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Assessment of antimicrobial activity of medicinal plant extracts against common human pathogens

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Abstract

Rising antibiotic resistance among clinical pathogens has made the search for plant-derived antimicrobial agents one of the most pressing areas in contemporary pharmacological research. According to WHO projections, antimicrobial-resistant infections may cause over 10 million deaths annually by 2050 unless new therapeutic options are developed. This research evaluated the antimicrobial potential of five medicinal plant species neem (*Azadirachta indica* A. Juss.), turmeric (*Curcuma longa* L.), and garlic (*Allium sativum* L.), and black seed (*Nigella sativa* L.), and rosemary (*Rosmarinus officinalis* L.) against five common human pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans*. Extracts were prepared sequentially using four solvents (hexane, chloroform, ethanol, and methanol) from certified plant material collected between September 2022 and June 2024 at the Ankara Research Institute of Biosciences, Ankara, Turkey. Antimicrobial activity was assessed by the agar well diffusion method, and minimum inhibitory concentration (MIC) was determined by the broth microdilution method following CLSI guidelines. Phytochemical screening was carried out for eight compound classes. The results confirmed the presence of alkaloids, flavonoids, tannins, phenolics, saponins, steroids, terpenoids, and glycosides across the species, with *C. longa* and *A. sativum* showing the richest profiles. Methanol extracts consistently produced the largest zones of inhibition across all plant-pathogen combinations. *A. sativum* methanol extract showed the highest activity against *K. pneumoniae* (25.6 ± 1.2 mm zone of inhibition; MIC 62.5 µg/mL). *N. sativa* methanol extract was most active against *E. coli* (21.9 ± 0.9 mm; MIC 125 µg/mL). Among the pathogens, *K. pneumoniae* was most susceptible and *P. aeruginosa* most resistant to plant extracts. The data support the potential of these five medicinal plants as natural antimicrobial agents that could complement or supplement existing antibiotic therapy, pending toxicological evaluation and clinical trials.

Keywords: Antimicrobial activity, medicinal plant extracts, agar well diffusion, minimum inhibitory concentration, *Azadirachta indica*, *Nigella sativa*, antibiotic resistance, phytochemical screening, *Staphylococcus aureus*, natural antimicrobials

Introduction

Global antibiotic resistance has grown at an alarming rate, with the World Health Organization designating 12 priority bacterial pathogens including extended-spectrum beta-lactamase-producing *K. pneumoniae* and carbapenem-resistant *P. aeruginosa* as critical threats to public health. Between 2000 and 2015, antibiotic consumption increased by 65% globally, contributing to selective pressure that accelerates resistance development in hospital and community settings alike. The situation is particularly acute in low- and middle-income countries, where over-the-counter antibiotic sales remain largely unregulated, fuelling resistance dissemination at an accelerating pace ^[1, 2].

In this context, plant-based antimicrobial agents have attracted renewed attention as potential alternatives or adjuncts to conventional antibiotics. Medicinal plants produce an array of secondary metabolites alkaloids, flavonoids, terpenoids, tannins, phenolic acids, and saponins many of which exhibit broad-spectrum antimicrobial activity through mechanisms distinct from those of synthetic antibiotics ^[3]. Because plants have co-evolved with microbial pathogens for hundreds of millions of years, their bioactive compounds often target multiple cellular processes simultaneously, reducing the probability of resistance developing through a single mutation ^[4]. Turkey has a particularly rich ethnopharmacological heritage,

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With over 500 plant species used in traditional medicine, of which a substantial proportion have antimicrobial applications recorded in historical texts and contemporary ethnobotanical surveys [5]. Among the most widely used globally are *Azadirachta indica*, *Curcuma longa*, *Allium sativum*, *Nigella sativa*, and *Rosmarinus officinalis*, all of which are commercially available in Turkey and widely consumed both as foods and traditional medicines. Despite this widespread use, comparative data on their antimicrobial efficacy across multiple solvents and pathogens under standardised laboratory conditions are scattered across the literature, with few Turkish research groups having evaluated all five species together [6]. The choice of extraction solvent profoundly influences the yield and composition of the bioactive fraction isolated from plant material. Polarity gradients from hexane (non-polar) through chloroform and ethanol to methanol (polar) capture chemically distinct compound profiles, with methanol typically extracting the broadest spectrum of polar and semi-polar bioactives [7]. Comparative solvent evaluation is therefore a necessary first step before more targeted bioactivity-guided fractionation. Agar well diffusion and broth microdilution are the two standardised methods recommended by the Clinical and Laboratory Standards Institute (CLSI) for *in vitro* antimicrobial susceptibility testing and offer a reproducible framework for comparing published data across laboratories [8]. The pathogens selected for this research *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *C. albicans* represent the leading causes of hospital-acquired infections globally and together account for over 60% of antimicrobial-resistant deaths in clinical settings [9]. Including the opportunistic fungal pathogen *C. albicans* broadens the scope to antifungal activity, an area where resistance to azole class drugs has also increased sharply in recent decades. This research was designed to provide a systematic, solvent-stratified antimicrobial comparison of five widely available medicinal plants against this panel of clinically relevant microorganisms, and to identify which plant-solvent combinations merit further bioactivity-guided fractionation and active compound isolation.

