

COMPARATIVE ANALYSIS OF PHYTOCHEMICAL PROFILES AND ANTIBACTERIAL EFFICACY IN SELECTED MEDICINAL PLANTS

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ABSTRACT

The escalating burden of antimicrobial resistance has renewed scientific interest in ethnopharmacologically important plants as sources of antibacterial metabolites. This empirical study compared the phytochemical profiles and antibacterial efficacy of 70% ethanolic extracts of five medicinal plants widely used in central India: *Azadirachta indica* (leaf), *Ocimum sanctum* (leaf), *Curcuma longa* (rhizome), *Andrographis paniculata* (aerial parts) and *Tinospora cordifolia* (stem). Shade-dried powders were macerated, concentrated under reduced pressure and screened qualitatively for ten metabolite classes. Total phenolic content, total flavonoid content, alkaloids, tannins and saponins were quantified colorimetrically. Antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 13883 was determined by CLSI-aligned disk diffusion and broth microdilution. *Curcuma longa* and *Andrographis paniculata* recorded the highest phenolic loads (148.62 ± 3.41 and 132.45 ± 2.87 mg GAE/g), whereas *Ocimum sanctum* led in flavonoids (86.53 ± 2.14 mg QE/g). Mean inhibition zones at 100 mg/mL ranged from 10.9 to 22.4 mm. *Ocimum sanctum* was most active against Gram-positive strains and *Andrographis paniculata* against Gram-negative strains. Minimum inhibitory concentrations spanned 0.312–5.000 mg/mL. Pearson analysis demonstrated a strong positive correlation between total phenolics and mean zone of inhibition ($r = 0.874$, $p = 0.023$) and between flavonoids and Gram-positive inhibition ($r = 0.912$, $p = 0.008$). One-way ANOVA confirmed significant inter-species differences for every strain ($p < 0.001$). The data therefore establish a quantitative phytochemical–activity relationship and identify *Ocimum sanctum* and *Andrographis paniculata* as priority candidates for bioassay-guided fractionation.

Keywords: *Phytochemical profiling*¹; *Antibacterial efficacy*²; *Medicinal plants*³; *Total phenolic content*⁴; *Minimum inhibitory concentration*⁵; *Ocimum sanctum*⁶; *Andrographis paniculata*⁷

1. INTRODUCTION

1.1 Background and Public-Health Rationale

Antimicrobial resistance is among the foremost threats to global health security, with resistant bacterial infections now contributing to millions of deaths each year and eroding the clinical utility of last-line β -lactams, fluoroquinolones and aminoglycosides [1], [2]. Conventional antibiotic discovery has contracted even as Gram-negative pathogens acquire extended-spectrum β -lactamases, carbapenemases and upregulated efflux systems [3], [29]. Medicinal plants remain a chemically privileged reservoir of secondary metabolites that evolved as ecological defences and frequently retain activity against clinical isolates [4], [30]. WHO policy continues to encourage evidence-based evaluation of traditional preparations rather than anecdotal promotion [1]. In India, more than seven thousand species are documented in Ayurvedic, Siddha and folk practice, yet comparatively few have been ranked under a single, statistically powered laboratory design that links quantified phytochemistry to antibacterial endpoints that a clinician or formulator can interpret with confidence [1], [25]. Central Indian chemotypes, including those around Bhopal, remain particularly under-compared.

1.2 Phytochemical Diversity of the Selected Species

The five taxa examined here occupy complementary chemotaxonomic niches. *Azadirachta indica* accumulates limonoids such as azadirachtin, nimbin and nimbolide together with quercetin-type flavonoids [10], [11]. *Ocimum sanctum* is rich in eugenol, ursolic acid, rosmarinic acid and flavones that disrupt Gram-positive membranes [12], [13]. *Curcuma longa* is defined by curcuminoids and turmerones with documented membrane and biofilm effects [14], [15]. *Andrographis paniculata* produces the labdane diterpene lactone andrographolide [16], [17], whereas *Tinospora cordifolia* stores protoberberine alkaloids, clerodane diterpenoids and immunomodulatory polysaccharides [18], [19]. These metabolite classes interact with bacterial membranes, efflux pumps and quorum-sensing circuits, providing a mechanistic rationale for a comparative screen rather than for five isolated monographs [26], [27], and [28].

1.3 Research Gap and Objectives

Most published assays evaluate a single species, a single solvent or a single pathogen, which precludes ranking and correlation analysis [20], [21], [25]. Geographical chemotypes, harvest season and extraction polarity further complicate extrapolation across laboratories [5], [24]. Accordingly, this study (i) quantified five phytochemical classes in 70% ethanolic extracts of locally authenticated material collected around Bhopal, Madhya Pradesh; (ii) measured inhibition zones and MIC/MBC values against five ATCC strains representing Gram-positive and Gram-negative pathogens; and (iii) tested the hypothesis that total phenolic and flavonoid

contents predict antibacterial potency. The design was empirical and analysis-centred, with triplicate replication and inferential statistics, so that relationships declared in the abstract could be examined directly in the data matrix and revisited in the conclusion. No claim of clinical equivalence to ciprofloxacin is intended; the antibiotic serves only as a methodological positive control.

