaloids were strongly positive in both Mayer's (acetone, ethanol) and Wagner's (ethanol, +++) tests, and quinones showed the broadest and most intense distribution of any class, reaching +++ in acetone and ++ in every other extract. Saponins, phlobatannins, fixed oils and phytosterols were not detected in bark. Overall, bark therefore represents a considerably richer reservoir of bioactive terpenoid, steroidal, glycosidic and quinonoid constituents than leaf tissue.

Table 2: Qualitative phytochemical screening of *G. zeylanicum* bark extracts.

Phytoconstituent	Aq	Et	Ac	EA	Chl
Carbohydrates (Benedict's/Fehling's)	+	+	–	–	++
Proteins & amino acids (Ninhydrin)	–	–	–	–	+
Fixed oils & fats	–	–	–	–	–
Gums & mucilage	–	++	–	–	++
Phenols & tannins (lead acetate)	–	++	++	–	–
Flavonoids (Shinoda/NaOH)	–	++	++	–	–
Glycosides (Keller–Killiani)	+	++	++	++	++
Glycosides (Liebermann)	–	–	++	–	–
Steroids	–	++	++	++	++
Alkaloids (Mayer's)	–	++	++	–	–
Alkaloids	–	++	–	–	–

Phytoconstituent	Aq	Et	Ac	EA	Chl
(Wagner's)		+			
Saponins	–	–	–	–	–
Phlobatannins	–	–	–	–	–
Terpenoids	+	++	++	++	++
Reducing sugars	–	++	–	–	–
Phytosterols	–	–	–	–	–
Quinones	+++	++	+++	++	++

Aq, aqueous; Et, ethanol; Ac, acetone; EA, ethyl acetate; Chl, chloroform. +++, abundant; ++, positive; +, slightly positive; –, not detected.

These results agree with reports that *Glochidion* species are consistently rich in terpenoids, steroids, flavonoids, phenolics, glycosides and alkaloids (Gao et al., 2022; Xiao et al., 2018), and with the repeated isolation of triterpenoids such as glochidonol, glochidiol and glochidone from congeneric species (Thang et al., 2011; Hasan et al., 2012; Tian et al., 2013). The strong Keller–Killiani positivity in bark is consistent with reports of phenolic and megastigmane glycosides in *G. zeylanicum* and allied taxa (Gao et al., 2022). The weaker-than-expected flavonoid/phenolic response, despite prior quantitative reports of substantial flavonoid and polyphenolic content in this species (Duangjan et al., 2019; Anantachoke et al., 2015), most likely reflects the limited sensitivity of colour-reaction screening relative to HPLC/LC–MS, or the occurrence of these compounds in conjugated glycosidic forms. The pronounced and previously under-reported quinone positivity may represent oxidative derivatives of phenolic precursors, consistent with an active phenolic-oxidation pathway already documented for the genus (Gao et al., 2022), and merits targeted follow-up.

4.3. Antimicrobial activity

The 70% ethanol and acetone extracts of both leaf and bark were screened against five Gram-positive bacteria, five Gram-negative bacteria and four fungal strains. All extracts showed clear, dose-dependent inhibition (1000 > 500 > 250 µg/well) that remained below the corresponding standard antibiotic/antifungal (20 µg/mL) at every concentration tested.

4.3.1. Activity against Gram-positive bacteria

Against Gram-positive organisms (Fig. 3), the acetone extracts of both leaf and bark were generally more active than the ethanol extracts. The leaf acetone extract retained the greatest activity, reaching 55.9% of the standard's inhibition against *Streptococcus mutans* and 54.6% against *Bacillus subtilis* at 1000 µg; the corresponding leaf ethanol extract reached 52.3% and 51.4% against the same organisms. Bark extracts followed the same ranking but with lower ceiling values (acetone: 49.0% against *B. subtilis*, 45.5% against *Staphylococcus aureus*; ethanol: 39.2% against *B. subtilis*). Across both tissues, *B. subtilis* and *S. mutans* were the most susceptible species, whereas *Bacillus cereus* and *Enterococcus faecalis* were comparatively resistant, with no inhibition detectable for either extract at 250 µg against these two organisms.

