Review on Phytochemical and Pharmacological activity of *Acalypha communis* and *acalypha malabarica*

Dhanya P[1]*, Rajasekaran S [1]

[1]Department of Pharmacology, PhD Scholar, Faculty of Pharmacy, Bhagwant University, Ajmer, Rajasthan, India
[2]Department of Pharmacology, Faculty of Pharmacy, Bhagwant University, Ajmer, Rajasthan, India
*Correspondence: dhanyanidheeshp@gmail.com

Abstract

Acalypha communis *and* Acalypha malabarica *(Euphorbiaceae) are allied taxa with uneven species-level evidence. This review consolidates phytochemistry, analytical methods, and pharmacology to guide future work.* A. communis: *Three cycloartane triterpenes—16α-hydroxymollic acid, 15α-hydroxymollic acid, and 7β,16β-dihydroxy-1,23-dideoxyjessic acid—are the only unequivocal isolates from aerial parts. In vitro they inhibit vancomycin-resistant enterococci (MICs 8–32 μg/mL), with additional MRSA activity for 16α-hydroxymollic acid; Gram-negative activity is poor, and a murine* Staphylococcus aureus *model was inactive. Given weak UV chromophores, UHPLC-CAD/ELSD, UHPLC–HRMS/MS, HPTLC with* p-*anisaldehyde–H_2SO_4, and diagnostic* ^{1}*H-NMR cyclopropyl signals constitute a pragmatic QC toolkit. Non-triterpenoid classes remain unverified at species level.* A. malabarica: *The most complete profile used successive Soxhlet extractions, with methanol richest in phenolics, flavonoids, and proanthocyanidins; FTIR corroborates a mixed phenolic/terpenoid/glycosidic matrix. GC–MS (EI) tentatively listed ~20 constituents, including a monoglyceride, an amide, and a cucurbitacin hit, but identities are provisional and prone to siloxane artifacts; LC-HRMS/MS plus NMR confirmation is required. Species-specific ethnomedicine is sparse; one community record documents the plant as an edible leafy green ("pitta kura"). A wound-healing study in rats is noted but needs critical appraisal.* ***Conclusions:*** A. communis *offers defined cycloartane benchmarks with constrained translational activity;* A. malabarica *presents chemically rich but unconfirmed leads. Priority directions include dermatophyte-focused bioassay-guided fractionation, wound-relevant anti-inflammatory and antioxidant assays, FBMN-guided dereplication, and validated quantitative methods aligned with ICH Q2(R2)/Q14. A minimal QC set—UHPLC-HRMS fingerprints with targeted XICs, UHPLC-CAD/ELSD semiquantitation, and HPTLC identities—will support reproducible ethnopharmacology and preclinical development. These steps will strengthen species-specific claims and enable rational development pathways.*

Keywords: *Acalypha communis; Acalypha malabarica*; phytochemistry; cycloartane triterpenes; 16α-hydroxymollic acid; 15α-hydroxymollic acid; 7β,16β-dihydroxy-1,23-dideoxyjessic acid; antimicrobial activity; vancomycin-resistant enterococci; wound healing; UHPLC–HRMS/MS; HPTLC.

1. Introduction

Acalypha L. (Euphorbiaceae: Acalyphoideae) is a pantropical, demonstrably monophyletic genus comprising roughly 450–500 species—the third-largest euphorb lineage after *Euphorbia* and *Croton*—with its center of diversity in the Neotropics, major secondary radiations in Africa (including numerous Madagascan endemics), and smaller but notable lineages in Asia and Oceania, a few species extending into warm-temperate regions [1]. Circumscription is supported by a coherent suite of characters: chiefly herbaceous to shrubby habit; simple, alternate leaves with evident stipules; unisexual, apetalous flowers; staminate flowers (typically 4–8 stamens) whose anthers elongate and twist after dehiscence; pistillate flowers with highly laciniate stigmas and a 3-locular capsule; and, in many taxa of subg. *Acalypha*, conspicuously accrescent pistillate bracts that envelop the developing fruits. Palynological work shows minute (≈9–22 µm), finely sculptured, brevicolporate pollen consistent with wind pollination and documents significant variation in exine and aperture traits that has informed infrageneric concepts [2–3]. Multilocus phylogenies now recognize four subgenera—*Acalypha*, *Linostachys* (s.s.), *Androcephala*, and *Hypandrae*—and reveal homoplasy in several traditional morphological groupings, motivating integrated use of macro- and micromorphology in regional treatments [4]. Established by Linnaeus (1753), the genus has a conserved type, *A. virginica* L., lectotypified by Small (1913); contemporary nomenclators (WFO, IPNI) and the Acalypha Taxonomic Information System compile ≈500 accepted names and synonymize historical segregates, providing a stable basis for ongoing monographic work [5–7]. *Acalypha communis* Müll.Arg. and *A. malabarica* Müll.Arg. are both accepted species, protologued in Linnaea 34 at pp. 23 and 42, respectively [7]. *A. communis*—a polymorphic South American taxon in the sect. Communes complex—has been lectotypified from Brazil (Minas Gerais; Widgren) and treated in modern revisions; principal synonyms include *Ricinocarpus communis* (Müll.Arg.) Kuntze and several Müller infraspecific names now united under a broadly circumscribed species with recognized subspp. (*communis, apicalis, paraguariensis, saltensis, tracheliifolia*) [8–12]. It consists of herbs to subshrubs with pistillate bracts typically glandular-ciliate and subspecific limits keyed by vestiture, leaf proportions, and bract characters; it flowers seasonally across Cerrado–Chaco–Pampa habitats from ~200–1200 m in Brazil, Paraguay, Argentina, Bolivia, and Uruguay, with most units assessed LC/NT in provisional catalogues. In contrast, *A. malabarica* is an Indian annual (peninsular India: Karnataka, Kerala, Maharashtra, with additional Deccan localities) whose type locality is "Malabar"; it is accepted in global nomenclators, with *Ricinocarpus malabaricus* (Müll.Arg.) Kuntze treated as synonym and no routine infraspecific ranks recognized in regional floras [13]. Diagnostic for *A. malabarica* is pistillate bracts with gland-tipped hairs and hispid capsules (vs. *A. lanceolata* with eglandular bracts and glabrous capsules), small ovate-lanceolate leaves, and axillary androgynous spikes; phenology is commonly Aug–Nov, and the

species occupies ruderal/open, often moist–shady lowland sites [14]. For identification, *A. communis* is best treated with the sectional revision and Brazilian synopsis providing keys and updated synonymy, whereas *A. malabarica* is reliably handled in eFlora of India and India Flora Online keys and herbarium portals [14].

1.1. Phytochemical Review of *Acalypha Communis*

Acalypha communis—phytochemical evidence to date—centers on three cycloartane-type triterpenes unequivocally isolated from the aerial parts: 16α-hydroxymollic acid, 15α-hydroxymollic acid, and 7β,16β-dihydroxy-1,23-dideoxyjessic acid. Structures were assigned by routine NMR/MS workflows with confirmatory derivatization, and antibacterial screening showed activity against vancomycin-resistant enterococci (MICs 8, 32, and 8 µg/mL, respectively), with additional inhibition of methicillin-resistant staphylococci by 16α-hydroxymollic acid; activity against Gram-negative bacteria was weak, and a murine Staphylococcus aureus infection model for the 7β,16β-dihydroxy-1,23-dideoxyjessic acid congener was negative—making these three molecules the current benchmark, species-attributed constituents and bioactivity data for A. communis [16]. Genus-level reviews emphasize that comparatively few Acalypha spp. have been chemically characterized in depth and list only these cycloartanes for A. communis, so extrapolating flavonoid/phenolic or essential-oil profiles from other congeners is not yet evidence-based [17]. All three metabolites belong to the 9β,19-cyclo-5α-lanostane (cycloartane) lineage—widely distributed in angiosperms and periodically linked to antibacterial effects—with the A. communis set differing mainly by C-15/C-16 hydroxylation and oxygenation on a jessic-acid–type side chain; very limited internal SAR hints at superior VRE potency for C-16 vs C-15 hydroxylation and restoration of 8 µg/mL VRE activity by the 7β,16β-dihydroxylated side chain, though without in-vivo efficacy under the single condition reported [16,18]. Beyond these triterpenes, species-specific data for A. communis remain sparse; notably, recent pharmacology shows crude A. communis extracts can reverse fluconazole resistance in Candida via efflux-pump inhibition, but the active constituents were not identified—underlining the need for targeted isolation beyond triterpenes [19]. For future work, a cycloartenol-origin triterpene pathway is plausible, and advanced UHPLC–HRMS/MS with in-source-fragment elimination, diagnostic-ion rules and feature-based molecular networking can rapidly flag cycloartane chemotypes in crude extracts, to be followed by 2D-NMR to resolve C-15/C-16 stereochemistry; parallel MRSA/VRE panels plus permeability/PK screens are essential given the in-vivo/ in-vitro disconnect seen so far [16, 20] As summarized in **Table 1**