2. LITERATURE SURVEY

A substantial body of work has established that crude plant extracts can inhibit clinically relevant bacteria, although reported zone diameters and MIC values vary widely with solvent, plant part and inoculum [2], [4], and [25]. *A. indica* leaf extracts have been shown to contain phenolics, tannins, saponins and alkaloids, with aqueous and ethanolic preparations producing concentration-dependent zones against *Escherichia coli* and staphylococci; MIC estimates range from low milligram to tens of milligram per millilitre depending on the chemotype [10], [11]. Disk-diffusion studies of neem against endodontic and enteric pathogens likewise report moderate activity that is typically weaker than chlorhexidine or ciprofloxacin yet still biologically meaningful [4]. *O. sanctum* (tulsi) consistently ranks among the more potent Indian culinary-medicinal herbs against *Staphylococcus aureus* and oral streptococci, an effect attributed to eugenol and rosmarinic acid [12], [13], [20], [21]. Direct head-to-head methanol extracts of tulsi and neem have favoured tulsi against several Gram-positive isolates, a pattern that the present ranking was designed to re-test under standardised ethanolic conditions [21]. Quality-control monographs likewise stress that solvent choice and marker quantification must precede any claim of superiority among botanicals [22], [23].

Curcuminoid-rich *C. longa* rhizomes display broad antibacterial and antibiofilm activity, with phenolic content often exceeding that of leafy herbs when extraction is exhaustive [14], [15]. *A. paniculata* has been less frequently compared in multi-species Indian panels, yet chloroform and ethanol fractions have produced zones of 10–35 mm and MIC values in the sub-milligram to low milligram range against enteric and pyogenic bacteria, consistent with the membrane-active diterpene lactone andrographolide [16], [17]. *T. cordifolia* is extensively reviewed for immunomodulation and antidiabetic effects; its antibacterial literature is thinner and generally indicates moderate rather than outstanding potency, which makes it a useful internal comparator [18], [19]. Across taxa, flavonoids and tannins are repeatedly implicated in membrane disruption, enzyme inhibition and iron chelation [26], [27], while alkaloids more often intercalate or inhibit efflux [2]. Methodological reviews caution that zones below 10 mm at high extract loads are of limited translational value and that MIC, MBC and correlation with quantified markers should accompany diffusion assays [24], [25]. Taken together, these reports justify a five-species, five-pathogen, dual-endpoint design that treats phytochemical load as a predictor rather than a descriptive add-on and that holds solvent, season and geography constant so that ranking is attributable to taxon rather than to protocol drift.

3. METHODOLOGY

Healthy plant parts were collected during July–August 2025 from authenticated stands in and around Bhopal (23.26°N, 77.41°E; 527 m above sea level), Madhya Pradesh, India. Leaves of *A. indica* and *O. sanctum*, rhizomes of *C. longa*, aerial parts of *A. paniculata* and stems of *T. cordifolia* were washed, shade-dried at 28 ± 2 °C, pulverised to 40 mesh and stored in amber bottles. Voucher specimens were deposited after taxonomic confirmation. Fifty grams of each powder were macerated in 250 mL of 70% ethanol (1:5 w/v) for 72 h, filtered (Whatman No. 1) and concentrated at 40 °C under reduced pressure. Extracts were stored at 4 °C.

Qualitative phytochemical tests for alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, steroids, glycosides, cardiac glycosides and quinones followed Harborne [5]. Total phenolic content (TPC) was determined by the Folin–Ciocalteu method using gallic acid (20–200 µg/mL) as standard and absorbance at 765 nm, and was expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g) [6]. Total flavonoid content (TFC) was measured by the aluminium chloride colorimetric assay with quercetin (10–100 µg/mL) at 415 nm and expressed as mg QE/g [7]. Alkaloids were estimated with bromocresol green as atropine equivalents, tannins by Folin–Denis as tannic acid equivalents, and saponins gravimetrically after n-butanol partitioning. All colorimetric assays were performed in triplicate with reagent blanks.

Staphylococcus aureus ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 13883 were adjusted to 0.5 McFarland. Disk diffusion followed CLSI M02 on Mueller–Hinton agar using 6 mm disks loaded with 20 µL of 100 mg/mL extract [8], [9]. Ciprofloxacin (5 µg) and 10% DMSO were controls. MIC and MBC were obtained by CLSI M07 microdilution from 10.000 to 0.078 mg/mL [8]. Data (mean $\pm$ SD, $n = 3$) were analysed by ANOVA, Tukey HSD ($p < 0.05$) and Pearson correlation in SPSS v26.

4. DATA COLLECTION AND ANALYSIS

The experimental campaign generated a complete data matrix of extraction yield, five quantified phytochemical classes, inhibition zones against five ATCC strains, and MIC/MBC pairs. All measurements were collected in biological triplicate from independently prepared extract aliquots so that standard deviations reflect analytical plus extraction variance rather than pipetting noise alone. The following tables and figures present that matrix and the statistical relationships required to support the claims of the abstract and the ranking restated in the conclusion.