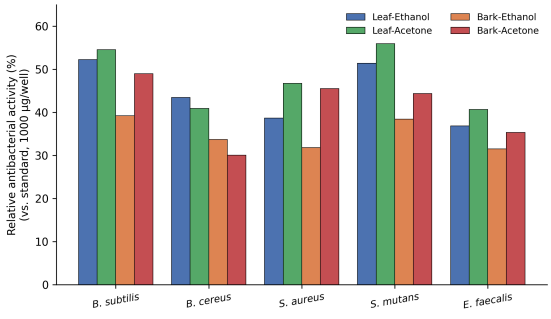

Fig. 3: Relative antibacterial activity (%, at 1000 µg/well, expressed relative to the standard antibiotic) of leaf and bark ethanol/acetone extracts of *G. zeylanicum* against five Gram-positive bacteria.

4.3.2. Activity against Gram-negative bacteria

Gram-negative susceptibility (Fig. 4) was more variable. Both leaf extracts achieved their highest relative activity against *Salmonella enterica* (67.8% for both ethanol and acetone at 1000 µg), followed by *Pseudomonas fluorescens* (46–49%) and *Escherichia coli* (47–49%). The bark acetone extract likewise peaked against *S. enterica* (65.6%) and *E. coli* (51.6%), but was completely inactive against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, both of which were only weakly inhibited by the bark ethanol extract (28.8% and 22.7%, respectively) and by the leaf extracts (39–43% and 20–36%). *K. pneumoniae* was the most resistant Gram-negative organism overall, and *P. aeruginosa* the second most resistant, consistent with their characteristically low outer-membrane permeability and efflux-pump activity.

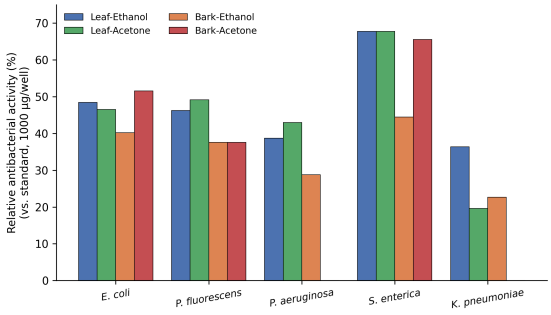

Fig. 4: Relative antibacterial activity (%, at 1000 µg/well, expressed relative to the standard antibiotic) of leaf and bark ethanol/acetone extracts of *G. zeylanicum* against five Gram-negative bacteria.

4.3.3. Antifungal activity

Antifungal action (Fig. 5) was narrow, being confined to the two *Candida* species; neither extract inhibited the filamentous fungi *Aspergillus niger* or *Aspergillus brasiliensis* at any concentration. Against *Candida albicans*, the ethanol extracts of leaf and bark were more effective than the corresponding acetone extracts (leaf-ethanol 52.1% vs. leaf-acetone 39.4%; bark-ethanol 35.2% vs. bark-acetone 30.9%, all at 1000 µg). The reverse pattern held for *Candida glabrata*, which was inhibited only by the acetone leaf extract (55.4%) among leaf samples, while bark ethanol and acetone extracts performed identically (60.8% each). The selective inhibition of yeast but not mould pathogens is consistent with the thicker, chitin- and melanin-rich cell wall and more efficient detoxification systems of *Aspergillus* spp. (Perfect, 2017).

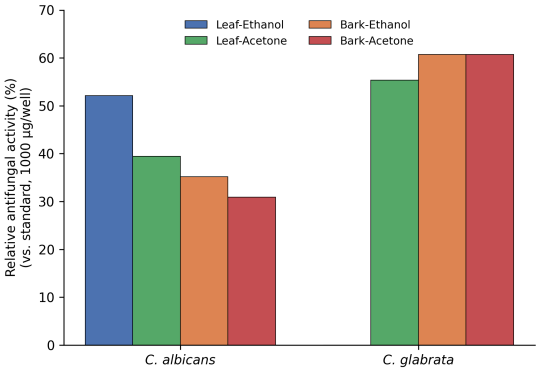

Fig. 5: Relative antifungal activity (%, at 1000 µg/well, expressed relative to the standard antifungal agent) of leaf and bark ethanol/acetone extracts of *G. zeylanicum* against *Candida albicans* and *Candida glabrata*.