Table 1: ***Phytochemical evidence for Acalypha communis (Euphorbiaceae).***

Category	Details
Taxon	*Acalypha communis* Müll.Arg. (Euphorbiaceae).
Plant part studied	Aerial parts (non-root tissues) are used for extraction and isolation
Major chemical class detected (species-level)	Cycloartane-type triterpenes (9β,19-cyclo-5α-lanostane skeleton)

Identified compounds	(i) 16α-hydroxymollic acid, (ii) 15α-hydroxymollic acid, (iii) 7β,16β-dihydroxy-1,23-dideoxyjessic acid — all isolated from *A. communis*.
Key structural features	Differences among the three congeners arise from site-specific hydroxylation at C-15 vs C-16, and side-chain oxygenation (jessic-acid type) in compound (iii).
Isolation & analytics (as reported)	Organic extraction → chromatographic purification → structure assignment by 1D/2D-NMR and MS, aided by derivatization.
Antibacterial activity (in vitro)	Against vancomycin-resistant enterococci (VRE): MICs = 8, 32, 8 μg/mL for compounds (i)–(iii), respectively; compound (i) also active vs MRSA (MIC 64 μg/mL). Activity vs Gram-negatives poor.
In vivo outcome	Compound (iii) did not protect mice in a *Staphylococcus aureus* infection model (single test).
SAR hints (very limited set)	Potency trend suggests C-16 OH (α) (compound i) > C-15 OH (α) (compound ii) vs VRE; adding 7β,16β-diol on a jessic-type side chain (compound iii) restores VRE MIC to 8 μg/mL but doesn't fix in-vivo efficacy.
Chemotaxonomic context	Cycloartanes are widespread in plants; *A. communis* provides one of the few species-tied examples within *Acalypha* with explicit structures
What's *not* yet shown for *A. communis*	No peer-reviewed, species-specific reports (to date) of flavonoid/phenolic/alkaloid profiles, essential-oil composition, or other non-triterpenoid isolates—beware of generic *Acalypha* lists being misapplied to *A. communis*.
Method tips for future work	Rapid dereplication: UHPLC-HRMS/MS with diagnostic ions + feature-based molecular networking, then full 2D-NMR; run bioassay-guided fractionation in parallel against VRE/MRSA; add early permeability & cytotox screens (e.g., hemolysis).
Development caveats	Typical triterpene issues (permeability/PK) likely explain the in-vivo failure despite decent VRE MICs; synergy testing with antibiotics and formulation approaches may help.

1.2. Analytical review of *Acalypha communis*

Targeted analytical review (A. communis). At present, the only species-tied analytes for *Acalypha communis* are three cycloartane-type triterpenes from the aerial parts—16α-hydroxymollic acid, 15α-hydroxymollic acid, and 7β,16β-dihydroxy-1,23-dideoxyjessic acid—originally isolated by classic organic extraction, chromatography, and full spectroscopic workup [21]. Given the weak UV chromophoricity of cycloartanes, quantitative and fingerprint work is most robust with mass-sensitive detectors (CAD/ELSD) and/or LC–HRMS profiles; modern dereplication for cycloartane-rich matrices should pair UHPLC–HRMS with diagnostic-ion rules and FBMN (ISFE–DIR–FBMN) to accelerate annotation while minimizing redundancy [24,25,28–32]. Orthogonal visualization and quick QC can be achieved by HPTLC on silica gel with *p*-anisaldehyde–H_2SO_4 derivatization, which reliably stains terpenoids, including triterpenes [33,36]. For structure confirmation, cycloartanes display characteristic cyclopropyl methylene resonances near δ 0.45 and 0.73 ppm in ^1H NMR—useful for early scaffold triage before full 2D-NMR [26]. Until commercial standards emerge, a practical quantitation strategy is to isolate one marker (e.g., 16α-hydroxymollic acid) for a CAD/ELSD response-factor curve and report congeners as "marker equivalents," with cross-validation by exact-mass LC–HRMS and HPTLC densitometry [28–31,33]. Method lifecycle should follow ICH Q2(R2) validation and align development/continual-improvement documentation with ICH Q14 principles [23,34]. Because in-vitro antibacterial activity is moderate and in-vivo efficacy has not been demonstrated, analytics should couple content/fingerprint readouts with exposure-relevant stability/solubility checks and enable synergy designs (e.g., antibiotic combinations) [21,22]. Finally, genus reviews emphasize that relatively few *Acalypha* spp. is chemically well-profiled; thus, non-triterpenoid classes remain data gaps for *A. communis* and are priorities for future analytical work as summarized in Table 2 [27].

Table 2: ***Analytical evidence for Acalypha communis (Euphorbiaceae).***

Category	Analytical review for *A. communis* (numbered citations)
Analytes of interest (species-tied)	Three cycloartane-type triterpenes from aerial parts: 16α-hydroxymollic acid, 15α-hydroxymollic acid, 7β,16β-dihydroxy-1,23-dideoxyjessic acid—the only compounds unambiguously isolated and reported to date specifically for *A. communis*
Sampling & extraction (what worked so far)	The original isolation used organic extraction of aerial parts, followed by chromatographic purification and full spectroscopic characterization (1D/2D-NMR, MS, derivatization)
Chromatography & detection—what to use	Because cycloartanes are weakly UV-absorbing, rely on HPLC with universal detectors (CAD/ELSD) for quantitation/fingerprints, or LC–HRMS for profiling: CAD/ELSD suit non-chromophore triterpenes and are widely used for plant triterpenoids. For quick orthogonal ID

	and QC, HPTLC with p-anisaldehyde–H_2SO_4 derivatization visualizes terpenoids (triterpenes appear blue–violet/red)
LC–MS/MS dereplication (cycloartanes)	Use UHPLC–HRMS with data-dependent MS/MS; for cycloartane-rich plants, a modern workflow combines in-source fragment elimination + diagnostic-ion rules + feature-based molecular networking (FBMN) to annotate large CT sets and reduce redundancy—directly applicable to *A. communis* profiling
Structure elucidation—diagnostic NMR	Cycloartanes show characteristic cyclopropane methylene protons ~δ 0.45 & 0.73 ppm in ^1H NMR, plus multiple tertiary methyl singlets—useful skeleton confirmation before full 2D work; these diagnostics are well-documented in cycloartane studies (e.g., *Actaea* CTs) and generalize to *A. communis* isolates
Quantitation strategy (markers & matrix)	Until commercial standards are available, isolate one marker (e.g., 16α-hydroxymollic acid) to prepare a single-point response-factor curve with CAD/ELSD, and report the others as 16α-hydroxymollic-acid equivalents (mass-sensitive detection). Cross-validate by LC–HRMS (exact mass) and HPTLC–densitometry after anisaldehyde derivatization
Method validation (regulatory baseline)	Follow ICH Q2(R2) (2023) for validation (specificity, linearity, accuracy, precision, range, LOD/LOQ, robustness). If you develop the method de novo, align development docs to ICH Q14 (2023) lifecycle principles
Bioactivity-linked analytics (context)	The three cycloartanes have moderate in-vitro antibacterial activity (VRE MICs 8–32 µg/mL; MRSA 64 µg/mL for one congener) but no in-vivo protection in a mouse *S. aureus* model; analytics should therefore capture potency-relevant exposure (stability, solubility) and enable synergy testing with antibiotics
Recommended QC fingerprint	(i) HPTLC: silica gel; non-polar→mid-polar mobile phase (e.g., toluene/ethyl acetate variants); derivatize with p-anisaldehyde–H_2SO_4, record Vis/UV-366 nm; (ii) UHPLC–CAD chromatographic profile (5–30 min gradient) stored as a lot-release fingerprint; (iii) LC–HRMS TIC + targeted XICs for the three markers
Data gaps	No peer-reviewed species-specific data yet for non-triterpenoid classes (flavonoids/alkaloids/volatiles), nor validated multi-analyte LC–MS quantitation—priority areas for future analytical work

1.3. Pharmacological review of Acalypha communis

A consolidated genus review records a species-level entry for Acalypha communis that notes traditional topical use against skin disorders in Argentina (San Luis province) and reports that methanolic aerial-part extracts show weak activity against yeasts and Aspergillus (MICs typically ≥1 mg mL^{-1}) but markedly better inhibition of dermatophytes (MIC ≈0.25–0.5 mg mL^{-1}), cohering with the skin-use rationale [38,42]. In the underlying survey, extracts were prepared from locally collected aerial parts and tested by CLSI/NCCLS microbroth protocols (M27-A2 for yeasts; M38-A for filamentous fungi) against a panel spanning *Candida*, *Cryptococcus, Aspergillus* spp., and dermatophytes, with the ethnomedically selected set outperforming randomly chosen plants—especially versus dermatophytes—strengthening the ethnomedical link for *A. communis* [42]. Complementing extract-level signals, three cycloartane-type triterpenes purified from *A. communis* (16α-hydroxymollic acid, 15α-hydroxymollic acid, and 7β,16β-dihydroxy-1,23-dideoxyjessic acid) display moderate in-vitro antibacterial potency against VRE (MICs 8–32 μg mL^{-1}) with modest MRSA activity for one congener but no protection in a mouse *S. aureus* model, underscoring development hurdles [37]. Such Gram-positive-biased activity is consistent with membrane-interacting mechanisms described for terpenoids and suggests follow-ups that couple MICs with membrane assays and antibiotic-synergy designs [39]. Regional taxonomic syntheses and checklists further document *A. communis* occurrences and voucher context in South America, providing geographic/collection scaffolding for ethnographic and pharmacognostic work [40–41]. Overall, safety/toxicity data remain species-specific gaps for *A. communis*; any topical development should incorporate early ADME/Tox and standardized QC fingerprints alongside dermatophyte-focused bioassay-guided fractionation to connect traditional use with defined chemistry as reported in **Table 3** [37–39,42]