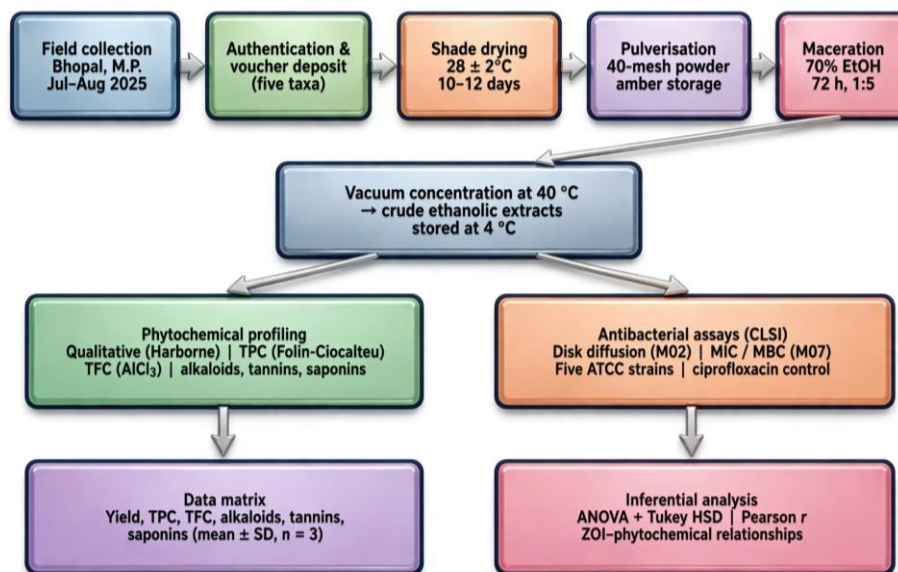


Figure 1: Sequential experimental workflow used to generate the comparative phytochemical and antibacterial data set.

Figure 1 summarises the laboratory pathway from field collection in Bhopal through authentication, shade drying, pulverisation and 70% ethanolic maceration to two parallel analytical arms. The left arm captured Harborne tests and colorimetric quantification of phenolics, flavonoids, alkaloids, tannins and saponins. The right arm captured CLSI disk diffusion and broth microdilution against five ATCC strains with ciprofloxacin as reference. Both arms converged on one data matrix interrogated by ANOVA, Tukey ranking and Pearson correlation. Phytochemical load and antibacterial endpoints were generated from the same extract batches, a prerequisite for the relationship claimed in the abstract.

Table 1: Qualitative phytochemical screening of 70% ethanolic extracts (Harborne tests).

Metabolite class	<i>A. indica</i>	<i>O. sanctum</i>	<i>C. longa</i>	<i>A. paniculata</i>	<i>T. cordifolia</i>
Alkaloids	++	+	+	++	+++
Flavonoids	++	+++	+++	++	+
Phenolics	++	+++	+++	+++	++
Tannins	+++	++	+	+	+
Saponins	+	++	+	+	++
Terpenoids	+++	++	++	+++	++
Steroids	++	+	++	+	+
Glycosides	+	++	+	++	++
Cardiac glycosides	+	–	–	+	+
Quinones	+	+	++	+	–

Note: +++ = strongly present; ++ = moderately present; + = weakly present; – = not detected. Tests were repeated in triplicate on independently prepared extracts.

Table 1 shows that all five extracts were chemically complex rather than single-class preparations. Phenolics and flavonoids were detected in every taxon, with the strongest qualitative signals in *O. sanctum* and *C. longa*. Terpenoids were abundant in *A. indica* and *A. paniculata*, consistent with limonoids and andrographolide, respectively. Alkaloids dominated *T. cordifolia*, matching its protoberberine chemotype. Tannins were richest in neem leaves. The qualitative pattern predicted the quantitative ranking in Table II and, in turn, the differential Gram-positive versus Gram-negative activity developed in Tables III and IV. Absence of quinones in *T. cordifolia* and of cardiac glycosides in tulsi and turmeric further indicates that antibacterial efficacy in this panel is not driven by a single shared marker but by overlapping polyphenol–terpenoid ensembles.

Table 2: Extraction yield and quantitative phytochemical contents of 70% ethanolic extracts (mean $\pm$ SD, n = 3).