4.4. General discussion

Taken together, the extraction and phytochemical data indicate that *G. zeylanicum* accumulates predominantly polar-to-moderately-polar metabolites, with bark providing a more chemically diverse (though lower-yielding) matrix than leaf, particularly with respect to terpenoids, steroids, glycosides and quinones. This chemical profile plausibly underlies the observed antimicrobial behaviour: acetone extracts, which recovered the strongest terpenoid, steroid, alkaloid and quinone responses, were consistently the more potent antibacterial fractions, whereas ethanol extracts – richer in glycosides and reducing sugars – tended to outperform acetone specifically against *C. albicans*. Such solvent-dependent antimicrobial variation is well documented (Cowan, 1999; Eloff, 1998) and is consistent with the known accumulation of hydrolysable tannins, ellagitannins, lignans, flavonoids and terpenoids across the Phyllanthaceae (Bagalkotkar et al., 2006; Calixto et al., 1998), compounds that act through membrane disruption, nucleic-acid and enzyme inhibition, metal chelation and induction of oxidative stress (Cowan, 1999; Daglia, 2012).

The greater overall susceptibility of Gram-positive bacteria is consistent with their lack of an outer lipopolysaccharide membrane, which otherwise restricts phytochemical penetration in Gram-negative species (Nikaido, 2003; Silhavy et al., 2010); the marked resistance of *P. aeruginosa* and *K. pneumoniae* further reflects their efflux-pump activity and β-lactamase production (Nikaido, 2003; Breijyeh et al., 2020). The present antimicrobial spectrum is broadly consistent with previous reports on *G. ellipticum*, *G. lanceolarium* and *G. velutinum*, which likewise contain phenolics, hydrolysable tannins, corilagin, geraniin, flavonoids and triterpenoids associated with moderate antibacterial and antioxidant activity (Quang et al., 2008; Lin et al., 2014), and with the broad-spectrum activity reported for related Phyllanthaceae such as *Phyllanthus emblica*, *P. amarus* and *P. niruri* (Bagalkotkar et al., 2006; Calixto et al., 1998) and for *Bridelia retusa*.

Although none of the crude extracts matched the potency of the reference antibiotic/antifungal agent, retention of 20–68% of standard activity by simple ethanol and acetone extracts is a promising starting point for bioassay-guided fractionation. Discrepancies with earlier quantitative phenolic/flavonoid data for this species (Duangjan et al., 2019; Anantachoke et al., 2015) most likely reflect differences in geographic origin, plant age, harvest season, extraction protocol, and the sensitivity of qualitative colour tests relative to instrumental analysis (Gobbo-Neto & Lopes, 2007). Future work combining LC–MS/HPLC dereplication with activity-guided fractionation of the acetone and ethanol bark extracts is warranted to isolate and identify the specific terpenoid, steroidal, quinonoid and glycosidic constituents responsible for the antibacterial and antifungal activities reported here.

Conclusion

This study systematically evaluated the phytochemical composition and antimicrobial potential of *Glochidion zeylanicum* leaf and bark extracts from the Eastern Ghats of Andhra Pradesh. Extraction yield increased with solvent polarity, while bark contained higher levels of bioactive phytochemicals than leaves. Acetone bark extracts exhibited the strongest antibacterial activity, particularly

against Gram-positive bacteria, whereas ethanol extracts showed better activity against *Candida albicans*. Gram-negative bacteria and filamentous fungi were comparatively resistant. Although the extracts were less potent than standard drugs, their promising bioactivity supports the ethnomedicinal importance of *G. zeylanicum* and warrants further isolation and characterization of its active antimicrobial constituents.

Funding

This research was funded by the Council of Scientific and Industrial Research.

Acknowledgements

The authors would like to express their sincere gratitude to the Department of Botany, Andhra University College of Science & Technology and for allowing us to use their Laboratories and supplying us with the microbes and all instruments. The authors gratefully acknowledge the Council of Scientific & Industrial Research (CSIR) for providing financial support to carry out this research.

