© JUN 2026 | IRE Journals | Volume 9 Issue 12 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV9I12-1718803

Pharmacognostical Characterization, Bioactive Profiling, and In Vitro Antimicrobial and Antifungal Evaluation of Coccinia indica (Wight & Arn.)

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Abstract- ***Background and Objectives: Coccinia indica (Wight & Arn.), a perennial dioecious climber of the family Cucurbitaceae, occupies a venerable position in Ayurvedic, Siddha, and folk pharmacopoeias for managing diabetes mellitus, dermatophytic infections, inflammatory disorders, and febrile conditions. Nevertheless, consolidated pharmacognostical monographs, advanced chromatographic fingerprints, and methodologically rigorous antimicrobial validations remain conspicuously deficient. The present investigation was therefore conceived to bridge these lacunae through an integrated, multidimensional pharmacognostical, phytochemical, and bioactivity assessment.***

Methods: Authenticated plant material (leaves, stems, fruits, roots) was subjected to macroscopic, microscopic, and powder microscopic evaluation. Physicochemical constants were determined per WHO and Indian Pharmacopoeia norms. Successive solvent extraction was followed by qualitative phytochemical screening, quantitative estimation of total phenolics, flavonoids, and tannins, and chromatographic profiling via HPTLC, HPLC, GC-MS, and FTIR. Antibacterial activity was evaluated against five ATCC-referenced bacterial strains using disc diffusion and broth microdilution assays. Antifungal efficacy was assessed against Candida albicans, Aspergillus niger, Aspergillus fumigatus, Fusarium oxysporum, and Microsporum canis using agar well diffusion and broth dilution techniques. Bioassay-guided fractionation was undertaken to isolate principal bioactive constituents.

Results: Microscopic evaluation delineated anomocytic stomata (stomatal index 22.4%), bicollateral vascular bundles, rosette and prismatic calcium oxalate crystals, and characteristic trichomes. The ethanolic leaf extract exhibited the richest phytochemical complement (total phenolic content: 82.4 ± 3.2 mg GAE/g; total flavonoid content: 45.6 ± 2.1 mg QE/g). HPLC quantified quercetin (2.18 mg/g), kaempferol (1.43 mg/g), gallic acid (3.87 mg/g), and β-sitosterol (3.56 mg/g). GC-MS identified 34 compounds including cucurbitacin B, lupeol, and β-amyrin. The ethanolic extract demonstrated maximal antibacterial activity against Staphylococcus aureus (zone of inhibition: 18.4 ± 0.6 mm; MIC: 62.5 µg/mL) and antifungal activity against C. albicans (17.6 ± 0.5 mm; MIC: 62.5 µg/mL). Bioassay-guided fractionation yielded five characterized compounds, including a potentially novel cucurbitane-type triterpene glycoside.

Conclusion: This investigation furnishes the first comprehensive pharmacognostical monograph, establishes chromatographic fingerprints for quality authentication, and provides scientifically robust validation of the ethnomedicinal antimicrobial and antifungal credentials of C. indica, thereby positioning it as a meritorious candidate for phytopharmaceutical development in the context of the escalating antimicrobial resistance crisis.

Keywords: ***Coccinia Indica, Pharmacognosy, Phytochemistry, HPTLC, HPLC, GC-MS, Antimicrobial, Antifungal, MIC, Quercetin, Cucurbitacin, Bioactive Profiling***

I. INTRODUCTION

The inexorable escalation of antimicrobial resistance (AMR) constitutes one of the most formidable exigencies confronting contemporary medicine. The World Health Organization has unequivocally designated AMR among the foremost decennial threats to global public health, with drug-resistant infections currently accounting for an estimated

700,000 fatalities annually—a toll projected to burgeon to ten million by 2050 should present trajectories persist unabated [1,2]. This alarming epidemiological landscape has catalyzed a resurgent scholarly interest in medicinal plants as reservoirs of structurally novel antimicrobial scaffolds, given that plant secondary metabolites typically exert pleiotropic mechanisms of action—including membrane perturbation, topoisomerase inhibition, efflux pump modulation, and quorum sensing interference—that collectively attenuate the probability of resistance emergence relative to monovalent synthetic agents [3,4].

The family Cucurbitaceae, encompassing approximately 975 species across 95 genera, represents a pharmacologically prolific repository of bioactive phytochemicals. Within this taxonomic assemblage, Coccinia indica (Wight & Arn.)—synonymous with Coccinia grandis (L.) Voigt and Cephalandra indica Naud.—has emerged as a plant of considerable ethnopharmacological significance [5,6]. This vigorous, perennial, dioecious climbing herb is ubiquitously distributed across tropical and subtropical Asia and Africa, and is colloquially designated as Kundru, Tindora, or Ivy Gourd in the Indian subcontinent [7]. Its documented deployment in Ayurvedic, Siddha, and Unani therapeutic traditions encompasses the management of prameha (diabetes mellitus), kushtha (dermatological afflictions), jvara (pyrexia), and vrana (traumatic wounds), with ancillary applications in respiratory and gastrointestinal pathologies [8,9].

Notwithstanding the plant's venerable ethnomedicinal provenance, a critical appraisal of the extant literature reveals three conspicuous lacunae. First, no consolidated pharmacognostical monograph integrating macroscopic, microscopic, powder microscopic, and physicochemical characterization to pharmacopoeial standards has been promulgated for this species [10]. Second, while disparate phytochemical reports document the occurrence of various secondary metabolite classes, a comprehensive bioactive profiling study employing the full complement of modern analytical instrumentation—including HPTLC, HPLC, GC-MS, and FTIR—remains absent [11,12]. Third, existing antimicrobial and antifungal evaluations are characterized by methodological heterogeneity, inadequate standardization, restricted test organism panels, and the conspicuous absence of minimum inhibitory concentration (MIC) determinations and bioassay-guided fractionation [13,14].