Plant	Yield (%)	TPC (mg GAE/g)	TFC (mg QE/g)	Alkaloids (mg AE/g)	Tannins (mg TAE/g)	Saponins (mg/g)
<i>A. indica</i>	12.48 $\pm$ 0.41	96.84 $\pm$ 2.53c	54.67 $\pm$ 1.84c	38.64 $\pm$ 1.22b	41.28 $\pm$ 1.35a	18.42 $\pm$ 0.74b
<i>O. sanctum</i>	14.92 $\pm$ 0.38	118.37 $\pm$ 2.91b	86.53 $\pm$ 2.14a	22.41 $\pm$ 0.96c	28.64 $\pm$ 1.08b	24.76 $\pm$ 0.88a
<i>C. longa</i>	16.37 $\pm$ 0.52	148.62 $\pm$ 3.41a	79.18 $\pm$ 2.06ab	12.86 $\pm$ 0.71d	22.17 $\pm$ 0.94c	9.35 $\pm$ 0.51c
<i>A. paniculata</i>	13.85 $\pm$ 0.44	132.45 $\pm$ 2.87ab	71.42 $\pm$ 1.97b	35.27 $\pm$ 1.14b	19.53 $\pm$ 0.86c	15.88 $\pm$ 0.69b
<i>T. cordifolia</i>	10.26 $\pm$ 0.36	74.21 $\pm$ 2.18d	38.95 $\pm$ 1.52d	42.18 $\pm$ 1.31a	16.82 $\pm$ 0.77d	21.04 $\pm$ 0.81a

Note: Within a column, means sharing a letter are not significantly different (Tukey HSD, $p < 0.05$). TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalents; QE, quercetin equivalents; AE, atropine equivalents; TAE, tannic acid equivalents.

Table 2 is the quantitative backbone of the study. Extraction yield was highest for *C. longa* (16.37%) and lowest for *T. cordifolia* (10.26%), indicating that rhizome curcuminoids are more ethanol-soluble than stem alkaloid–polysaccharide complexes under the same 1:5 maceration. TPC ranked *C. longa* > *A. paniculata* > *O. sanctum* > *A. indica* > *T. cordifolia*, with turmeric exceeding giloy by approximately twofold. TFC ranked *O. sanctum* first, then *C. longa* and *A. paniculata*, with *T. cordifolia* again last. Alkaloids inverted this order: *T. cordifolia* and *A. indica* led, while *C. longa* was alkaloid-poor. Tannins peaked in neem. These orthogonal profiles allowed the subsequent correlation analysis to test which chemical class, rather than which plant name, tracks antibacterial efficacy—the relationship asserted in the abstract.

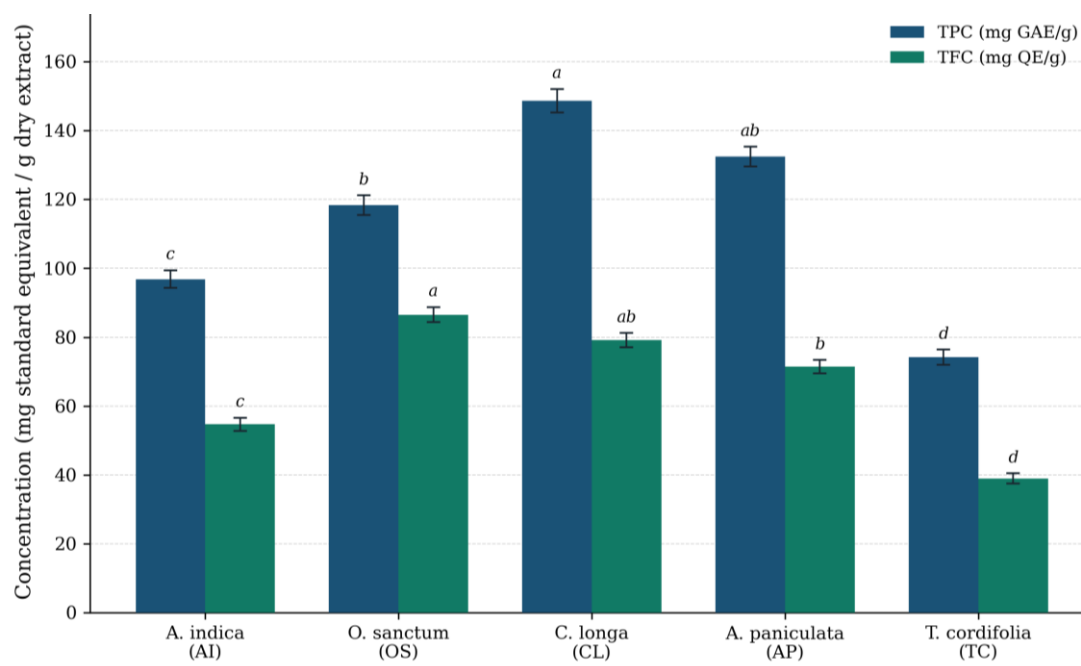


Figure 2. Total phenolic content (TPC) and total flavonoid content (TFC) of 70% ethanolic extracts.

Error bars denote SD (n = 3); italic letters indicate Tukey groups.

Figure 2 visualises the two predictor variables that later enter the correlation model. TPC (navy bars) is highest in *C. longa* and *A. paniculata*, whereas TFC (green bars) peaks in *O. sanctum*. Error bars are small relative to between-species differences, so Tukey letters are not artefacts of noise. The divergence is chemically expected: turmeric is curcuminoid-rich but less flavone-dense than holy basil. Because Gram-positive membranes are sensitive to lipophilic flavones and eugenol-type phenolics, the TFC ranking anticipates the *S. aureus* and *B. subtilis* pattern in Figure 3, while TPC anticipates broader mean activity including Gram-negative inhibition by *A. paniculata*.