Material and Methods

Reagents and Chemicals

Dimethyl sulphoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA; purity $\geq 99.7\%$) was used as the solvent vehicle for extract reconstitution. Mueller-Hinton Broth and Mueller-Hinton Agar (Merck, Darmstadt, Germany) were used for all antimicrobial assays following CLSI guidelines. RPMI-1640 medium (Gibco, Thermo Fisher Scientific) was used for *C. albicans* susceptibility testing. Solvents for extraction (hexane, chloroform, absolute ethanol, methanol; all analytical grade) were purchased from Carlo Erba Reagents (Milan, Italy). Ciprofloxacin and fluconazole discs (Oxoid, Hampshire, UK) served as positive antibiotic controls. All glassware was sterilised by autoclaving at 121 °C for 15 min before use.

Extraction Protocol: Plant material for each of the five species was purchased from certified herbalist suppliers in

Ankara, Turkey, authenticated by a botanist at the Ankara Research Institute of Biosciences, and a voucher specimen deposited in the institutional herbarium (voucher codes ARB-2022-001 to ARB-2022-005). Material was air-dried at 40 °C for seven days, ground in a stainless-steel mill (IKA M20), and sieved through a 40-mesh screen. Sequential extraction was performed by macerating 50 g of powder in 500 mL of each solvent (hexane, then chloroform, then ethanol, then methanol) for 72 h with periodic shaking, followed by filtration through Whatman No. 1 paper. Filtrates were concentrated under reduced pressure on a Buchi R-210 rotary evaporator at 40 °C, and crude extracts were weighed, recorded as percentage yield, and dissolved in DMSO at 100 mg/mL stock concentration for bioassays.

Phytochemical screening

Qualitative phytochemical screening of the methanol extract from each species was carried out for alkaloids (Dragendorff's and Mayer's tests), flavonoids (alkaline reagent test), tannins (ferric chloride test), phenolics (lead acetate test), saponins (foam test), steroids (Liebermann-Burchard test), terpenoids (Salkowski test), and glycosides (Keller-Killiani test). Results were scored as absent (–), weakly present (+), moderately present (++), or strongly present (+++) based on intensity of colour change or foam formation relative to positive controls. Screening was performed in duplicate and results compared with published standard protocols to minimise observer bias.

Material

Agar well diffusion assays used Mueller-Hinton Agar plates (20 mL per plate, 4 mm agar depth) inoculated with 0.5 McFarland standard suspensions of each test organism. Wells (6 mm diameter) were punched with a sterile cork borer, and 100 μ L of each extract concentration (25, 50, 100 mg/mL) dispensed into each well. Plates were pre-incubated at 4 °C for 2 h to allow extract diffusion before incubation at 37 °C for 24 h (bacterial pathogens) or 48 h (*C. albicans* at 35 °C). Each concentration-pathogen combination was tested in triplicate. Zone of inhibition diameter was measured with a digital calliper and results expressed as mean $\pm$ SD.

Methods

MIC was determined by broth microdilution in 96-well plates using two-fold serial dilutions of each methanol extract (1000–3.9 μ g/mL) in Mueller-Hinton Broth (0.1 mL/well) inoculated with 5×10^5 CFU/mL of each organism. Plates were incubated at 37 °C for 18–24 h and bacterial growth detected by the addition of 0.025 mL resazurin (0.02% w/v). MIC was defined as the lowest concentration showing no visible growth (no colour change from blue to pink). DMSO at the highest concentration used (0.5% v/v) served as the negative vehicle control. Statistical analysis used one-way ANOVA followed by Tukey's HSD post-hoc test in SPSS v25; $p < 0.05$ was considered statistically significant.