Table 3*:* Pharmacological / ethnopharmacological evidence for Acalypha communis

Category	Details
Taxon & region	*Acalypha communis* Müll.Arg. (Euphorbiaceae); native across the Cerrado–Chaco–Pampa belt of S. America (Argentina, Bolivia, Brazil, Paraguay); several subspp. recognized in recent synopses.
Documented ethnomedicinal use	Traditional topical use for skin disorders (e.g., San Luis, Argentina); aligns with extract activity pattern against dermatophytes.
Usual plant parts / preparations (reported)	Aerial parts: methanolic extracts screened in vitro versus fungi (yeasts, *Aspergillus*, dermatophytes).
Extract-level pharmacology (antifungal)	Methanolic aerial-part extracts showed weak activity vs yeasts/*Aspergillus* but notable inhibition of dermatophytes (typical MIC range ≈ 0.25–0.5 mg·mL^{-1} in CLSI assays). [

Isolated compounds (species-tied)	Three cycloartane-type triterpenes: 16α-hydroxymollic acid, 15α-hydroxymollic acid, 7β,16β-dihydroxy-1,23-dideoxyjessic acid (from aerial parts; structures by 1D/2D-NMR, MS, derivatization).
In-vitro antibacterial activity (purified)	Against VRE: MICs 8, 32, 8 μg·mL^{-1} (for 16α-OH, 15α-OH, jessic-type diol, respectively). 16α-hydroxymollic acid also active vs MRSA (MIC 64 μg·mL^{-1}). Poor vs Gram-negatives.
In-vivo outcome (purified)	No protection in a mouse *Staphylococcus aureus* infection model for the jessic-type diol (despite decent VRE MIC). [
Mechanistic context (class-based)	Triterpenoid terpenes often perturb bacterial membranes/envelopes and can modulate permeability/efflux → explains Gram-positive bias and Gram-negative weakness; *direct MoA for these three* not yet published.
Safety / translational notes	No species-specific tox data; class issues likely include solubility/permeability, protein binding. Development should pair bioactivity with early ADME/Tox (hemolysis, mammalian cytotox, microsomal stability, PPB) and consider topical routes for dermatophytosis.
Analytical markers for QC (helpful for ethnopharmacology)	Use the three cycloartanes as markers; profile by UHPLC-HRMS/MS (targeted XICs), semi-quantify by UHPLC-CAD/ELSD, and verify by HPTLC (p-anisaldehyde–H_2SO_4).
Evidence gaps	No species-tied data yet for anti-inflammatory, antioxidant, antiviral, antidiabetic, wound-healing.
Priority next steps	(i) Bioassay-guided fractionation against dermatophytes to link folk use to defined actives; (ii) MoA (membrane assays, time-kill) + synergy (vancomycin/linezolid/daptomycin) for VRE/MRSA; (iii) ADME/Tox + simple PK; (iv) Standardize topical extracts with a minimal QC fingerprint; (v) ethically document dose/route in living traditions.

1.4. Extraction evidence for Acalypha communis

Acalypha communis extractions to date are species-tied to the aerial parts, typically dried ≤45 °C, milled (≈20–40 mesh), and subjected to organic extraction; these workflows yielded the only confirmed constituents—three cycloartane-type triterpenes (16α-hydroxymollic acid, 15α-hydroxymollic acid, and 7β,16β-dihydroxy-1,23-dideoxyjessic acid) after chromatography and full spectroscopic elucidation [43]. Crude methanolic aerial-part extracts have been screened by CLSI microbroth methods: activity is weak against yeasts/Aspergillus but notable against dermatophytes (typical MIC ≈ 0.25–0.5 mg mL^{-1}), consistent with genus-level ethnomedical context [44–45]. For fractionation, a Kupchan-style liquid–liquid scheme remains the workhorse (waterified MeOH → n-hexane → CH_2Cl_2/$CHCl_3$ → EtOAc → n-

BuOH), with numerous "modified Kupchan" precedents in natural products workflows [46–47]. On plates, triterpenes visualize reliably with p-anisaldehyde–H_2SO_4 (blue-violet/reddish bands); scale-up typically proceeds via silica flash to semi-prep HPLC [52, 57]. Because cycloartanes are weakly UV-absorbing, non-chromophoric quantitation and QC fingerprints should favor UHPLC-CAD/ELSD (mass-sensitive) complemented by UHPLC–HRMS/MS profiling; pairing diagnostic-ion rules with feature-based molecular networking (FBMN) accelerates dereplication of cycloartane chemotypes [55–56, 58–59]. Two pragmatic tracks are: (A) isolate-oriented (optional brief defat; EtOAc/MeOH extraction; Kupchan partition; flash + semi-prep HPLC; HRMS + 1D/2D-NMR) and (B) bioactive-crude (70–90% MeOH maceration/sonication; quick hexane de-wax; standardize DER/solids% and HPTLC/UHPLC fingerprints; CLSI MICs) [43, 45–47, 52, 57]. "Green"/accelerated options—UAE**,** MAE**,** PLE/ASE**,** and $SCCO_2$ (with 5–10% EtOH modifier, pressure-stepped)—offer time/yield gains and cleaner matrices; report solvent grade, L/S, time, temperature, particle size, agitation, cycles, partition volumes, and % recovery to ensure reproducibility [48–51, 62–64]. Minimum validation/QC should align with ICH Q14 (development) and Q2(R2) (validation), using UHPLC–HRMS TIC + targeted XICs for the three markers, UHPLC-CAD/ELSD area% (reporting others as 16α-hydroxymollic-acid equivalents until standards are available), and HPTLC identity after anisaldehyde derivatization as summarised on **Table 4** [43, 52, 55–56, 60–61].

Table 4: **Extraction Evidence for *Acalypha Communis***

Category	**Extraction review for A. communis (numbered citations)**
Plant material & parts	Aerial parts used in all species-tied studies to date. Dry ≤45 °C; mill to 20–40 mesh; record moisture/drug:solvent ratio. [1–3]
What has been extracted (species-level)	Three cycloartane triterpenes—16α-hydroxymollic acid, 15α-hydroxymollic acid, 7β,16β-dihydroxy-1,23-dideoxyjessic acid—isolated after organic extraction + chromatography; structures by 1D/2D-NMR and MS.
Extracts tested as crude	MeOH aerial-part extracts screened in vitro (CLSI) for antifungals: weak vs yeasts/Aspergillus, notable vs dermatophytes (typical MIC ~0.25–0.5 mg mL^{-1}).
Baseline extraction (from literature)	Organic maceration/Soxhlet of aerial parts (non-polar→mid-polar); chromatographic purification to isolates; direct MeOH macerates for antifungal screening.
Polarity logic (why these solvents)	Cycloartanes are non-/mid-polar and weakly UV-absorbing → enrich with hexane/DCM/EtOAc/MeOH steps; crude antifungal use favors MeOH 70–100% to keep mid-polar matrices (possible synergy).

Recommended partitioning (workhorse)	Kupchan partition of MeOH extract: waterified MeOH → n-hexane → DCM/$CHCl_3$ → EtOAc → n-BuOH, tracking terpenoids (hexane/DCM rich).
Chromatography & visualization	Silica gel/flash → semi-prep HPLC. TLC/HPTLC with p-anisaldehyde–H_2SO_4 for triterpenes (blue-violet/red bands).
Analytics tied to extraction	For profiling/dereplication: UHPLC–HRMS/MS (data-dependent) + FBMN to annotate cycloartanes; for quantitation/fingerprints of non-chromophoric triterpenes: UHPLC-CAD/ELSD; orthogonal HPTLC ID.
Two practical workflows	(A) Isolates: (i) Defat (brief hexane, optional); (ii) Extract: EtOAc or MeOH; (iii) Kupchan partition; (iv) Flash silica; (v) Semi-prep HPLC; (vi) Confirm by HRMS + 1D/2D-NMR. [1,4,18] • (B) Bioactive crude (dermatophytes): (i) MeOH 70–90% maceration/sonication; (ii) Optional quick hexane wash to de-wax; (iii) Standardize DER, solids %, and HPTLC/UHPLC fingerprints; (iv) CLSI MICs.
"Green"/accelerated options	UAE (20–40 kHz; EtOAc/MeOH/i-PrOH) improves yield/time; MAE for polar crudes (monitor T to avoid artifacts); PLE/ASE (60–120 °C; 10–15 MPa; EtOAc/EtOH) for reproducible exhaustives; $scCO_2$ (+5–10% EtOH) for non-polar triterpenes, step-fractionate by pressure.
Process variables to report	Solvent (grade), L/S ratio, time, temperature, particle size, agitation, number of cycles, partition volumes, % solvent recovery. (Good reporting practice)
QC fingerprint (minimum set)	(i) UHPLC–HRMS TIC + targeted XICs for the 3 markers; (ii) UHPLC-CAD/ELSD area% (report others as 16α-hydroxymollic-acid equivalents until standards available); (iii) HPTLC identity after anisaldehyde derivatization.
Validation framework	Develop under ICH Q14; validate (specificity, linearity, accuracy, precision, range, LOD/LOQ, robustness) per ICH Q2(R2).
Common pitfalls & fixes	Over-defatting can strip triterpenes (keep short); heavy chlorophyll/wax complicates columns (apply hexane chill/quick wash); prolonged heat → degradation (prefer UAE/PLE over long Soxhlet).
Safety & scale-up notes	Prefer EtOH/i-PrOH over chlorinated solvents for scale; if targeting topicals, include residual-solvent checks and irritation screens; note class issues (solubility/permeability) that may affect downstream pharmacology.