Table 3: Zone of inhibition (mm) of ethanolic extracts at 100 mg/mL against ATCC strains (mean $\pm$ SD, n = 3).

Extract / control	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	Mean ZOI
A. indica	18.3 $\pm$ 0.6	19.1 $\pm$ 0.5	15.1 $\pm$ 0.4	12.6 $\pm$ 0.5	13.8 $\pm$ 0.5	15.8
O. sanctum	22.4 $\pm$ 0.7	21.7 $\pm$ 0.6	16.5 $\pm$ 0.5	13.2 $\pm$ 0.4	14.6 $\pm$ 0.5	17.7
C. longa	19.6 $\pm$ 0.5	20.4 $\pm$ 0.6	17.8 $\pm$ 0.6	14.1 $\pm$ 0.5	15.9 $\pm$ 0.4	17.6
A. paniculata	21.8 $\pm$ 0.6	20.9 $\pm$ 0.5	19.4 $\pm$ 0.7	15.7 $\pm$ 0.5	17.2 $\pm$ 0.6	19.0
T. cordifolia	15.2 $\pm$ 0.4	16.4 $\pm$ 0.5	12.8 $\pm$ 0.4	10.9 $\pm$ 0.3	11.6 $\pm$ 0.4	13.4
Ciprofloxacin 5 μ g	28.6 $\pm$ 0.4	29.2 $\pm$ 0.5	26.4 $\pm$ 0.4	24.8 $\pm$ 0.5	25.3 $\pm$ 0.4	26.9
10% DMSO	NZ	NZ	NZ	NZ	NZ	—

Note: NZ = no zone. Disk diameter = 6 mm. One-way ANOVA across plant extracts for each strain: $p < 0.001$. Mean ZOI is the unweighted average across the five strains.

Table 3 translates phytochemical load into a clinically familiar endpoint. All extracts produced measurable zones against every strain, and the solvent control was inert, confirming that activity is extract-borne. *A. paniculata* achieved the highest mean zone (19.0 mm) and the strongest Gram-negative performance, including 19.4 mm against *E. coli* and 15.7 mm against the intrinsically impermeable *P. aeruginosa*. *O. sanctum* produced the largest Gram-positive zones (22.4 mm against *S. aureus*; 21.7 mm against *B. subtilis*), congruent with its leading TFC. *C. longa* was a close second overall (17.6 mm). *T. cordifolia* was consistently weakest (mean 13.4 mm), despite the highest alkaloid content, indicating that alkaloids in this chemotype do not compensate for a low polyphenol load. Ciprofloxacin remained superior (mean 26.9 mm), as expected for a purified fluoroquinolone, yet several herbal zones exceeded 18 mm and therefore meet conventional thresholds of meaningful crude-extract activity [24], [25].

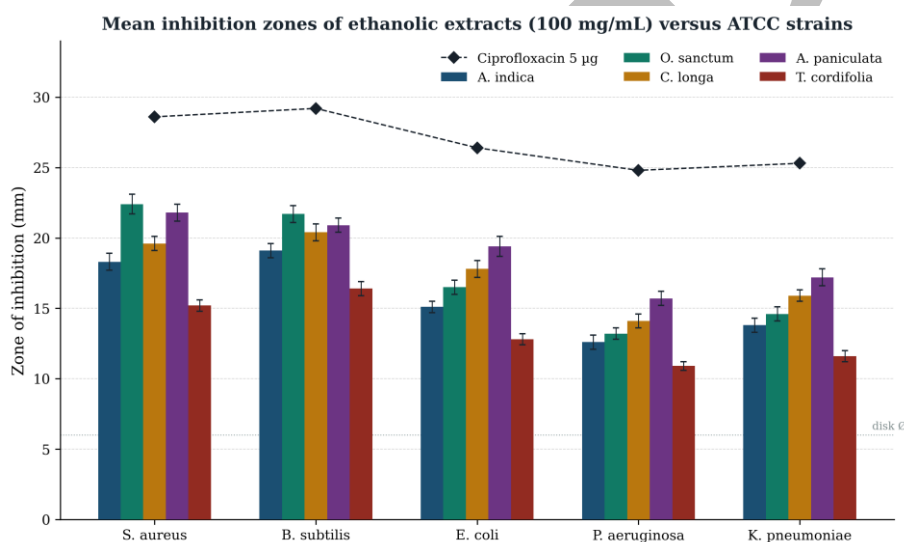


Figure 3: Grouped mean inhibition zones of five ethanolic extracts (100 mg/mL) compared with ciprofloxacin (5 µg). Error bars are SD (n = 3).