References

Abubakar, A. R., & Haque, M. (2020). Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences, 12(1), 1. https://doi.org/10.4103/jpbs.jpbs_175_19

Ajanal, M., Gundkalle, M. B., & Nayak, S. U. (2012). Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Ancient Science of Life, 31(4), 198. https://doi.org/10.4103/0257-7941.107361

Anantachoke, N., Lomarat, P., Praserttirachai, W., Khammanit, R., & Mangmool, S. (2015). Antioxidant and antibacterial activities of Glochidion hypoleucum. Asian Pacific Journal of Tropical Biomedicine, 5(7), 544–549. https://doi.org/10.1016/j.apjtb.2015.04.012

Ang-Lee, M. K., Moss, J., & Yuan, C. S. (2001). Herbal medicines and perioperative care. JAMA, 286(2), 208–216.

Bagalkotkar, G., Sagineedu, S. R., Saad, M. S., & Stanslas, J. (2006). Phytochemicals from Phyllanthus niruri Linn. and their pharmacological properties: A review. Journal of Pharmacy and Pharmacology, 58(12), 1559–1570.

Baquero, F. (1997). Gram-positive resistance: Challenge for the development of new antibiotics. Journal of Antimicrobial Chemotherapy, 39(Suppl A), 1–6. https://doi.org/10.1093/jac/39.suppl_1.1

Bauer, A., & Brönstrup, M. (2014). Industrial natural product chemistry for drug discovery and development. Natural Product Reports, 31(1), 35–60.

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of Gram-negative bacteria to current antibacterial agents and approaches to resolve it. Molecules, 25(6), 1340.

Calixto, J. B., Santos, A. R. S., Cechinel Filho, V., & Yunes, R. A. (1998). A review of the plants of the genus Phyllanthus: Their chemistry, pharmacology and therapeutic potential. Medicinal

Research Reviews, 18(4), 225–258.

Chopra, I., Hodgson, J., Metcalf, B., & Poste, G. (1996). New approaches to the control of infections caused by antibiotic-resistant bacteria. An industry perspective. Journal of the American Medical Association, 275(5), 401–403.

Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564–582.

Daglia, M. (2012). Polyphenols as antimicrobial agents. Current Opinion in Biotechnology, 23(2), 174–181.

Demirkapu, M., & Yanan, S. (2021). Alkaloids and their pharmacological effects. Pharmacognosy Reviews, 15(29), 45–52.

Duangjan, C., et al. (2019). Phenolic contents and antioxidant activities of Glochidion zeylanicum. Industrial Crops and Products, 132, 544–551.

Duangjan, C., et al. (2021). Cytotoxic and biological activities of *Glochidion zeylanicum* extracts against human cancer cell lines. Biomedicine & Pharmacotherapy, 139, 111588.

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica, 64(8), 711–713.

Gao, R., Xu, X., Sun, H., et al. (2022). Norbisabolane-type sesquiterpenoid derivatives, benzofuran lignans and a phenolic glycoside from Glochidion wilsonii. Phytochemistry, 204, 113447.

Gobbo-Neto, L., & Lopes, N. P. (2007). Medicinal plants: Factors of influence on the content of secondary metabolites. Química Nova, 30(2), 374–381.

Gupta, P. D., & Birdi, T. J. (2017). Development of botanicals to combat antibiotic resistance. Journal of Ayurveda and Integrative Medicine, 8(4), 266–275. https://doi.org/10.1016/j.jaim.2017.05.004

Gupta, R., et al. (2005). Medicinal plant diversity and their conservation. Indian Journal of Traditional Knowledge, 4(3), 234–245.

Hajar, R., et al. (2017). Warfarin: Historical perspective. Heart Views, 18(1), 1–4.

Hasan, A. A., Azam, A. T. M. Z., Hasan, C. M., & Haque, M. R. (2012). Triterpenoids and steroids isolated from aerial parts of Glochidion multiloculare. Pakistan Journal of Scientific and Industrial Research A: Physical Sciences, 55(3), 163–168.

Hassan, B. A. R. (2012). Medicinal plants (importance and uses). Pharmaceutica Analytica Acta, 3(10), 1–2.

Jiang, X., Wang, Y., & Zhang, L. (2024). Advances in metabolomics-based identification of plant secondary metabolites using LC–HRMS/MS. Phytochemistry Reviews, 23, 45–68.