1.5. Phytochemistry of *Acalypha malabarica*

The first species-tied profile applied successive Soxhlet extraction (petroleum ether → chloroform → ethyl acetate → acetone → methanol) and found methanol to be the richest fraction, reporting totals of phenols (≈240 mg/g, GAE), flavonoids (≈620 mg/g, QE), and proanthocyanidins (≈530 mg/g, catechin eq.), with qualitative tests positive for alkaloids, phenolics, flavonoids, tannins, glycosides, steroids, and terpenoids; FTIR bands (e.g., O–H ~3291 cm^{-1}, aliphatic C–H ~2916–2970 cm^{-1}, C=O ~1737–1740 and 1601–1650 cm^{-1}) supported a mixed phenolic/terpenoid/glycosidic matrix [65]. GC–MS (methanolic extract) tentatively annotated ~20 constituents (NIST matching), frequently highlighting 2-myristoylglycinamide, cucurbitacin B, and 1-monolinoleoylglycerol among fatty-acid derivatives and other features, but without isolation or NMR confirmation—hence identities remain provisional [65]. Two analytical cautions apply: (i) EI–GC–MS library matches alone are not definitive and should be verified by LC–HRMS(/MS) plus 1D/2D-NMR; and (ii) cyclic/linear siloxanes (diagnostic ions m/z 73, 147, 207, 281, 355) commonly arise from septa/column bleed or vial elastomers and can appear as "ghost peaks," so blanks and hardware controls are essential [66–67]. In practice, reports of high-mass triterpenoids (e.g., cucurbitacins) from GC–MS should be treated as hypotheses until orthogonally confirmed, consistent with broader guidance on the limits of library-only identifications [68]. Overall, *A. malabarica* shows chemically rich, polar leaf extracts (MeOH ≫ other solvents) with FTIR-level functional corroboration and a tentative GC–MS roster; rigorous phytochemical proof now requires bioassay-guided isolation and orthogonal structural confirmation of any proposed markers as shown in **Table 5** [65–68].

Table 5: **Phytochemical evidence for *Acalypha malabarica*)**.

Category	Details (numbered citations)
Scope & source	First species-tied, lab-scale profile (leaves) using successive Soxhlet extraction plus qualitative tests, TPC/TFC/TPA quantitation, FTIR, and GC–MS.
Plant part & locality	Leaves, collected in Telangana, India; shade-dried, milled (0.3 mm), stored at 4 °C until analysis.
Extraction solvents & method	Soxhlet (6 h each) in rising polarity: petroleum ether (60 °C) → chloroform (61 °C) → ethyl acetate (77 °C) → acetone (56 °C) → methanol (65 °C).
Qualitative phytochemicals (screen)	Alkaloids, phenols, flavonoids, tannins, glycosides (incl. cardio-glycosides), steroids & terpenoids, saponins, proteins/amino acids, carbohydrates, phytosterols detected across solvent fractions (details per test).
Quantitative totals (best solvent)	Methanol extract highest: TPC 240 mg/g (GAE); TFC 620 mg/g (QE); TPA 530 mg/g (catechin eq.). Other solvents lower (trend MeOH > acetone/EtOAc > chloroform > pet. ether).

FTIR: key bands & assignments	3291 cm^{-1} (O–H stretch, H-bonded); 2916–2970 cm^{-1} (aliphatic C–H); 1737 cm^{-1} (C=O); 1650–1601 cm^{-1} (C=O/C=C in aromatics); 1371 cm^{-1} (O–H bend); 1256–1340 cm^{-1} (C–N); 1024–1100 cm^{-1} (C–O–C/phosphate); ~563/506 cm^{-1} (halo); 486 cm^{-1} (S–S).
GC–MS overview (MeOH extract)	20 compounds annotated (NIST matching). Headliners and peak areas (%): 2-myristoylglycinamide (RT 30.040; 0.78%; antimicrobial tag), cucurbitacin B (30.520; 0.85%; antitumor tag), 1-monolinoleoylglycerol (31.340; 1.31%; antimicrobial/antioxidant tag), plus fatty-acid esters; siloxanes also reported.
Analytical cautions	Library-only GC–MS IDs are tentative—confirm with LC-HRMS/MS and 1D/2D-NMR; NIST matches can misassign without orthogonal data [4]. Siloxane peaks (e.g., ions m/z 73, 147, 207, 281, 355) often arise from septa/column bleed—treat cyclic siloxanes as likely artifacts unless validated
What's established	Rich polar chemistry in MeOH (phenols/flavonoids/proanthocyanidins) with FTIR corroboration; tentative GC–MS roster provides leads but no isolated, NMR-confirmed molecules from this species yet.
Gaps	No dose–response bioassays tied to the MeOH extract in this study; no structure-confirmed isolates; siloxanes included in paper's list (likely non-biogenic).
Next steps (rigorous profile)	(i) Bioassay-guided fractionation (antioxidant/antimicrobial panels) → isolate actives; (ii) LC-HRMS/MS + NMR confirmation for cucurbitacin/monoglyceride/amide claims; (iii) QC: UHPLC-HRMS targeted XICs + HPTLC visualizations for phenolics/terpenoids; (iv) Blank/bleed controls to suppress siloxane artifacts.
Genus's context (for expectations, not substitution)	*Acalypha* spp. widely report phenolics/terpenoids and topical uses; however, do not impute congener chemistry to *A. malabarica* without species-level confirmation.

1.6. Analytical review of *Acalypha malabarica*

The most complete, species-tied leaf workup applies successive Soxhlet extraction (petroleum ether → chloroform → ethyl acetate → acetone → methanol), with the methanolic fraction proving richest in polar metabolites (high TPC/TFC/TPA) and yielding a diagnostic FTIR profile (O–H, aliphatic C–H, carbonyl and heteroatom bands), together supporting a phenolic/flavonoid/proanthocyanidin-leaning pool [69]. GC–MS (EI) tentatively listed ~20 constituents (e.g., 2-myristoylglycinamide, cucurbitacin B, 1-monolinoleoylglycerol), but without isolation or NMR confirmation—hence these IDs should be treated as hypotheses pending orthogonal LC–HRMS(/MS) and full 1D/2D-NMR verification; NIST guidance and

methods (AMDIS/deconvolution, library search) underline that library matches alone are insufficient for definitive identification [72–73]. Practical chromatographic hygiene also matters: siloxane "ghost peaks" from septa/column bleed are common and produce characteristic ion series (e.g., *m/z* 73, 147, 207, 281, 355), so blanks, fresh liners/septa, and monitoring diagnostic ions are essential [70–71,76]. For modern profiling/dereplication beyond the anchor paper, pair UHPLC–HRMS/MS (DDA/DIA) with Feature-Based Molecular Networking (GNPS/GNPS2) to cluster related ions, separate isomers, and prioritize candidates before isolation [73,75]. When moving from discovery to QC (fingerprints/marker assays), develop and validate methods under ICH **Q2(R2)** and align documentation with current regulatory adoption (FDA) as reported in **Table 6** [76–77].

Table 6: Analytical review of *Acalypha malabarica*

Aspect	**Details**
Sample & extraction	Successive Soxhlet: petroleum ether → chloroform → ethyl acetate → acetone → methanol.
Metabolite-rich fraction	Methanolic extract shows highest polar load (↑TPC/↑TFC/↑TPA).
FTIR (diagnostic bands)	Broad O–H; aliphatic C–H; carbonyl (C=O); heteroatom-associated bands → consistent with phenolics, flavonoids, proanthocyanidins.
GC–MS (EI) survey	~20 tentative constituents reported (e.g., 2-myristoylglycinamide, cucurbitacin B, 1-monolinoleoylglycerol), without isolation/NMR. Treat as putative only.
ID caution (libraries)	NIST/AMDIS deconvolution + library hits alone are insufficient → require orthogonal confirmation (LC–HRMS(/MS), 1D/2D-NMR).
Artefacts / "ghost" peaks	Siloxane bleed common; characteristic ions m/z 73, 147, 207, 281, 355. Use blanks, fresh liners/septa; monitor diagnostic ions.
Modern profiling / dereplication	Pair UHPLC–HRMS/MS (DDA/DIA) with Feature-Based Molecular Networking (GNPS/GNPS2) to cluster analogs, separate isomers, prioritize targets before isolation.
From discovery → QC	Build fingerprints/marker assays from confirmed IDs; develop methods per ICH Q2(R2) (specificity, linearity, range, accuracy, precision, LOD/LOQ, robustness).
Regulatory alignment	Ensure documentation aligns with current FDA adoption/expectations for analytical validation packages.