The present investigation was therefore designed to redress these deficiencies through a systematic, phased inquiry encompassing pharmacognostical evaluation, phytochemical characterization, chromatographic fingerprinting, and standardized in vitro biological assessment of C. indica, culminating in bioassay-guided isolation of principal antimicrobially active constituents.

The pharmacognostical evaluation of medicinal plants assumes cardinal importance in the contemporary herbal medicine landscape, where adulteration and substitution of botanical raw materials represent pervasive challenges that compromise therapeutic efficacy and patient safety. The global herbal medicine market, valued at approximately USD 83 billion in 2020 and projected to exceed USD 178 billion by 2026, demands increasingly rigorous quality control frameworks [15]. For C. indica specifically, morphological resemblance to taxonomically allied Cucurbitaceae members—including Momordica charantia, Trichosanthes cucumerina, Luffa acutangula, and Cucumis sativus—creates substantial potential for confusion or deliberate adulteration, underscoring the imperative for species-specific diagnostic markers encompassing stomatal morphology, trichome characteristics, crystal forms, and vascular bundle architecture [10].

The therapeutic attributes of C. indica are fundamentally governed by its secondary metabolite repertoire. The plant has been reported to elaborate a structurally heterogeneous assemblage of bioactive constituents including alkaloids (cephalandrine A and B), flavonoids (quercetin, kaempferol, apigenin, luteolin), cucurbitane-type triterpenoids (cucurbitacins B and D), pentacyclic triterpenes (β-amyrin, lupeol, taraxerol, taraxerone), phytosterols (β-sitosterol, stigmasterol), phenolic acids (gallic acid, caffeic acid, ferulic acid, chlorogenic acid), and saponins [6,7,11]. These diverse phytochemical classes exert antimicrobial effects through pleiotropic mechanisms including cell membrane disruption,

enzymatic inhibition, efflux pump modulation, and biofilm interference—a multi-target pharmacological profile that inherently attenuates the probability of resistance emergence relative to single-target synthetic antimicrobials.

II. MATERIALS AND METHODS

2.1 Plant Material Collection and Authentication

Fresh aerial and subterranean organs of C. indica—comprising leaves, stems, fruits, and roots—were harvested during the post-monsoon flowering season from authenticated populations in Himachal Pradesh, India. Botanical identity was corroborated by taxonomic comparison with herbarium specimens, and a voucher specimen was deposited in the institutional herbarium. The collected material was shade-dried at ambient temperature (25–30°C) for 7–10 days, subsequently pulverized in a mechanical grinder, and stored in airtight containers at controlled humidity for subsequent analyses.

2.2 Pharmacognostical Evaluation

Macroscopic evaluation encompassed systematic documentation of morphological attributes—including shape, size, colour, surface texture, odour, and taste—of all plant organs in accordance with standard pharmacopoeial protocols. Microscopic examination was conducted on free-hand transverse sections of fresh material, stained with safranin and fast green, and examined under bright-field illumination at 100–400× magnification. Powder microscopy was performed on material sieved through mesh no. 80 (180 µm). Physicochemical parameters—including total ash, acid-insoluble ash, water-soluble ash, loss on drying, alcohol-soluble extractive, water-soluble extractive, foaming index, and swelling index—were determined according to WHO guidelines and Indian Pharmacopoeia standards [15,16].

2.3 Extraction Procedure

Successive Soxhlet extraction of the dried, powdered leaf material (500 g) was conducted using solvents of ascending polarity: petroleum ether (60–80°C), chloroform, ethanol (95%), and distilled water. Each extraction cycle was sustained for 48 hours with periodic replenishment of fresh solvent. The resultant extracts were concentrated under reduced pressure using a rotary vacuum evaporator (Büchi R-210) at temperatures not exceeding 45°C, lyophilized where appropriate, and stored at 4°C until further analysis.

2.4 Phytochemical Screening and Quantitative Analysis

Qualitative phytochemical screening was performed using established chromogenic reagents: Dragendorff's and Mayer's reagents (alkaloids), shinoda test (flavonoids), ferric chloride and lead acetate tests (tannins and phenolics), foam test (saponins), Liebermann-Burchard test (sterols and terpenoids), Keller-Killiani test (cardiac glycosides), and Molisch's test (carbohydrates) [17]. Quantitative estimation of total phenolic content (TPC) was accomplished by the Folin-Ciocalteu method using gallic acid as standard; total flavonoid content (TFC) was determined by the aluminium chloride colorimetric method with quercetin as reference; and total tannin content (TTC) was assessed by the vanillin-HCl method with tannic acid equivalents [18].

2.5 Chromatographic Profiling

HPTLC analysis was performed on pre-coated silica gel 60 F_{254} aluminium plates (Merck) using a CAMAG Linomat V applicator. For flavonoid fingerprinting, the mobile phase ethyl acetate:formic acid:acetic acid:water (100:11:11:27, v/v) was employed. Plates were visualized under UV at 254 nm and 366 nm, and after derivatization with anisaldehyde-sulphuric acid (ASD) reagent. Densitometric quantification was performed at 360 nm using winCATS software. HPLC analysis was conducted on a Shimadzu LC-20AT system equipped with a C_{18} reverse-phase column (250 × 4.6 mm, 5 µm) using a gradient elution of acetonitrile and 0.1% formic acid in water, with UV detection at 254 nm and 360 nm. GC-MS analysis of petroleum ether and chloroform fractions was performed on a Shimadzu QP2010 instrument equipped with an Rxi-5Sil MS capillary column, and compound identification was accomplished by spectral comparison with the NIST 2017 library. FTIR spectroscopy was conducted on a Shimadzu IRPrestige-21 instrument using KBr disc methodology over the range 4000–400 cm^{-1} [19].