Kensler, T. W., Wakabayashi, N., & Biswal, S. (2007). Cell survival responses to environmental stresses via the Keap1–Nrf2–ARE

pathway. Annual Review of Pharmacology and Toxicology, 47, 89–116. https://doi.org/10.1146/annurev.pharmtox.46.120604.141046

Khameneh, B., Iranshahy, M., Soheili, V., & Bazzaz, B. S. F. (2019). Review on plant antimicrobials: A mechanistic viewpoint. Antimicrobial Resistance & Infection Control, 8, 118. https://doi.org/10.1186/s13756-019-0559-6

Lin, L. C., Wang, M. N., Tseng, T. Y., Sung, J. S., & Tsai, T. H. (2014). Phytochemical constituents and biological activities of species belonging to the genus Glochidion. Journal of Traditional and Complementary Medicine, 4(1), 1–9.

Linh, N. N., Hop, N. Q., Nhung, P. T. T., Dao, P. T. B., Manh, V. Q., Pham, T. V., & Son, N. T. (2024). Glochidion species: A review on phytochemistry and pharmacology. Natural Product Communications, 19(9). https://doi.org/10.1177/1934578X241276962

Liu, J., et al. (2020). Artemisinin and its derivatives. Pharmacology & Therapeutics, 216, 107650.

Madsen, H., & Bertelsen, F. (1995). Historical perspectives of medicinal plants. Journal of Ethnopharmacology, 48(2), 89–94.

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., & Yolken, R. H. (1995). Manual of Clinical Microbiology (6th ed.). American Society of Microbiology Press.

Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. Microbiology and Molecular Biology Reviews, 67(4), 593–656.

Otsuka, H., et al. (2003). Megastigmane glucosides and flavonoid glycosides from Glochidion zeylanicum. Phytochemistry, 62(6), 957–962.

Perfect, J. R. (2017). The antifungal pipeline: A reality check. Nature Reviews Drug Discovery, 16(9), 603–616.

Pesic, M. (2015). Role of medicinal plants in modern medicine. Botanical Review, 81(3), 1–10.

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. Pharmacognosy Reviews, 6(11), 1–5.

Phillipson, J. D. (2001). Phytochemistry and medicinal plants. Phytochemistry, 56(3), 237–243.

Qiu, J. (2007). Traditional medicine: A culture in the balance. Nature, 448, 126–128.

Quang, T. H., Ngan, N. T. T., Minh, C. V., et al. (2008). Phenolic constituents from Glochidion species and their biological activities. Chemical and Pharmaceutical Bulletin, 56, 1469–1472.

Rasoanaivo, P., et al. (2011). Medicinal plants in malaria treatment. Malaria Journal, 10(Suppl 1), S5.

Raunsai, P., et al. (2022). Phytochemical profiling and biological activities of Glochidion zeylanicum. Plants, 11(5), 623.

Ravi, S., et al. (2022). Preliminary phytochemical analysis and in

vitro antioxidant activity of Glochidion ellipticum Wight. Biomedicine, 42(1), 148–153.

Rios, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100(1–2), 80–84.

Sandhu, D. S., & Heinrich, M. (2005). The use of health foods and remedies. Phytotherapy Research, 19(8), 633–637.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011). Extraction, isolation, and characterization of bioactive compounds from plants' extracts. African Journal of Traditional, Complementary and Alternative Medicines, 8(1). https://doi.org/10.4314/ajtcam.v8i1.60483

Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. Cold Spring Harbor Perspectives in Biology, 2(5), a000414.

Stojanoski, N. (1999). Development of health culture in ancient civilizations. Medicinski Pregled, 52(9–10), 367–371.

Teoh, E. S. (2016). Medicinal Orchids of Asia. Springer.

Thang, T. D., Kuo, P. C., Yu, C. S., Yang, M. L., Doong, S. L., Son, N. T., & Wu, T. S. (2011). Chemical constituents of the leaves of Glochidion obliquum and their bioactivity. Archives of Pharmacal Research, 34(3), 383–389. https://doi.org/10.1007/s12272-011-0305-5

Tian, J. M., Fu, X. Y., Zhang, Q., He, H. P., Gao, J. M., & Hao, X. J. (2013). Chemical constituents from Glochidion assamicum. Biochemical Systematics and Ecology, 48, 288–292.

Xiao, W., Li, R., Huang, S., & Zhou, Y. (2018). Norbisabolane sesquiterpenoids from Glochidion coccineum. Fitoterapia, 127, 1–6. https://doi.org/10.1016/j.fitote.2018.02.005

Zargari, A. (1992). Medicinal Plants. Tehran University Press.