1.7. Ethnopharmacology review of *Acalypha malabarica*

Floristic portals fix the taxon and range (peninsular India, Western Ghats–Deccan belt) and are presently more informative on identity and habitat than on remedies [78,79]. Verified, species-specific ethnomedicinal records remain sparse; however, a community food-systems study from Zaheerabad (Telangana) lists *A. malabarica* among edible leafy greens ("pitta kura"), documenting dietary—not therapeutic—use and thereby providing a culturally grounded starting point for later biomedical hypotheses [80]. Contemporary laboratory chemistry on leaves (successive Soxhlet; MeOH richest; FTIR and GC–MS screening) supports a polar-leaning metabolite pool consistent with antioxidant/anti-inflammatory plausibility, but without species-tied isolates or dose–response pharmacology [81]. A wound-healing study in Wistar rats has been listed in the October 2024 issue of *Biochemical and Cellular Archives*; pending an accessible abstract/PDF, effect sizes and histology endpoints cannot be independently summarized and should be interpreted cautiously [82]. Genus-level syntheses for *Acalypha* (and a new South-Asian review) provide broad mechanistic context but do not substitute for primary, species-level data on *A. malabarica* [83,84]. Congeneric lines of plausibility include rodent wound-healing with *A. indica* and topical antifungal activity of *A. wilkesiana*, which map mechanistically onto skin-use hypotheses (ROS scavenging, collagen deposition, dermatophyte control) but should be treated strictly as hypotheses for *A. malabarica* until confirmed [85,86]. In sum: what is currently supported for *A. malabarica* is (i) clear taxonomy and distribution, (ii) documented food use in at least one South-Indian community, and (iii) a phenolic-rich MeOH leaf chemistry profile; what is not yet supported are specific therapeutic claims, validated pharmacology, safety, and isolated actives as summarized in **Table 7**

Table 7: Ethnopharmacology review of *Acalypha malabarica*

Category	Details
Taxon & range	*Acalypha malabarica* Müll.Arg. (Euphorbiaceae); small annual herb native to peninsular India (Western Ghats–Deccan). Identity/range documented in Indian floras/portals.
Vernacular & parts used	Leaves most commonly referenced in analytical work and local food-system notes; aerial parts used for extractions.
Documented ethnomedicinal/food use	Species-specific medicinal records are scarce; one community study lists the plant as an edible leafy green ("pitta kura")—dietary ethnobotany rather than therapy. Treat unsourced "traditional uses" with caution until village-level documentation is available.
Extraction & chemistry (species-tied)	Successive Soxhlet (petroleum ether → chloroform → ethyl acetate → acetone → methanol); MeOH extract richest in phenolics, flavonoids, proanthocyanidins; FTIR indicates mixed phenolic/terpenoid/glycosidic matrix; GC–MS (EI) lists ~20 tentative constituents from MeOH (e.g., 2-myristoylglycinamide, cucurbitacin B, 1-monolinoleoylglycerol).

Analytical cautions	GC–MS library-only IDs are tentative without standards or orthogonal proof; siloxane peaks (m/z 73, 147, 207, 281, 355) often arise from septa/column bleed—run blanks and confirm by LC-HRMS/MS + NMR before drawing pharmacological conclusions.
Extract-level pharmacology (species-tied)	A Wistar rat wound-healing study of leaf preparations is reported (journal ToC/title available), suggesting interest in topical repair; dosing, effect sizes, and histology endpoints require direct paper review before firm conclusions.
Biological plausibility (context, not proof)	Phenolic-rich MeOH extract supports antioxidant/anti-inflammatory hypotheses; congeners show topical activities (e.g., *A. indica*—wound healing; *A. wilkesiana*—antifungal for superficial mycoses), but do not impute these to *A. malabarica* without species-specific data.
Safety/toxicity (knowns/unknowns)	No *species-specific* tox or irritation data found; any topical development should include skin irritation/sensitization, cytotoxicity, and basic genotox screens.
Evidence grade (current)	Chemistry: moderate (MeOH extract, FTIR, preliminary GC–MS) [• Ethnography: low (dietary record only) • Pharmacology: provisional (wound-healing paper noted, details pending)
Next steps (actionable)	1) Obtain and critically appraise the wound-healing study; 2) Run bioassay-guided fractionation (antioxidant/anti-inflammatory, dermatophyte panels) on MeOH extract; 3) Confirm any GC–MS leads via LC-HRMS/MS + 1D/2D-NMR; 4) Implement QC fingerprinting (UHPLC-HRMS targeted XICs + HPTLC anisaldehyde for phenolics/terpenoids); 5) Build a minimal safety package for topical use.

1.8. Extraction review of *Acalypha malabarica*

Reported species-tied work uses shade-dried, milled (~0.3 mm) leaves that were cold-stored before analysis, then extracted by successive Soxhlet (≈6 h each; petroleum ether → chloroform → ethyl acetate → acetone → methanol), with methanol yielding the richest fraction; that methanolic extract displayed high TPC/TFC/TPA values, FTIR bands consistent with a mixed phenolic/terpenoid/glycosidic matrix, and ~20 tentative GC–MS (EI) constituents [87]. For scale-up, a Kupchan solvent–solvent partition of crude MeOH (waterified 9:1) into n-hexane → CH_2Cl_2/$CHCl_3$ → EtOAc → n-BuOH is a practical workhorse that is well-documented in natural-products workflows [92]. "Green/accelerated" options can shorten timelines or reduce solvent: ultrasound-assisted extraction (UAE) in MeOH/EtOH/EtOAc/i-PrOH (20–40 kHz) [88], microwave-assisted extraction (MAE) for polar matrices [89], pressurized/accelerated solvent extraction (PLE/ASE; 60–120 °C; 10–15 MPa) for reproducible exhaustives [90], and supercritical CO_2 (±5–10% EtOH modifier) for non-polars with facile solvent removal [91]. Minimum analytics to pair with extraction should include

LC–HRMS(/MS) profiling with targeted XICs for confirmed markers and orthogonal NMR on isolates; when using GC–MS, run replicate blanks and monitor characteristic siloxane ions (e.g., *m/z* 73/147/207/281/355) to flag bleed/ghost peaks, and treat library-only identifications as hypotheses until confirmed—consistent with best-practice guidance from instrument vendors and NIST as summarized in **Table 8** [93–95].

Table 8: Extraction review of *Acalypha malabarica*

Category	**Extraction review of *A. malabarica***
Plant part & preprocessing (reported)	Leaves, shade-dried, milled (~0.3 mm), cold-stored prior to analysis.
Extraction scheme actually used (species-tied)	Successive Soxhlet (6 h each) in rising polarity: petroleum ether → chloroform → ethyl acetate → acetone → methanol; MeOH gave the richest extract.
What the extracts contained (analytic readouts)	MeOH leaf extract showed high phenolics/flavonoids/proanthocyanidins (quantified) and FTIR bands consistent with a mixed phenolic/terpenoid/glycosidic matrix; GC–MS (EI) listed ~20 tentative constituents from MeOH.
GC–MS identification caveats (good practice)	Library-only calls are preliminary; confirm candidates by LC-HRMS(/MS) and 1D/2D-NMR to avoid false positives. [9] Also watch for siloxane artifacts (typical ions m/z 73, 147, 207, 281, 355); run blanks and maintain columns/liners.
Workhorse fractionation (when scaling beyond Soxhlet)	Apply Kupchan solvent–solvent partition to a crude MeOH extract: waterify MeOH (9:1) → partition n-hexane → CH_2Cl_2/$CHCl_3$ → EtOAc → n-BuOH; track fractions analytically.
"Green/accelerated" extraction upgrades	UAE (20–40 kHz; MeOH/EtOH/EtOAc/i-PrOH) to shorten time & reduce solvent [2]; MAE for fast recovery from polar matrices ; ASE/PLE (60–120 °C; 10–15 MPa) for reproducible exhaustives; SFE ($scCO_2$ ± 5–10% EtOH) for non-polar lipids/terpenoids with easy solvent removal
Two practical routes (fit to current data)	A) Phenolic-rich crude (aligns with [1]): 70–90% MeOH maceration/sonication or UAE → minimal clean-up (brief hexane de-wax) → quantify TPC/TFC/TPA → LC-HRMS profile. B) Non-polar enrichment (prospect for lipids/terpenoids): SFE (40–60 °C; 30–40 MPa; 5–10% EtOH modifier) or EtOAc/hexane extraction → Kupchan → monitor by LC-HRMS.
Analytics to pair with extraction (minimum)	LC-HRMS/MS for profiling + targeted XICs of confirmed markers; orthogonal NMR on isolates; for quick checks, HPTLC (visualization reagent by lab standard) and replicate

	blank GC–MS runs to flag siloxanes before trusting EI library hits.
Reporting essentials	Always log solvent grade, L/S ratio, time, temperature, particle size, agitation, number of cycles, and any partition volumes so batches are reproducible and comparable across labs. (Good practice aligned with reviews
Common pitfalls & fixes	Over-interpreting GC–MS: defer claims until LC-HRMS/NMR ; bleed/ghost peaks: replace septa/liners, re-condition columns, monitor diagnostic siloxane ions ; over-defatting: keep hexane steps short to avoid losing neutral terpenoids.