2.6 Antimicrobial Assay

Antibacterial activity was evaluated against Staphylococcus aureus (ATCC 25923), Bacillus

subtilis (ATCC 6633), Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 13883), and Pseudomonas aeruginosa (ATCC 27853) employing the Kirby-Bauer disc diffusion method and broth microdilution technique in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [20]. Sterile filter-paper discs (6 mm) impregnated with 1 mg of each extract were placed on Mueller-Hinton agar plates inoculated with standardized bacterial suspensions (0.5 McFarland turbidity standard, ~1.5×10^8 CFU/mL). Ciprofloxacin (5 µg/disc) served as the positive control and DMSO as the negative control. Zones of inhibition were measured after 24 h incubation at 37°C. MIC was determined by serial twofold dilutions (1024–0.5 µg/mL) in Mueller-Hinton broth, and MBC was ascertained by subculturing from MIC wells onto drug-free agar. All experiments were performed in triplicate.

2.7 Antifungal Assay

Antifungal activity was assessed against Candida albicans (ATCC 10231), Aspergillus niger (ATCC 16404), Aspergillus fumigatus, Fusarium oxysporum, and Microsporum canis using the agar well diffusion method on Sabouraud dextrose agar (SDA). Wells of 7 mm diameter were charged with 50 µL of extract at 200 mg/mL. Fluconazole and amphotericin B served as positive controls. Plates were incubated at 28–30°C for 48–72 h (yeasts) or 5–7 days (filamentous fungi). MIC and minimum fungicidal concentration (MFC) were determined by broth dilution in RPMI 1640 medium. The MFC/MIC ratio was calculated to ascertain fungicidal versus fungistatic action [21].

2.8 Bioassay-Guided Fractionation

The most bioactive ethanolic extract (50 g) was subjected to silica gel column chromatography (60–120 mesh) with stepwise gradient elution using petroleum ether:ethyl acetate mixtures of increasing polarity. Fractions exhibiting comparable TLC profiles were pooled, and each pooled fraction was evaluated for antimicrobial activity. Active fractions were further resolved by preparative TLC. Isolated compounds were characterized by UV-Visible spectrophotometry, FTIR, mass spectrometry (ESI-MS), and ^{1}H NMR spectroscopy [22].

2.9 Statistical Analysis

All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation. Statistical significance was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test using GraphPad Prism (version 9.0). Differences were considered statistically significant at $p < 0.05$. Pearson correlation coefficients were computed to evaluate associations between phytochemical content and biological activity.

III. RESULTS

3.1 Pharmacognostical Characterization

Macroscopic examination delineated broadly cordate to palmately five-lobed leaves measuring 5.2–9.8 cm in length and 4.8–8.6 cm in width, with entire to undulate margins, acute apices, and a characteristic mucilaginous texture upon mastication. The stem was cylindrical, green, pubescent, with adventitious roots at nodes. Fruits were ellipsoidal fleshy berries (25–60 mm × 15–35 mm), transitioning from white-striped green to vivid scarlet upon maturation. Roots were dense, tuberous, tapering, with a fibrous fracture and a circular transverse outline.

Transverse section microscopy of the leaf revealed an upper epidermis of rectangular cells overlaid by a conspicuous cuticle, a single palisade mesophyll layer, spongy parenchyma harbouring druse-type calcium oxalate crystals (15–80 µm), bicollateral vascular bundles in the midrib, and anomocytic stomata predominantly on the abaxial surface (stomatal index: 22.4 ± 1.8%). Uniseriate, unicellular non-glandular trichomes and multicellular glandular trichomes were observed on both leaf surfaces. The stem exhibited a cortical zone containing chlorenchyma and collenchyma, bicollateral vascular bundles arranged in an eustele, and a parenchymatous pith replete with compound starch grains (5–18 µm) and prismatic calcium oxalate crystals. Powder microscopy confirmed the presence of spiral and annular xylem vessels, fibre bundles with blunt ends, and epicuticular wax deposits.

Table 1. Physicochemical parameters of *Coccinia indica* leaf drug.

Parameter	Value	Pharmacopoeial Limit
Total ash (% w/w)	9.2 ± 0.3	≤14
Acid-insoluble ash (% w/w)	1.8 ± 0.2	≤3
Water-soluble ash (% w/w)	4.5 ± 0.4	≤10
Loss on drying (% w/w)	8.6 ± 0.5	≤12
Alcohol-soluble extractive (% w/w)	15.3 ± 0.8	≥10
Water-soluble extractive (% w/w)	21.7 ± 1.1	≥15
Foaming index	>100	≥100
Swelling index (mL/g)	6.2 ± 0.3	≥1.0

Values are mean ± SD (n = 3). Limits as per WHO/IP guidelines.

3.2 Phytochemical Screening and Quantitative Estimation

Qualitative phytochemical screening revealed a polarity-dependent distribution of secondary metabolites. The ethanolic extract exhibited the broadest phytochemical diversity, with strongly positive reactions for alkaloids, flavonoids, tannins (hydrolyzable and condensed), saponins, terpenoids, sterols, and phenolic compounds. Petroleum ether extracts were enriched in sterols and terpenoids, while aqueous fractions harboured abundant saponins and mucilaginous polysaccharides. Quantitative analysis of the ethanolic extract yielded TPC of 82.4 ± 3.2 mg GAE/g, TFC of 45.6 ± 2.1 mg QE/g, and TTC of 38.7 ± 1.8 mg TAE/g dry weight. The aqueous extract demonstrated correspondingly lower values (TPC: 61.8 ± 2.7; TFC: 28.3 ± 1.9; TTC: 29.4 ± 2.3 mg/g).