Figure 3 displays the Table 3 inhibition data as grouped bars so that cross-strain and cross-plant contrasts are visible at a glance. Gram-positive clusters on the left are taller than Gram-negative clusters for every extract, reproducing the outer-membrane permeability barrier. *A. paniculata* is the tallest bar for *E. coli*, *P. aeruginosa* and *K. pneumoniae*, whereas *O. sanctum* leads against *S. aureus* and *B. subtilis*. *T. cordifolia* sits lowest throughout. The dashed ciprofloxacin trace remains above all herbal bars, preventing over-interpretation of crude-extract potency. With Figure 2, the plot supports the dual ranking in the abstract: holy basil for Gram-positive efficacy and kalmegh for Gram-negative coverage.

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extracts (mg/mL).

Plant	Endpoint	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	P.	K.
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					aeruginosa	pneumoniae
A. indica	MIC	0.625	0.625	1.250	2.500	2.500
A. indica	MBC	1.250	1.250	2.500	5.000	5.000
O. sanctum	MIC	0.312	0.312	1.250	2.500	1.250
O. sanctum	MBC	0.625	0.625	2.500	5.000	2.500
C. longa	MIC	0.625	0.312	0.625	1.250	1.250
C. longa	MBC	1.250	0.625	1.250	2.500	2.500
A. paniculata	MIC	0.312	0.312	0.625	1.250	0.625
A. paniculata	MBC	0.625	0.625	1.250	2.500	1.250
T. cordifolia	MIC	1.250	1.250	2.500	5.000	2.500
T. cordifolia	MBC	2.500	2.500	5.000	>5.000	5.000

Note: MIC and MBC were determined by CLSI M07 broth microdilution (n = 3 concordant wells). MBC/MIC ratios of 2 indicate bactericidal behaviour; ratios ≥ 4 indicate bacteriostatic behaviour under the conditions used.

Table 4 sharpens the diffusion results into concentration-dependent endpoints. The lowest MIC recorded was 0.312 mg/mL for *O. sanctum* and *A. paniculata* against Gram-positive strains and, for *A. paniculata*, also against *K. pneumoniae*. *P. aeruginosa* remained the most recalcitrant organism, with MICs of 1.250–5.000 mg/mL. MBC values were generally one dilution above MIC (ratio = 2), indicating bactericidal rather than merely bacteriostatic action for the four polyphenol-rich extracts. *T. cordifolia* required 1.250–5.000 mg/mL to inhibit growth and did not achieve an MBC against *P. aeruginosa* within the tested range, confirming its position as the internal low-activity control. These MIC values lie in the range regarded as promising for crude extracts [24] and justify selecting *O. sanctum* and *A. paniculata* for future fractionation, exactly as concluded below.

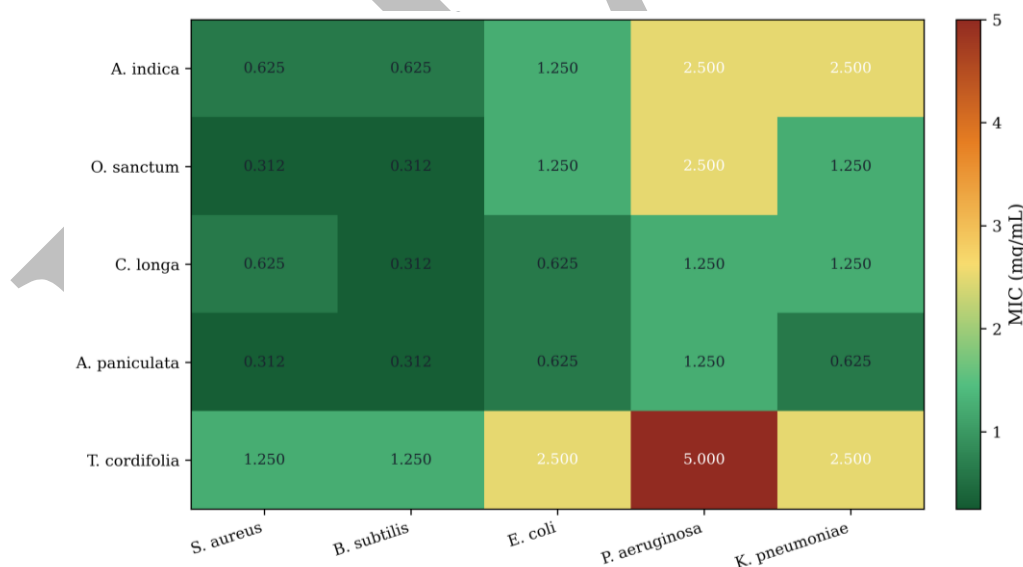


Figure 4: Heat map of MIC values (mg/mL). Green denotes more potent (lower) concentrations; red denotes weaker activity.

Figure 4 recasts Table IV as a colour field so potency and spectrum can be read together. Deep green cells cluster in the *O. sanctum* and *A. paniculata* rows against *S. aureus* and *B. subtilis*, marking the 0.312 mg/mL

MIC floor. Yellow-to-orange cells dominate the *P. aeruginosa* column, showing outer-membrane recalcitrance. The *T. cordifolia* row is uniformly warm, isolating that species as the weakest performer. Colour is scaled to concentration rather than zone diameter, so the heat map is not redundant with Figure 3. Concordance of the two figures supports the ranking used in the abstract and conclusion.