2. Summary

Acalypha communis and *A. malabarica* are accepted Euphorbiaceae species with clear but uneven evidence bases. For *A. communis,* the only unambiguous species-level isolates are three cycloartane triterpenes—16α-hydroxymollic acid, 15α-hydroxymollic acid, and 7β,16β-dihydroxy-1,23-dideoxyjessic acid—from aerial parts. These show moderate, Gram-positive-biased antibacterial activity in vitro (VRE MICs 8–32 µg/mL; MRSA activity for one congener) but no efficacy in a mouse *S. aureus* model. Extract-level antifungal data favor dermatophytes over yeasts/*Aspergillus,* aligning with limited skin-use notes. Analytically, weak UV chromophores necessitate UHPLC-CAD/ELSD for semi-quantitation, UHPLC–HRMS/MS for profiling, HPTLC (*p*-anisaldehyde–H_2SO_4) for identity, and diagnostic cyclopropyl ^1H-NMR signals. For *A. malabarica,* the most complete leaf workup used successive Soxhlet; methanol extracts were richest (high phenols/flavonoids/proanthocyanidins) with FTIR corroboration. GC–MS (EI) tentatively listed ~20 constituents, but identities lack orthogonal confirmation and may include siloxane artifacts. Species-specific ethnomedicine is sparse (documented food use as “pitta kura”); a wound-healing rat study is noted but requires critical appraisal.

3. Conclusion

A. communis currently offers well-defined cycloartane benchmarks yet limited translational promise without solving exposure/permeability. Non-triterpenoid chemistry remains a major gap. *A. malabarica* presents chemically rich, polar extracts and plausible antioxidant/anti-inflammatory potential, but its pharmacology is preliminary and compound identities remain provisional. Across both species, rigor in dereplication, structural proof, and validated analytics is the rate-limiting step for credible ethnopharmacological development.

Future scope

Bioassay-guided isolation: Link *A. communis* dermatophyte activity and *A. malabarica* wound/skin hypotheses to purified actives; run MRSA/VRE panels, dermatophyte MICs, time-kill, and synergy (vancomycin/linezolid/daptomycin). Advanced dereplication: Pair UHPLC–HRMS/MS (DDA/DIA) with feature-based molecular networking; create targeted XICs for confirmed markers; avoid over-interpreting EI library matches. Orthogonal structure confirmation: 1D/2D-NMR for all leads; confirm or refute cucurbitacin/monoglyceride/amide calls in *A. malabarica*; expand beyond triterpenes in *A. communis*. Pharmaceutics & ADME/Tox: Early permeability, solubility, stability, protein-binding, cytotoxicity, and irritation testing; consider topical delivery/formulation strategies. Standardization & QC: Implement ICH Q2(R2)/Q14-aligned methods—UHPLC-HRMS fingerprints (targeted XICs), UHPLC-CAD/ELSD semi-quant, HPTLC identity; report full process variables. Field and taxonomy: Voucher specimens, georeferenced collections, and ethically documented local uses to anchor biomedical claims, especially for *A. malabarica*

4. Reference

1. Levin, G. A., Cardinal-McTeague, W. M., Steinmann, V. W., & Sagun, V. G. (2022). Phylogeny, classification, and character evolution of *Acalypha* (Euphorbiaceae: Acalyphoideae). *Systematic Botany, 47*(2), 477–497. https://doi.org/10.1600/036364422X16512572275034
2. Sagun, V. G., Levin, G. A., & van der Ham, R. W. J. M. (2010). Pollen morphology and ultrastructure of *Acalypha* (Euphorbiaceae). *Review of Palaeobotany and Palynology, 160*(3–4), 121–129.
3. Levin, G. A., & Simpson, M. G. (1994). Phylogenetic implications of pollen ultrastructure in *Acalypha* (Euphorbiaceae). *American Journal of Botany, 81*(2), 188–195. h
4. Cardiel, J. M., & Muñoz-Rodríguez, P. (2012). Synopsis of *Acalypha* L. (Euphorbiaceae) of continental Ecuador. *Phytotaxa, 69*, 1–37.
5. World Flora Online. (2025). *Acalypha* L. (taxon page). Retrieved September 4, 2025.
6. International Plant Names Index (IPNI). (n.d.). *Acalypha virginica* L. (type information). Retrieved September 4, 2025.
7. Acalypha Taxonomic Information System (ATIS). (2025). About *Acalypha*—history and typification (lectotype *A. virginica* designated by Small, 1913). Retrieved September 4, 2025.
8. Cardiel, J. M., & Muñoz-Rodríguez, P. (2012). Synopsis of *Acalypha* L. (Euphorbiaceae) of continental Ecuador. *Phytotaxa, 69*, 1–37.
9. Cardiel, J. M., Muñoz-Rodríguez, P., & Muñoz Garmendia, F. (2013). Revised taxonomy and nomenclature of *Acalypha* sect. *Communes* (Euphorbiaceae). *Taxon, 62*(6), 1295–1303.
10. Cardiel, J. M. (2022). Updated synopsis of *Acalypha* (Euphorbiaceae, Acalyphoideae) from Brazil. *Plant Systematics and Evolution, 308*, 1–38.

11. Müller Argoviensis, J. (1865). Euphorbiaceae (protologues of *Acalypha communis* p. 23 and *Acalypha malabarica* p. 42). *Linnaea, 34*, .
12. World Flora Online. (n.d.). *Acalypha malabarica* Müll.Arg. (taxon page). Retrieved September 4, 2025, f
13. . — GBIF Backbone. Retrieved September 4, 2025, from https://www.gbif.org/
14. eFlora of India. (n.d.). *Acalha malabarica* Müll.Arg. (species account). Retrieved September 4, 2025, from https://efloraofindia.com/
15. India Flora Online (IISc–JCB). (n.d.). *Acalypha malabarica* (species page with distribution/phenology). Retrieved September 4, 2025, from https://indiaflora.in/
16. Gutiérrez-Lugo, M.-T., Singh, M. P., Maiese, W. M., & Timmermann, B. N. (2002). New antimicrobial cycloartane triterpenes from *Acalypha communis*. *Journal of Natural Products, 65*(6), 872–875. https://doi.org/10.1021/np020044g. PubMed
17. Seebaluck, R., Gurib-Fakim, A., & Mahomoodally, F. (2015). Medicinal plants from the genus *Acalypha* (Euphorbiaceae)—A review of their ethnopharmacology and phytochemistry. *Journal of Ethnopharmacology, 159*, 137–157. https://doi.org/10.1016/j.jep.2014.10.040. PubMed
18. Cappiello, F., Loffredo, M. R., Del Plato, C., Cammarone, S., Casciaro, B., Quaglio, D., Mangoni, M. L., Botta, B., & Ghirga, F. (2020). The revaluation of plant-derived terpenes to fight antibiotic-resistant infections. *Antibiotics, 9*(6), 325. https://doi.org/10.3390/antibiotics9060325. PMC
19. Gil, F., Laiolo, J., Bayona-Pacheco, B., Cannon, R. D., Ferreira-Pereira, A., & Carpinella, M. C. (2022). Extracts from Argentinian native plants reverse fluconazole resistance in *Candida* species by inhibiting the efflux transporters Mdr1 and Cdr1. *BMC Complementary Medicine and Therapies, 22*, 264. https://doi.org/10.1186/s12906-022-03745-4. BioMed Central
20. Yu, T., Chen, J.-M., Liu, W., Zhao, J.-Q., Li, P., Liu, F.-J., Jiang, Y., & Li, H.-J. (2024). In-depth characterization of cycloartane triterpenoids and discovery of species-specific markers from three *Cimicifuga* species guided by ISFE–DIR–FBMN. *Journal of Chromatography A, 1728*, 465015. https://doi.org/10.1016/j.chroma.2024.465015
21. Gutiérrez-Lugo, M.-T., Singh, M. P., Maiese, W. M., & Timmermann, B. N. (2002). New antimicrobial cycloartane triterpenes from *Acalypha communis*. *Journal of Natural Products, 65*(6), 872–875. PubMed
22. Cappiello, F., Loffredo, M. R., Del Plato, C., et al. (2020). The revaluation of plant-derived terpenes to fight antibiotic-resistant infections. *Antibiotics, 9*(6), 325. PubMedPMC
23. International Council for Harmonisation. (2023). *ICH Q2(R2): Validation of analytical procedures* (Step 5 guideline). European Medicines Agency (EMA)
24. Yu, T., Chen, J.-M., Liu, W., Zhao, J.-Q., Li, P., Liu, F.-J., Jiang, Y., & Li, H.-J. (2024). In-depth characterization of cycloartane triterpenoids and discovery of species-specific markers from three *Cimicifuga* species guided by ISFE–DIR–FBMN. *Journal of Chromatography A, 1728*, 465015. PubMed
25. Yu, T., Chen, J.-M., Liu, W., et al. (2024). In-depth characterization of cycloartane triterpenoids… (article record/abstract). *Journal of Chromatography A, 1728*, 465015. ScienceDirect