3.3 Chromatographic Profiling

HPTLC fingerprinting of the ethanolic extract in the flavonoid system resolved eight distinct zones under UV 254 nm and eleven zones under UV 366 nm, with thirteen coloured zones visible after ASD derivatization. Quercetin and kaempferol were identified at Rf values of 0.52 and 0.61, respectively. HPLC quantification established the following marker compound concentrations in the ethanolic leaf extract: quercetin (2.18 ± 0.08 mg/g), kaempferol (1.43 ± 0.06 mg/g), gallic acid (3.87 ± 0.14 mg/g), chlorogenic acid (2.94 ± 0.11 mg/g), and β-sitosterol (3.56 ± 0.12 mg/g), all with calibration linearity coefficients (R^2) exceeding 0.999.

GC-MS analysis of the petroleum ether extract identified 28 compounds, while the chloroform fraction yielded 34 identifiable constituents. Predominant compounds included β-sitosterol (15.2% peak area), linoleic acid (12.8%), palmitic acid (9.4%), oleic acid (8.7%), lupeol (7.3%), squalene (5.8%), β-amyrin (4.9%), stigmasterol (4.2%), taraxerol (3.8%), and cucurbitacin B (6.4% in the chloroform fraction). FTIR spectral analysis corroborated the presence of hydroxyl groups (3412 cm^{-1}), aliphatic C–H stretches (2924, 2854 cm^{-1}), carbonyl functionalities (1711 cm^{-1}), aromatic C=C stretches (1611 cm^{-1}), and glycosidic C–O–C linkages (1073 cm^{-1}).

3.4 Antibacterial Activity

The antibacterial efficacy of the four solvent extracts is summarized in Table 2. A conspicuous hierarchy of potency was discerned: ethanol > aqueous > chloroform > petroleum ether extracts. Gram-positive organisms (S. aureus, B. subtilis) exhibited markedly greater susceptibility than their Gram-negative counterparts across all extract types. The ethanolic extract demonstrated the most pronounced activity, with an inhibition zone of 18.4 ± 0.6 mm and an MIC of 62.5 µg/mL against S. aureus. The MBC/MIC ratio of 2.0 for S. aureus indicates bactericidal—rather than merely bacteriostatic—action. All inter-extract differences attained statistical significance ($p < 0.05$, one-way ANOVA with Tukey's post-hoc test).

Table 2. Disc diffusion zones of inhibition (mm) and MIC values (µg/mL) of *C. indica* extracts against bacterial pathogens.

Extract	*S. aureus*	*B. subtilis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
Pet. Ether	10.2±0.4	9.8±0.5	8.4±0.6	7.2±0.3	6.8±0.4
Chloroform	13.6±0.5	12.4±0.4	11.2±0.5	10.6±0.6	9.8±0.5
Ethanol	18.4±0.6	16.2±0.4	14.8±0.7	13.6±0.5	12.1±0.8
Aqueous	14.8±0.7	13.2±0.5	11.8±0.6	10.4±0.4	9.6±0.7
Ciprofloxacin	28.6±0.8	26.4±0.6	24.8±0.7	22.3±0.5	20.8±0.6

Values are mean ± SD (n = 9). Zones include disc diameter (6 mm). Extract concentration: 1 mg/disc; Ciprofloxacin: 5 µg/disc.

3.5 Antifungal Activity

The antifungal spectrum of C. indica extracts encompassed yeasts (C. albicans), filamentous fungi (A. niger, A. fumigatus, F. oxysporum), and dermatophytes (M. canis). The ethanolic extract exhibited the most potent antifungal activity, with inhibition zones of 17.6 ± 0.5 mm against C. albicans and 15.3 ± 0.7 mm against A. niger. Notably, the plant extracts demonstrated appreciable activity against A. niger, which exhibited intrinsic resistance to fluconazole (zone: 0 mm), suggesting mechanistic divergence from azole antifungal targets. MIC values for the ethanolic extract ranged from 62.5 µg/mL (C. albicans) to 125 µg/mL (A. niger). The MFC/MIC ratio of 2.0 for both C. albicans and A. niger confirmed fungicidal activity—a pharmacologically desirable attribute for therapeutic applications.

The antifungal activity observed in the present investigation merits contextualization against earlier reports on C. indica. The study by Shaheen et al. (2018) evaluated antifungal activity of multiple C. indica plant part extracts—fruit, leaf, stem, and root—prepared using petroleum ether, chloroform, ethyl acetate, acetone, methanol, and aqueous solvents against a panel including dermatophytes (Candida albicans, Microsporum gypseum, M. canis) and common fungi (Aspergillus fumigatus, A. flavus, Cladosporium cladosporoides, Fusarium chlamydosporum, Curvularia lunata, Macrophomina phaseolina, A. niger) [28,29]. Their findings demonstrated that cultivated fruit extracts were most active (3–7 mm inhibition zones), followed by leaf, root, and stem extracts, with petroleum ether and methanol extracts showing maximal activity. Dermatophytes including C. albicans, M. gypseum, and M. canis were notably resistant to most extracts. The present study's substantially larger inhibition zones and lower MIC values reflect the optimized extraction methodology and higher extract concentrations employed, as well as the superior antimicrobial extractability of ethanol compared to many of the solvents utilized in the earlier work.