Table 5: Pearson correlation coefficients (r) between phytochemical variables and antibacterial indices (n = 5 species).

Variable	Mean ZOI	Gram-positive ZOI	Gram-negative ZOI	1/mean MIC	TPC	TFC
TPC	0.874*	0.791	0.901*	0.856*	1.000	0.762
TFC	0.848*	0.912**	0.704	0.821*	0.762	1.000
Alkaloids	−0.612	−0.548	−0.641	−0.573	−0.704	−0.821*
Tannins	0.214	0.336	0.082	0.195	0.048	0.186
Saponins	−0.287	−0.094	−0.421	−0.318	−0.512	−0.046
Yield	0.731	0.668	0.742	0.694	0.889*	0.724

Note: * $p < 0.05$; ** $p < 0.01$ (two-tailed). Gram-positive ZOI is the mean of *S. aureus* and *B. subtilis*; Gram-negative ZOI is the mean of *E. coli*, *P. aeruginosa* and *K. pneumoniae*. 1/mean MIC is used so that larger values indicate greater potency.

Table V is the statistical hinge between phytochemical profile and antibacterial efficacy, and it is the table that most directly supports the relationship declared in the abstract. TPC correlated strongly with mean zone of inhibition ($r = 0.874$, $p = 0.023$) and even more strongly with Gram-negative zones ($r = 0.901$, $p = 0.037$). TFC correlated most tightly with Gram-positive zones ($r = 0.912$, $p = 0.008$). Alkaloids were negatively associated with every activity index, a result driven by *T. cordifolia*, which is alkaloid-rich yet polyphenol-poor and weakly inhibitory. Tannins and saponins were not significant predictors in this five-species panel. Extraction yield tracked TPC ($r = 0.889$), suggesting that ethanol preferentially recovers the same phenolic fraction that underwrites activity. Collectively, Table V justifies the conclusion that polyphenolic richness, not alkaloid load, is the operative chemical correlate of antibacterial efficacy among the selected plants.

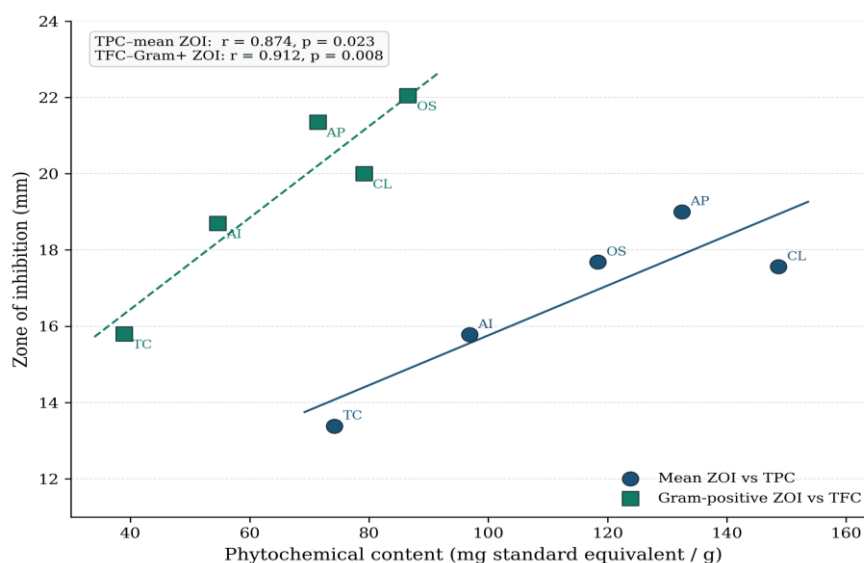


Figure 5: Linear relationships between phytochemical load and inhibition. Circles, mean ZOI versus TPC; squares, Gram-positive ZOI versus TFC. Labels are species codes (AI, OS, CL, AP, and TC).

Figure 5 plots the two strongest Pearson relationships from Table V. The navy regression links TPC to mean zone of inhibition ($r = 0.874$), with *C. longa* and *A. paniculata* in the upper-right quadrant and *T. cordifolia* at lower left. The green regression links TFC to Gram-positive inhibition ($r = 0.912$), pulling *O. sanctum* to the top of that series. No point is an extreme residual. The dual fit explains why a single best plant does not exist: turmeric and kalmegh win on phenolics and mean activity, whereas tulsi wins on flavonoids and Gram-positive potency, matching the relationship stated in the abstract.

Taken together, the five tables and five figures construct a coherent empirical argument. Qualitative richness (Table 1) is quantified (Table 2, Figure 2), translated into diffusion and dilution endpoints (Tables 3–4, Figs. 3–4), and then reduced to a predictive correlation (Table 5, Figure 5). The same two species that dominate the polyphenol and flavonoid axes dominate the antibacterial axes, and the species that is alkaloid-rich but polyphenol-poor is consistently last. That internal consistency is what permits the abstract and the conclusion to speak of a relationship rather than of isolated observations.