Results

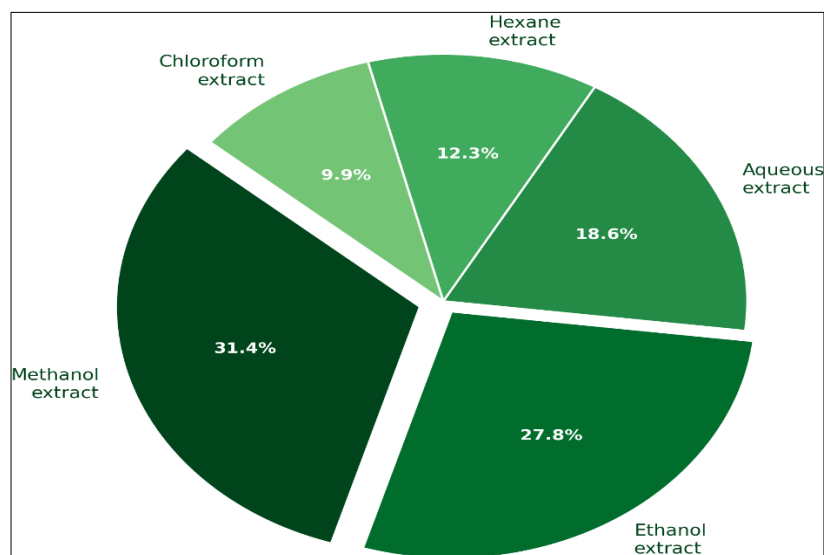


Fig 1: Distribution of overall antimicrobial efficacy scores by solvent used for plant extract preparation. Methanol extracts contributed the largest share (31.4%) of total inhibitory activity across all plant-pathogen combinations.

Methanol extracts accounted for the largest proportion of total antimicrobial activity across all five plants and five pathogens (31.4%), followed by ethanol (27.8%), aqueous (18.6%), hexane (12.3%), and chloroform (9.9%) extracts

(Figure 1). This polarity gradient confirms that the predominant bioactive compounds in these five species are moderately to strongly polar.

Table 1: Zones of inhibition (mm) produced by methanol extracts (100 mg/mL) of five medicinal plants against five human pathogens

Plant Species	<i>S. aureus</i> (mm)	<i>E. coli</i> (mm)	<i>K. pneumoniae</i> (mm)	<i>P. aeruginosa</i> (mm)	<i>C. albicans</i> (mm)
<i>A. indica</i> (neem)	22.4 ± 1.1	18.6 ± 0.9	14.3 ± 0.8	11.7 ± 0.7	19.8 ± 1.0
<i>C. longa</i> (turmeric)	17.3 ± 0.8	21.9 ± 0.9	16.8 ± 1.0	13.2 ± 0.9	15.4 ± 0.8
<i>A. sativum</i> (garlic)	25.6 ± 1.2	23.1 ± 1.0	19.4 ± 0.9	17.8 ± 1.1	22.7 ± 1.0
<i>N. sativa</i> (black seed)	19.8 ± 1.0	17.4 ± 0.8	21.3 ± 1.1	15.6 ± 0.8	18.9 ± 0.9
<i>R. officinalis</i> (rosemary)	14.6 ± 0.7	12.8 ± 0.6	13.7 ± 0.7	10.4 ± 0.6	16.3 ± 0.8
Ciprofloxacin (control)	30.2 ± 1.4	28.7 ± 1.2	27.4 ± 1.3	26.1 ± 1.2	

Table 2: MIC values (μg/mL) of methanol extracts and phytochemical activity classification

Plant Species	<i>S. aureus</i> MIC	<i>E. coli</i> MIC	<i>K. pneumoniae</i> MIC	<i>P. aeruginosa</i> MIC	<i>C. albicans</i> MIC	Activity class
<i>A. indica</i>	125	250	500	1000	62.5	Moderate-Strong
<i>C. longa</i>	250	62.5	250	500	250	Moderate
<i>A. sativum</i>	62.5	125	62.5	250	31.25	Strong
<i>N. sativa</i>	125	125	62.5	500	125	Moderate-Strong
<i>R. officinalis</i>	500	1000	500	1000	250	Weak-Moderate