26. Imai, A., Lankin, D. C., Nikolić, D., Ahn, S., van Breemen, R. B., Farnsworth, N. R., McAlpine, J. B., Chen, S.-N., & Pauli, G. F. (2016). Cycloartane triterpenes from the aerial parts of *Actaea racemosa*. *Journal of Natural Products, 79*(3), 541–554. PubMed
27. Seebaluck, R., Gurib-Fakim, A., & Mahomoodally, M. F. (2015). Medicinal plants from the genus *Acalypha* (Euphorbiaceae): Ethnopharmacology and phytochemistry. *Journal of Ethnopharmacology, 159*, 137–157. ScienceDirect
28. Baker, T. R., Mahony, C., Sica, V. P., & Little, J. G. (2018). A multi-detector chromatographic approach (UHPLC-UV/CAD/HRMS) for characterization and semiquantitation of botanical constituents to enable *in silico* safety assessment. *Analytical and Bioanalytical Chemistry, 410*, 4401–4415. PMC
29. Sun, J., Wu, F., Zhang, L., Wang, Z., & Yang, C. (2021). Determination of triterpenoids and phenolic acids from *Sanguisorba officinalis* by HPLC-ELSD and its application. *Molecules, 26*, 4769. PMC
30. Eom, H. Y., Park, S.-Y., Kim, M. K., Suh, J. H., Yeom, H., Min, J. W., Kim, U., Lee, J., Youm, J.-R., & Han, S. B. (2010). Comparison between evaporative light-scattering detection and charged-aerosol detection for the analysis of saikosaponins. *Journal of Chromatography A, 1217*(26), 4347–4354. PubMed
31. Acworth, I. N. (2017). *Charged aerosol detection*. Wiley. Wiley Online Library
32. Vehovec, T., & Obreza, A. (2010). Review of operating principle and applications of the charged aerosol detector. *Journal of Chromatography A, 1217*(10), 1549–1556. PubMed
33. Agatonovic-Kustrin, S., Gegechkori, V., & Morton, D. W. (2019). HPTLC–bioautography for the evaluation of antioxidant and antimicrobial potency of plant extracts. *Journal of Planar Chromatography*, 32, 267–279. (Includes *p*-anisaldehyde–H_2SO_4 derivatization guidance for terpenoids.) PMC
34. International Council for Harmonisation. (2024). *ICH Q14: Analytical procedure development* (Step 5; implementation and training materials). U.S. Food and Drug AdministrationICH Database
35. *Journal of Natural Products*—Table of contents, Volume 65, Issue 6 (2002) (issue containing Gutiérrez-Lugo et al.). ACS Publications
36. Agatonovic-Kustrin, S., Ristivojević, P., Gegechkori, V., Dragojević-Marta, A., & Morton, D. W. (2021). HPTLC and ATR/FTIR fingerprinting of antioxidant plant extracts: Method development and validation. *Molecules, 26*(19), 6064. (Demonstrates anisaldehyde derivatization and densitometry transferable to triterpene/sterol QC.)
37. Gutiérrez-Lugo, M.-T., Singh, M. P., Maiese, W. M., & Timmermann, B. N. (2002). New antimicrobial cycloartane triterpenes from *Acalypha communis*. *Journal of Natural Products, 65*(6), 872–875. https://doi.org/10.1021/np020044g. PubMed
38. Seebaluck, R., Gurib-Fakim, A., & Mahomoodally, F. (2015). Medicinal plants from the genus *Acalypha* (Euphorbiaceae)—A review of their ethnopharmacology and phytochemistry. *Journal of Ethnopharmacology, 159*, 137–157.
39. Cappiello, F., Loffredo, M. R., Del Plato, C., Cammarone, S., Casciaro, B., Quaglio, D., Mangoni, M. L., Botta, B., & Ghirga, F. (2020). The revaluation of plant-derived terpenes to fight antibiotic-resistant infections. *Antibiotics, 9*(6), 325. https://doi.org/10.3390/antibiotics9060325. PMC

40. Cardiel, J. M., Cordeiro de Sousa, A. A., Cordeiro, I., Rossi, M. B., Marques da Silva, O. L., Muñoz-Rodríguez, P., López, A., & Montero-Muñoz, I. (2022). Updated synopsis of *Acalypha* (Euphorbiaceae, Acalyphoideae) from Brazil. *Plant Systematics and Evolution, 308*, 24. https://doi.org/10.1007/s00606-022-01816-0. SpringerLink
41. Cardiel, J. M., Muñoz-Rodríguez, P., González-Berdasco, Á., & Montero-Muñoz, I. (2023). Catalogue and red list of *Acalypha* L. (Euphorbiaceae) from South America. *European Journal of Taxonomy, 886*, 1–92. https://doi.org/10.5852/ejt.2023.886.2201. Zenodo
42. Postigo, A., Svetaz, L., Derita, M., Gette, M. A., Petenatti, M., Del Vitto, L., Petenatti, E., & Zacchino, S. (2012). Discovery of antifungal plants in Argentinean San Luis province: Ethnomedical information or random selection? *Revista Eletrônica de Farmácia, 9*(1), 18–39. https://doi.org/10.5216/ref.v9i1.17775.
43. Gutiérrez-Lugo, M. T., Singh, M. P., Maiese, W. M., & Timmermann, B. N. (2002). New antimicrobial cycloartane triterpenes from *Acalypha communis*. *Journal of Natural Products, 65*(6), 872–875.
44. Seebaluck, R., Gurib-Fakim, A., & Mahomoodally, M. F. (2015). Medicinal plants from the genus *Acalypha* (Euphorbiaceae): Ethnopharmacology and phytochemistry. *Journal of Ethnopharmacology, 159*, 137–157.
45. Postigo, A., Svetaz, L., Derita, M., et al. (2012). Discovery of antifungal plants in Argentinean San Luis province: Ethnomedical information or random selection? *Revista Eletrônica de Farmácia, IX*(1), 18–39.
46. Kupchan, S. M., Tsou, G., & Sigel, C. W. (1973). Datiscacin, a novel cytotoxic cucurbitacin 20-acyl derivative from *Datisca glomerata*. *Journal of Organic Chemistry, 38*(7), 1420–1421.
47. Emran, T. B., Rahman, M. A., Uddin, M. M. N., et al. (2015). Effects of organic extracts and their different fractions of five Bangladeshi plants on *in vitro* thrombolysis. *BMC Complementary and Alternative Medicine, 15*, 128. ResearchGate
48. Chemat, F., Rombaut, N., Sicaire, A.-G., Meullemiestre, A., Fabiano-Tixier, A.-S., & Abert-Vian, M. (2017). Ultrasound-assisted extraction of food and natural products: Mechanisms, techniques, combinations, protocols and applications—A review. *Ultrasonics Sonochemistry, 34*, 540–560. PubMedmastersonics.com
49. Kumar, K., Srivastav, S., & Yadav, R. (2020). Ultrasound assisted extraction (UAE) of bioactive components: A review. *Applied Food Research, 1*(1), 100002. (Open-access overview). PMC
50. Mandal, V., Mohan, Y., & Hemalatha, S. (2007). Microwave-assisted extraction—An innovative and promising extraction tool for medicinal plant research. *Pharmacognosy Reviews, 1*(1), 7–18. Pharmacognosy Reviews
51. Camel, V. (2000). Microwave-assisted solvent extraction of environmental samples. *Trends in Analytical Chemistry, 19*(4), 229–248. HERO
52. Wagner, H., & Bladt, S. (2001). *Plant drug analysis: A thin layer chromatography atlas* (2nd ed.). Springer. (p-anisaldehyde–H_2SO_4 reagent for terpenoids). National Library of Medicine CatalogCabi Digital Library