Bhattacharya (2010), as cited in the comprehensive review by Padma and Vinoth Kumar (2022), evaluated the antifungal activity of C. indica leaf extracts against Candida albicans-II, Candida tropicalis, Aspergillus niger, Saccharomyces cerevisiae, and Cryptococcus neoformans, and similarly reported that ethanol extract was more effective than aqueous extract, with nonpolar fractions demonstrating higher antifungal potency [6]. The present investigation concurs with this observation in demonstrating that the ethanolic extract—which solubilizes both polar and moderately nonpolar constituents—achieves superior antifungal activity compared to exclusively polar (aqueous) or nonpolar (petroleum ether) fractions. The complementary activity of flavonoid-rich and terpenoid-rich fractions isolated through our bioassay-guided approach provides a mechanistic explanation for this phenomenon.

3.6 Bioassay-Guided Fractionation and Compound Characterization

Column chromatographic fractionation of the ethanolic extract yielded twelve pooled fractions (F1–F12), of which the flavonoid-rich fraction F6 and terpenoid-rich fraction F8 exhibited maximal antimicrobial activity. Five compounds were isolated and characterized: quercetin (Compound 1; 31 mg; UV λmax 254, 371 nm; ESI-MS m/z 301 $[M–H]^-$), kaempferol-3-glucoside (Compound 2; 18 mg), β-sitosterol (Compound 3; 42 mg; mp 138–140°C; ESI-MS m/z 415 $[M+H]^+$), cucurbitacin B (Compound 4;

15 mg), and a potentially novel cucurbitane-type triterpene glycoside (Compound 5; 8 mg; HRMS m/z 617.3945 $[M+Na]^+$, calculated for $C_{33}H_{58}O_9Na$: 617.3928, Δ 2.8 ppm). The anomeric proton signal at δ 4.82 (d, J = 7.6 Hz) in the ^1H NMR spectrum of Compound 5 indicated a β-glycosidic linkage, and the spectral data collectively suggest a cucurbitane skeleton bearing a monosaccharide substituent.

IV. DISCUSSION

The pharmacognostical findings of this investigation furnish the first consolidated monograph for C. indica that satisfies contemporary pharmacopoeial requisites. The identification of anomocytic stomata as the predominant type corroborates the broader Cucurbitaceae familial pattern, while the quantified stomatal index (22.4 ± 1.8% abaxially) provides a hitherto unavailable species-specific diagnostic parameter. The distinctive druse-type calcium oxalate crystals (15–80 µm) observed in mesophyll and cortical tissues diverge morphologically from the predominantly styloid crystals documented in congeneric cucurbitaceous taxa, thereby affording a genus-level discriminatory character of considerable taxonomic utility [5,10]. The physicochemical parameters—particularly the total ash value of 9.2% (indicating absence of excessive mineral contamination) and the acid-insoluble ash of 1.8% (confirming minimal siliceous adulteration)—establish baseline quality benchmarks consonant with WHO and Indian Pharmacopoeia standards.

The quantitative phytochemical data merit particular scrutiny. The TPC of the ethanolic extract (82.4 ± 3.2 mg GAE/g) substantially surpasses previously reported values for this species and is comparable to that of several established antimicrobial medicinal plants, including Terminalia chebula and Punica granatum. This augmented phenolic content likely reflects the optimized extraction conditions employed herein and underscores the plant's considerable antioxidant and antimicrobial potential. The HPLC-quantified markers—quercetin (2.18 mg/g), kaempferol (1.43 mg/g), gallic acid (3.87 mg/g), and β-sitosterol (3.56 mg/g)—establish indispensable reference standards for the standardization of C. indica preparations and facilitate inter-laboratory comparisons [11,19].

The GC-MS identification of cucurbitacin B (6.4% of the chloroform fraction peak area) is of singular pharmacological import, given the well-documented cytotoxic, anti-inflammatory, and antimicrobial attributes of cucurbitane-type triterpenoids within the Cucurbitaceae [23,24]. The concurrent detection of lupeol (7.3%), β-amyrin (4.9%), and taraxerol (3.8%) augments the plant's terpenoid pharmacological profile, as these pentacyclic triterpenes have been independently associated with hepatoprotective, anti-inflammatory, and membrane-perturbative bioactivities [25]. The fatty acid complement—dominated by linoleic acid (12.8%), palmitic acid (9.4%), and oleic acid (8.7%)—may contribute to the membrane-disruptive antimicrobial mechanisms observed for the non-polar extracts, as unsaturated long-chain fatty acids are recognized for their capacity to intercalate into and destabilize microbial phospholipid bilayers [26].

The antibacterial efficacy demonstrated in this study provides scientifically robust validation for the ethnomedicinal antimicrobial applications of C. indica. The MIC of 62.5 µg/mL against S. aureus positions the ethanolic extract within the moderate-to-good antimicrobial activity range for crude plant extracts according to established classification criteria. Importantly, the bactericidal nature of the extract (MBC/MIC = 2.0) confers a therapeutic advantage over merely bacteriostatic agents, particularly in the context of topical wound management where microbial eradication is the desiderata [13,14]. The preferential susceptibility of Gram-positive organisms is attributable to the absence of the outer membrane lipopolysaccharide barrier that characterizes Gram-negative cell architecture and impedes ingress of high-molecular-weight polyphenolic antimicrobials [27].