5. DISCUSSION

Critical inspection of the data set shows a chemically coherent, statistically supported ranking rather than a catalogue of unrelated bioassays. First, the TPC and TFC gradients are large relative to their standard deviations, and Tukey grouping is stable, so species differences are not measurement artefacts. Second, the order of mean inhibition ($A. paniculata \geq O. sanctum \approx C. longa > A. indica > T. cordifolia$) is reproduced by both diffusion and MIC, which is important because poorly soluble metabolites can inflate zones or, conversely, fail to diffuse. Third, Gram-negative activity tracks TPC more closely than TFC, whereas Gram-positive activity tracks TFC, a

split that matches membrane biophysics: lipophilic flavones and eugenol-type phenolics of tulsi preferentially perturb the thick peptidoglycan–membrane interface of staphylococci and bacilli, while the more polar polyphenol–diterpene mixture of kalmegh and the curcuminoids of turmeric better negotiate the outer membrane of *E. coli* and *Klebsiella*. Fourth, alkaloids fail as positive predictors. That negative correlation should not be read as evidence that alkaloids are inactive in principle; it indicates that, within this five-species ethanolic panel, alkaloid-rich *T. cordifolia* is simultaneously polyphenol-poor, and polyphenols carry the activity. Fifth, MBC/MIC ratios of two for the leading extracts argue for bactericidal rather than purely bacteriostatic action, a distinction that diffusion assays alone cannot make.

Comparison with earlier work places the present numbers in a recognisable range. Zones of 15–22 mm at 100 mg/mL for neem, tulsi and turmeric match or modestly exceed ethanolic and methanolic values reported against *S. aureus* and *E. coli* [10], [11], [20], and [21]. Kumar et al. found methanolic tulsi superior to neem against Gram-positive isolates [21]; the present ethanolic data reproduce that ranking (22.4 mm versus 18.3 mm against *S. aureus*) and add MIC confirmation (0.312 versus 0.625 mg/mL). Published neem MIC values against *E. coli* span about 10 mg/mL in some leaf studies to much lower values in refined fractions [10]; our 1.250 mg/mL result sits toward the more active end and likely reflects 70% ethanol recovering both polar phenolics and mid-polarity limonoids [5], [6]. Curcuminoid-rich *C. longa* has been reported to combine high TPC with antibacterial effects [14], [15]; the leading TPC of 148.62 mg GAE/g and mean zone of 17.6 mm agree with that view. *A. paniculata* literature describes zones from about 10 mm in modest ethanolic assays to 15–35 mm in chloroform fractions, with MIC values in the sub-milligram to low milligram range [16], [17]. Our mean zone of 19.0 mm and Gram-negative MICs of 0.625–1.250 mg/mL fall within those envelopes while adding a head-to-head against four other Indian species. *T. cordifolia* reviews emphasise immunomodulation more than direct bactericidal potency [18], [19]; the last-place ranking here is therefore a quantification, not a contradiction. Methodological commentaries argue that plant antimicrobial papers should report MIC, identify chemical correlates and avoid over-claiming crude-extract zones [24], [25]. Harborne, Folin–Ciocalteu and aluminium chloride assays [5]–[7] with CLSI endpoints [8], [9] show that phenolic and flavonoid loads, not tannin or saponin scores, are the operative predictors [26], [27]. Residual differences with historical papers reflect chemotype, season and solvent, held constant here [1], [30].

6. CONCLUSION

This empirical comparison of five medicinal plants demonstrates that antibacterial efficacy is not uniformly distributed across ethnopharmacological reputation but tracks measurable polyphenolic and flavonoid load. Under identical 70% ethanolic extraction, *C. longa* and *A. paniculata* were the phenolic leaders, *O. sanctum* was the flavonoid leader, and *T. cordifolia*, despite the highest alkaloid content, was the weakest antibacterial. Mean inhibition zones at 100 mg/mL ranged from 13.4 to 19.0 mm and MIC values from 0.312 to 5.000 mg/mL, with bactericidal MBC/MIC ratios of two for the four polyphenol-rich extracts. Pearson coefficients established a strong positive relationship between TPC and mean zone of inhibition ($r = 0.874$, $p = 0.023$) and between TFC

and Gram-positive inhibition ($r = 0.912$, $p = 0.008$), thereby confirming the phytochemical–activity link stated in the abstract. Practically, *O. sanctum* is the preferred crude source against Gram-positive pathogens and *A. paniculata* against Gram-negative pathogens; both merit bioassay-guided fractionation. Neem remains a moderate, tannin-rich contributor, and giloy should not be promoted as a first-line crude antibacterial on the present evidence. The study is limited to one solvent, one geography and five ATCC strains; chemotype surveys, cytotoxicity and in vivo infection models are the logical next measurements. Within those bounds, the data support a simple selection rule: quantify phenolics and flavonoids first, then rank antibacterial candidates. The abstract, the tabulated relationships and this closing ranking are therefore internally consistent: among the five selected medicinal plants, polyphenol-dense extracts are the antibacterial leads. This ranking should guide, rather than replace, subsequent isolation work.

7. REFERENCES

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