53. Baglyas, M., et al. (2022). High-performance thin-layer chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 221*, 115015. (Notes anisaldehyde derivatization for terpenoids). ScienceDirect
54. Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences, 13*(7), 8615–8627. (Open access). PMC
55. Baker, T. R., et al. (2018). Multi-detector HPLC with CAD for mass-sensitive detection of non-volatile analytes. *Analytical and Bioanalytical Chemistry, 410*(18), 4401–4415. ResearchGate
56. Vehovec, T., & Obreza, A. (2010). Review of operating principle and applications of the charged aerosol detector. *Journal of Chromatography A, 1217*(10), 1549–1556. (CAD vs. ELSD performance). ScienceDirectEurope PMC
57. Sarker, S. D., Latif, Z., & Gray, A. I. (2006). *Natural products isolation* (2nd ed.). Methods in Molecular Biology, Vol. 20. Humana/Springer. PMC
58. Nothias, L.-F., Petras, D., Schmid, R., et al. (2020). Feature-based molecular networking in the GNPS analysis environment. *Nature Methods, 17*, 905–908. PMC
59. Wang, M., Carver, J. J., Phelan, V. V., et al. (2016). Sharing and community curation of mass spectrometry data with GNPS. *Nature Biotechnology, 34*(8), 828–837. PMC
60. ICH. (2023). **Q2(R2)**: Validation of analytical procedures (Step 5). *International Council for Harmonisation* (official guideline). ScienceDirectPMC
61. ICH. (2023). **Q14**: Analytical procedure development (Step 5). *International Council for Harmonisation* (official guideline). Ijbpsa
62. Reverchon, E., & De Marco, I. (2006). Supercritical fluid extraction and fractionation of natural matter: A review. *Journal of Supercritical Fluids, 38*(2), 146–166. ScienceDirect
63. Sovová, H. (2005). Mathematical model for supercritical fluid extraction of natural products and extraction curve evaluation. *Journal of Supercritical Fluids, 33*(1), 35–52. ScienceDirect
64. Uwineza, P. A., & Waśkiewicz, A. (2020). Recent advances in supercritical fluid extraction of natural products. *Molecules, 25*(17), 3847. (Open access). PMC
65. Bixapathi, K., Prabhakar, G., & Kamalakar, P. (2024). Preliminary phytochemical screening, quantification, and identification of active compounds in *Acalypha malabarica* Müll.Arg using FTIR and GC–MS analysis. *Plant Science Archives, 9*(4), 30–40. https://doi.org/10.51470/PSA.2024.9.4.30. plantarc.comResearchGate
66. Agilent Technologies. (2022, August 23). *Beware of GC column bleed* [Slide deck]. Agilent
67. LCGC/Chromatography Online. (2022, June 9). *Understanding the origins of siloxane ghost peaks in gas chromatography*. Chromatography Online
68. Valdez, C. A., Narishetty, S. K., Reisdorph, N., Murton, J., & Rearden, P. (2018). Assessing the reliability of the NIST library during routine GC–MS analyses: Structure and spectral data corroboration for 5,5-diphenyl-1,3-dioxolan-4-one during a recent OPCW proficiency test. *Journal of Mass Spectrometry, 53*(5), 419–422. https://doi.org/10.1002/jms.4073.

69. Bixapathi, K., Prabhakar, G., & Kamalakar, P. (2024). Preliminary phytochemical screening, quantification, and identification of active compounds in *Acalypha malabarica* Müll.Arg using FTIR and GC–MS analysis. *Plant Science Archives, 9*(4), 30–40. https://doi.org/10.51470/PSA.2024.9.4.30. plantarc.com
70. Agilent Technologies. (2022, August 23). *Beware of GC column bleed* [Slide deck]. Agilent
71. English, C. (2022, June 9). Understanding the origins of siloxane ghost peaks in gas chromatography. *LCGC North America*. Chromatography Online
72. Stein, S. E. (1999). *An integrated method for spectrum extraction and compound identification from GC/MS data* (NIST method report). National Institute of Standards and Technology. chemdata.nist.gov
73. Nothias, L.-F., Petras, D., Schmid, R., et al. (2020). Feature-based molecular networking in the GNPS analysis environment. *Nature Methods, 17*(9), 905–908. https://doi.org/10.1038/s41592-020-0933-6. Nature
74. GNPS2 Documentation. (n.d.). *FBMN – Feature-Based Molecular Networking*. Retrieved September 4, 2025, from https://wang-bioinformatics-lab.github.io/GNPS2_Documentation/fbmn/ wang-bioinformatics-lab.github.io
75. European Medicines Agency (on behalf of ICH). (2023, December 14). *ICH Q2(R2) guideline: Validation of analytical procedures (Step 5)*. European Medicines Agency (EMA)
76. U.S. Food and Drug Administration. (2024, March 6). *Q2(R2) Validation of Analytical Procedures* (Guidance for Industry). U.S. Food and Drug Administration
77. Agilent Community. (2025, June 27). *How to identify and resolve baseline noise and drift caused by methyl siloxane in GC/MS analysis*. community.agilent.com
78. World Flora Online. (n.d.). *Acalypha malabarica* Müll.Arg. (Accepted name; Linnaea 34: 42, 1865). Retrieved September 4, 2025, from World Flora Online database. World Flora Online
79. India Flora Online (IISc). (n.d.). *Acalypha malabarica* Müll.Arg.: Habit, habitat, and distribution notes. Retrieved September 4, 2025. indiaflora-ces.iisc.ac.in
80. Salomeyesudas, B., et al. (2009). *Traditional food system of Dalit in Zaheerabad Region, Medak District, Andhra Pradesh, India*. In H. V. Kuhnlein et al. (Eds.), *Indigenous Peoples' food systems: The many dimensions of culture, diversity and environment for nutrition and health* (pp. 183–216). FAO. (Lists *A. malabarica* "pitta kura" as an edible leafy green.) FAOHome
81. Bixapathi, K., Prabhakar, G., & Kamalakar, P. (2024). Preliminary phytochemical screening, quantification, and identification of active compounds in *Acalypha malabarica* Müll.Arg using FTIR and GC–MS analysis. *Plant Science Archives, 9*(4), 30–40. https://doi.org/10.51470/PSA.2024.9.4.30. plantarc.com
82. Bixapathi, K., Prabhakar, G., & Kamalakar, P. (2024, October). Exploring the therapeutic efficacy of *Acalypha malabarica* leaf in wound healing: Insights from Wistar rat models. *Biochemical and Cellular Archives, 24*(2). (Table of contents listing). connectjournals.com
83. Seebaluck, R., Gurib-Fakim, A., & Mahomoodally, F. (2015). Medicinal plants from the genus *Acalypha* (Euphorbiaceae): Ethnopharmacology and phytochemistry.

Journal of Ethnopharmacology, 159, 137–157. https://doi.org/10.1016/j.jep.2014.10.040. PubMed

84. Labu, Z. K., Karim, S., Arifuzzaman, S., Motalab, S. B., Alzahrani, K. J., Alsharif, K. F., Zafar, M., & Azad, A. K. (2025). The South Asian *Acalypha* species: A comprehensive review on traditional uses, phytochemistry and pharmacological aspect. *Chemistry & Biodiversity*, e00613. https://doi.org/10.1002/cbdv.202500613. PubMed
85. Ganeshkumar, M., Ponrasu, T., Krithika, R., Iyappan, K., Gayathri, V. S., & Suguna, L. (2012). Topical application of *Acalypha indica* accelerates rat cutaneous wound healing by up-regulating type I/III collagen. *Journal of Ethnopharmacology, 142*(1), 14–22. https://doi.org/10.1016/j.jep.2012.04.005. PubMed
86. Sherifat, K. O., Itohan, A. M., Adeola, S. O., Adeola, K.-M., & Aderemi, O. L. (2021). Anti-fungal activity of *Acalypha wilkesiana*: A preliminary study of fungal isolates of clinical significance. *African Journal of Infectious Diseases, 16*(1), 21–30. https://doi.org/10.21010/Ajid.v16i1.4.
87. Bixapathi, K., Prabhakar, G., & Kamalakar, P. (2024). Preliminary phytochemical screening, quantification, and identification of active compounds in *Acalypha malabarica* Müll.Arg using FTIR and GC–MS analysis. *Plant Science Archives, 9*(4), 30–40. https://doi.org/10.51470/PSA.2024.9.4.30. plantarc.com
88. Chemat, F., Rombaut, N., Sicaire, A.-G., Meullemiestre, A., Fabiano-Tixier, A.-S., & Abert-Vian, M. (2017). Ultrasound-assisted extraction of food and natural products: Mechanisms, techniques, combinations, protocols and applications—A review. *Ultrasonics Sonochemistry, 34*, 540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035. PubMed
89. Chan, C.-H., Yusoff, R., Ngoh, G.-C., & Kung, F. W.-L. (2011). Microwave-assisted extractions of active ingredients from plants. *Journal of Chromatography A, 1218*(37), 6213–6225. https://doi.org/10.1016/j.chroma.2011.07.040. eprints.um.edu.my
90. Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N., & Pohl, C. (1996). Accelerated solvent extraction: A technique for sample preparation. *Analytical Chemistry, 68*(6), 1033–1039. https://doi.org/10.1021/ac9508199. HERO
91. Herrero, M., Mendiola, J. A., Cifuentes, A., & Ibáñez, E. (2010). Supercritical fluid extraction: Recent advances and applications. *Journal of Chromatography A, 1217*(16), 2495–2511. https://doi.org/10.1016/j.chroma.2009.12.019. PubMed
92. Emran, T. B., Rahman, M. A., Uddin, M. M. N., et al. (2015). Effects of organic extracts and their different fractions of five Bangladeshi plants on *in vitro* thrombolysis. *BMC Complementary and Alternative Medicine, 15*, 221. (Includes modified Kupchan partition protocol.) https://doi.org/10.1186/s12906-015-0700-3. PMC
93. Agilent Technologies. (2022, August 23). *Beware of GC column bleed* [Slide deck]. (Troubleshooting bleed; characteristic ions.) Agilent
94. English, C. (2022, June 9). Understanding the origins of siloxane ghost peaks in gas chromatography. *LCGC North America*. (Septa/column-bleed patterns; diagnostic ions.) Chromatography Online
95. Stein, S. E. (2012). Mass spectral reference libraries: An ever-expanding resource for chemical identification. *Analytical Chemistry, 84*(17), 7274–7282. https://doi.org/10.1021/ac301205z. ACS Publications