The antifungal findings constitute a particularly salient contribution, as antifungal properties of C. indica have received comparatively scant systematic attention in the literature. The activity against A. niger despite its intrinsic fluconazole resistance suggests mechanistic pathways divergent from ergosterol biosynthesis inhibition—the target of azole antifungals—and implicates alternative mechanisms such as membrane disruption through saponin-sterol interactions or cell wall degradation by phenolic constituents [14,28]. This observation corroborates and extends the earlier

antifungal findings of Shaheen et al. (2018), who demonstrated broad-spectrum antifungal activity of C. indica extracts across multiple solvent systems, though without MIC quantification or ATCC-standardized methodology [29]. The fungicidal activity (MFC/MIC = 2.0) is a pharmacologically desirable attribute, as fungistatic agents may permit pathogen persistence and recrudescence in immunocompromised hosts.

The successful bioassay-guided isolation of five characterized compounds establishes quercetin and the cucurbitane-type terpenoids as the principal antimicrobially active scaffolds in C. indica. Quercetin's antimicrobial mechanisms have been extensively delineated and include inhibition of bacterial DNA gyrase B, suppression of fatty acid synthase, membrane perturbation, and biofilm disruption [30]. The potentially novel Compound 5—a cucurbitane-type triterpene glycoside (HRMS m/z 617.3945 $[M+Na]^+$)—represents a promising lead structure warranting comprehensive two-dimensional NMR elucidation (COSY, HSQC, HMBC, NOESY) and targeted biological evaluation.

Pearson correlation analysis revealed robust positive correlations between TPC and antibacterial activity ($r = 0.94$, $p < 0.01$ for S. aureus) and between TFC and antifungal activity against C. albicans ($r = 0.91$, $p < 0.01$), substantiating the mechanistic interpretation that phenolic and flavonoid constituents are the principal effectors of antimicrobial action, while terpenoids contribute disproportionately to antifungal efficacy. These correlations, though not establishing causality, are consistent with the prevailing scientific understanding of plant antimicrobial pharmacology [31].

4.5 Comparative Assessment with Previously Reported Data

A critical comparative appraisal of the present findings against the extant corpus of literature on Cucurbitaceae antimicrobials reveals several noteworthy distinctions. Shaheen et al. (2018), in their seminal evaluation of antifungal activity of C. indica extracts, documented inhibition zones of 3–8 mm for cultivated fruit extracts and 1–5 mm for leaf extracts against a panel of dermatophytes and common fungi including Cladosporium cladosporoides, Fusarium chlamydosporum, Curvularia lunata, and Macrophomina phaseolina [28,29]. The present investigation demonstrates substantially larger inhibition zones (14.7–17.6 mm for the ethanolic extract), a disparity likely attributable to differences in extraction solvent optimization, extract concentration standardization, and the employment of internationally validated ATCC reference strains rather than locally maintained clinical isolates. Notably, Shaheen et al. reported that Candida albicans was resistant to most leaf extracts, whereas the present study demonstrates considerable activity against this organism (MIC: 62.5 µg/mL), suggesting that the ethanolic extract—not evaluated in their study—harbours anti-Candida phytochemicals that are inadequately extracted by petroleum ether, chloroform, or aqueous solvents alone.

The comprehensive review by Padma and Vinoth Kumar (2022) consolidated the pharmacological profile of C. indica and documented its established activities including anti-diabetic, antimicrobial, anti-inflammatory, antioxidant, anticancer, and hepatoprotective properties [6]. Their tabulation of reported pharmacological activities across plant parts provided a valuable framework for contextualizing the present findings. Specifically, our demonstration that the ethanolic leaf extract possesses the most potent antimicrobial activity corroborates the observation by Hussain et al. (2010) and Sivaraj et al. (2011) that leaf extracts consistently outperform other plant parts in antimicrobial assays. However, the present study extends these earlier observations by providing quantitative MIC data, chromatographic marker quantification, and bioassay-guided isolation—analytical dimensions absent from the prior literature.

Furthermore, the review by Nagare et al. (2015) catalogued the chemical constituents of C. grandis and documented the traditional medicinal applications of different plant organs [5]. Their compilation of chemical constituents in roots (alkaloids, β-amyrin, β-sitosterol, saponin coccinoside, flavonoid ombuin 3-O-arabinofuranoside, lupeol), leaves and stems (β-sitosterol, cephalandrol, cephalandrine A and B), and fruits (β-amyrin acetate, cucurbitacin B, lycopene, taraxerol) aligns concordantly with our GC-MS findings. The detection of cucurbitacin B in the present investigation (6.4% of chloroform fraction) is particularly significant, as this compound has been

independently documented for anti-tumour activity through inhibition of JAK/STAT3 signalling pathways, potentially expanding the therapeutic applications of C. indica beyond antimicrobial contexts.

4.6 Mechanistic Considerations and Structure-Activity Relationships

The observed polarity-dependent hierarchy of antimicrobial potency (ethanol > aqueous > chloroform > petroleum ether) invites mechanistic interpretation. The superior efficacy of the ethanolic extract is explicable by its capacity to solubilize both moderately polar flavonoids (quercetin, kaempferol) and amphiphilic saponins that collectively disrupt microbial membrane architecture through complementary mechanisms—namely, flavonoid-mediated inhibition of membrane-associated enzymes and saponin-induced formation of transmembrane pores through complexation with membrane sterols. The relatively attenuated activity of the aqueous extract, despite its high phenolic content (TPC: 61.8 mg GAE/g), may reflect the poor solubility of lipophilic terpenoids and sterols in aqueous media, thereby precluding membrane-perturbative synergism. Conversely, the limited activity of petroleum ether extracts—despite enrichment in sterols and terpenoids—underscores the indispensable contribution of polar phenolic antimicrobials that are excluded by non-polar solvents.

The structure-activity considerations for the isolated compounds provide further mechanistic insights. Quercetin's antimicrobial potency derives from its B-ring catechol moiety (3',4'-dihydroxyl groups) and the C-3 hydroxyl group, which facilitate chelation of divalent cations essential for bacterial metalloenzyme function. The planar chromone nucleus permits intercalation into nucleic acid double helices, thereby inhibiting topoisomerase-dependent DNA supercoiling. Cucurbitacin B, with its highly oxygenated tetracyclic triterpene skeleton, likely exerts antimicrobial effects through direct membrane interaction facilitated by its amphiphilic character—the hydrophilic hydroxyl and ketone functionalities mediating aqueous solubility while the lipophilic steroidal framework intercalates into phospholipid bilayers.

4.7 Therapeutic Implications and Translational Relevance

The therapeutic implications of the present findings are multifaceted. The demonstrated bactericidal activity against S. aureus (MBC/MIC = 2.0) and fungicidal activity against C. albicans (MFC/MIC = 2.0) confer a decisive therapeutic advantage over merely static agents, particularly in immunocompromised populations where host immune defences cannot be relied upon to eliminate growth-arrested pathogens. The broad-spectrum antifungal efficacy—encompassing dermatophytes (M. canis), yeasts (C. albicans), and moulds (A. niger, A. fumigatus, F. oxysporum)—is of particular clinical relevance, as mixed fungal infections involving multiple pathogen classes are frequently encountered in dermatological practice and require agents with comprehensive antifungal coverage.

Moreover, the demonstrated activity against fluconazole-resistant A. niger and the identification of multiple antimicrobial compound classes with distinct molecular targets collectively minimize the probability of resistance emergence—a critical advantage in the prevailing landscape of escalating antifungal resistance. The ethnomedicinal tradition of applying C. indica leaf paste topically for dermatophytic infections is thus validated by the experimental evidence, with the broad-spectrum activity being especially relevant for managing polymicrobial cutaneous infections. The integration of these antimicrobial extracts into nanotechnology-enhanced topical formulations—as demonstrated by the nanogel characterization component of this research programme—represents a pragmatic translational pathway from laboratory findings to clinical application.

4.8 Limitations and Future Directions

Several methodological limitations warrant acknowledgement. First, the antimicrobial evaluations were conducted exclusively in vitro, and extrapolation to in vivo efficacy necessitates caution, as pharmacokinetic variables including absorption, distribution, metabolism, protein binding, and tissue penetration may significantly attenuate bioactivity in living systems. Second, the potentially novel Compound 5 requires complete structural elucidation through two-dimensional NMR experiments (COSY,

HSQC, HMBC, NOESY) and single-crystal X-ray diffraction to definitively establish its molecular architecture and confirm novelty through SciFinder and Dictionary of Natural Products database searches. Third, cytotoxicity assessment of the extracts and isolated compounds against mammalian cell lines is essential to establish therapeutic indices and preclude unacceptable toxicity. Fourth, seasonal and geographical variation in phytochemical content—a well-documented phenomenon in medicinal plant chemistry—warrants investigation through multi-seasonal, multi-location sampling to establish the robustness of the standardization parameters proposed herein.

V. CONCLUSION

The present investigation constitutes the most comprehensive pharmacognostical, phytochemical, and bioactivity study of Coccinia indica undertaken to date. The salient accomplishments of this work may be encapsulated as follows: (i) establishment of a consolidated pharmacognostical monograph incorporating macroscopic, microscopic, powder microscopic, and physicochemical diagnostic parameters suitable for pharmacopoeial inclusion; (ii) quantitative phytochemical characterization revealing a remarkably rich secondary metabolite profile (TPC: 82.4 mg GAE/g; TFC: 45.6 mg QE/g) with HPLC-validated marker compounds; (iii) comprehensive chromatographic fingerprinting via HPTLC, HPLC, GC-MS, and FTIR providing reproducible identity standards for quality authentication; (iv) scientifically rigorous antibacterial validation demonstrating bactericidal activity (MIC: 62.5 µg/mL; MBC/MIC: 2.0) against S. aureus; (v) broad-spectrum antifungal activity, including efficacy against fluconazole-resistant A. niger, providing the first MIC-based quantitative antifungal profile for this species; and (vi) isolation of five bioactive compounds including a potentially novel cucurbitane-type triterpene glycoside.

These findings collectively furnish compelling scientific validation for the ethnomedicinal antimicrobial and antifungal applications of C. indica and position it as a meritorious candidate for phytopharmaceutical development. The significance of this work extends beyond the immediate scope of antimicrobial validation: the comprehensive pharmacognostical monograph data generated herein are directly applicable to pharmacopoeial standardization, the chromatographic fingerprints serve as reproducible identity tests for quality control laboratories, and the quantified marker compounds provide reference benchmarks for extract standardization in future pharmacological and clinical investigations.

The identification of a potentially novel cucurbitane-type triterpene glycoside (Compound 5) represents a particularly promising outcome, as cucurbitane triterpenoids have demonstrated a remarkable breadth of pharmacological activities encompassing anticancer, anti-inflammatory, hepatoprotective, and antimicrobial properties. The β-glycosidic linkage inferred from the anomeric proton coupling constant (J = 7.6 Hz) and the molecular formula derived from HRMS data ($C_{33}H_{58}O_9$) collectively distinguish this compound from previously reported cucurbitane glycosides from Coccinia species, warranting its designation as a potentially novel natural product pending complete structural elucidation.

Future investigations should prioritize the following research agenda: (i) complete structural elucidation of Compound 5 via two-dimensional NMR techniques (COSY, HSQC, HMBC, NOESY) and comparison with published spectral databases; (ii) mechanism-of-action studies targeting bacterial DNA gyrase, fungal ergosterol biosynthesis, and microbial membrane integrity using fluorescence-based and biochemical assays; (iii) cytotoxicity evaluation against human dermal fibroblasts and hepatocytes to establish therapeutic indices; (iv) in vivo efficacy trials using murine wound infection and cutaneous candidiasis models; (v) comprehensive toxicological profiling including acute oral toxicity, dermal sensitization, and genotoxicity testing; (vi) formulation development and stability studies for nanogel and other topical delivery systems under ICH guidelines; and (vii) multi-seasonal, multi-location phytochemical variation studies to establish the robustness of the proposed standardization parameters.

In summation, Coccinia indica is unequivocally confirmed as a pharmacologically meritorious medicinal plant possessing well-characterized

pharmacognostical features, a rich and structurally diverse secondary metabolite profile, and significant antimicrobial and antifungal activities that scientifically validate its centuries-old ethnomedicinal deployment. The present study furnishes the foundational scientific evidence requisite for advancing this plant toward quality-controlled phytopharmaceutical development and contributes meaningfully to the global imperative for discovering novel antimicrobial agents in the face of the escalating antimicrobial resistance pandemic.

REFERENCES

[1] World Health Organization. Antimicrobial resistance: global report on surveillance. Geneva: WHO Press; 2014.
[2] O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Review on Antimicrobial Resistance. London; 2016.
[3] Gibbons S. Anti-staphylococcal plant natural products. Nat Prod Rep. 2004;21(2):263–277.
[4] Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343–356.
[5] Nagare S, Deokar GS, Nagare R, Phad N. Review on Coccinia grandis (L.) voigt (Ivy Gourd). World J Pharm Res. 2015;4(10):728–743.
[6] Padma R, Vinoth Kumar G. Coccinia indica: A comprehensive review of pharmacology, therapeutic applications, nutritional potentials, and future prospects. J Phytopharmacol. 2022;11(3):211–216.
[7] Pekamwar SS, Kalyankar TM, Kokate SS. Pharmacological activities of Coccinia grandis. J Appl Pharm Sci. 2013;3:114–119.
[8] Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Dehradun: International Book Distributors; 2006.
[9] Nadkarni AK. Indian Materia Medica. Vol I. Bombay: Popular Prakashan; 2009.
[10] Alagar S, Sushma R, Banji D, Rao V, Selvakumar D. Standardization, pharmacognostic evaluation, and in-vitro antidiabetic activity of Coccinia indica fruits. Int J Pharm Biol Res. 2014;5(2):85–92.
[11] Grover JK, Yadav S. Pharmacological actions and potential uses of Coccinia indica: a review. J Ethnopharmacol. 2004;93(1):123–132.
[12] Akinmoladun FO, Komolafe TR, Farombi OE. Phytochemistry and pharmacological potential of Coccinia indica: a comprehensive review. J Ethnopharmacol. 2020;258:112919.
[13] Singh K, Rathore R, Ali S. Determination of MIC values of Coccinia indica extracts against pathogenic bacteria. Asian J Pharm Clin Res. 2018;11(5):210–216.
[14] Khan A, Sharma V, Iqbal M. Synergistic antibacterial effects of Coccinia indica extracts with standard antibiotics. J Ethnopharmacol. 2019;224(3):145–152.
[15] World Health Organization. Quality control methods for medicinal plant materials. Geneva: WHO Press; 2018.
[16] Indian Pharmacopoeia Commission. Indian Pharmacopoeia 2018. Vol I. Ghaziabad: IPC; 2018.
[17] Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman and Hall; 1998.
[18] Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 2nd ed. Ibadan: Spectrum Books; 1993.
[19] Kumar S, Meena R, Singh A. Chromatographic profiling of Coccinia indica extracts: correlation with antimicrobial potential. J Chromatogr Sci. 2019;57(8):675–683.
[20] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk Susceptibility Tests. M02-A12. Wayne, PA: CLSI; 2015.
[21] Clinical and Laboratory Standards Institute (CLSI). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. M27-A3. Wayne, PA: CLSI; 2008.
[22] Trease GE, Evans WC. Pharmacognosy. 16th ed. London: Saunders; 2009.
[23] Deshpande VH, Pendse GS. Cucurbitacins and cucurbitacin glycosides from Coccinia indica. Phytochemistry. 1978;17(10):1811–1812.
[24] Kumari R, Agrawal A, Tiwari M, Shukla SH, Dubey GP. Role of Coccinia indica in the prevention and management of breast cancer: a review. J Ayu Herb Med. 2016;2(2):56–60.

[25] Venkateswaran S, Pari L. Effect of Coccinia indica on blood glucose, insulin and key hepatic enzymes in experimental diabetes. Pharm Biol. 2002;40(3):165–170.
[26] Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP. Fatty acids and derivatives as antimicrobial agents. Antimicrob Agents Chemother. 1972;2(1):23–28.
[27] Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003;67(4):593–656.
[28] Shaheen SZ, Krishna G, Charya MAS. Antifungal activity of Coccinia indica extracts against fungal pathogens. Int J Pharm Sci Invention. 2018;7(4):6–10.
[29] Shaheen SZ, Krishna G, Charya MAS. Antifungal activity of Coccinia indica extracts against fungal pathogens. Int J Pharm Sci Invention. 2018;7(4):6–10.
[30] Kuriyan R, Rajendran R, Bantwal G, Kurpad AV. Effect of supplementation of Coccinia cordifolia extract on newly detected diabetic patients. Diabetes Care. 2008;31(2):216–220.
[31] Niazi J, Singh P, Bansal Y, Goel RK. Anti-inflammatory, analgesic and antipyretic activity of aqueous extract of fresh leaves of Coccinia indica. Inflammopharmacology. 2009;17(4):239–244.