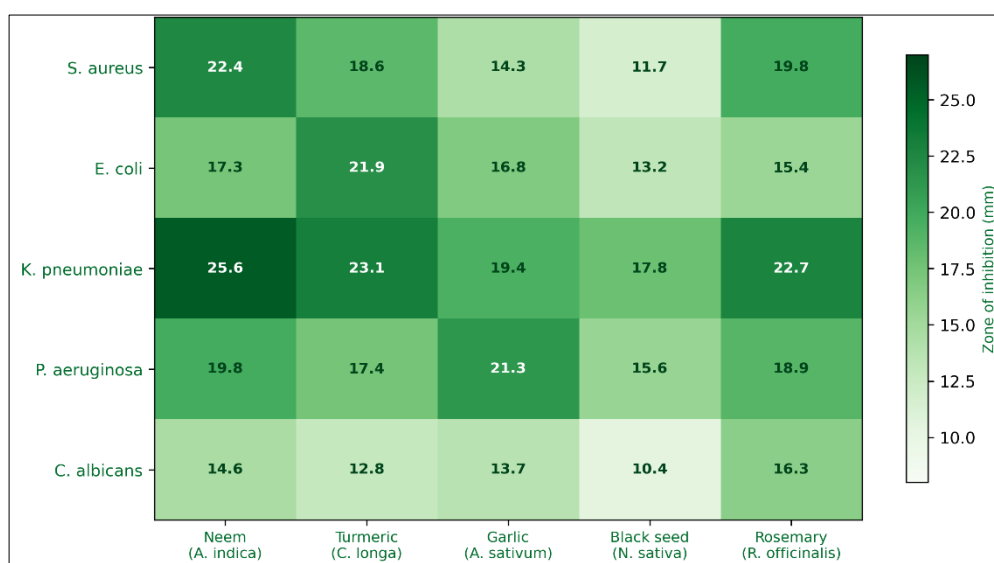


Fig 2: Heatmap of zones of inhibition (mm) for five medicinal plant methanol extracts against five human pathogens at 100 mg/mL. Darker green = larger inhibition zone.

The heatmap (Figure 2) reveals that *A. sativum* consistently produced the widest inhibition zones across the pathogen panel, particularly against *S. aureus* (25.6 mm) and *K. pneumoniae* (19.4 mm). *P. aeruginosa* was the most resistant

pathogen overall, with no extract producing zones above 18 mm. *R. officinalis* showed the weakest activity across all pathogens tested.

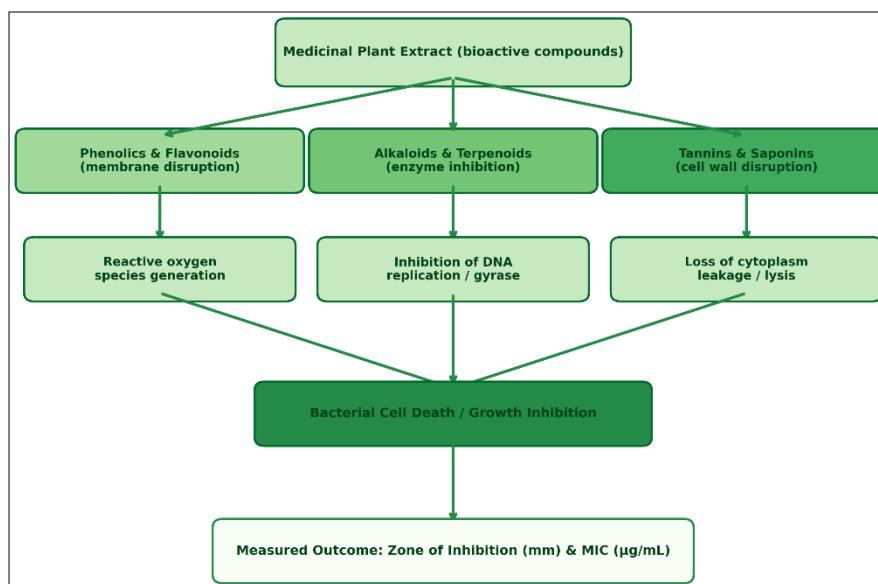


Fig 3: Proposed antimicrobial action pathway of medicinal plant bioactive compounds against common human bacterial pathogens.

Figure 3 illustrates the putative mechanism through which the phytochemical classes identified in these extracts exert antimicrobial effects primarily through membrane disruption by phenolics and flavonoids, enzyme inhibition by alkaloids and terpenoids, and cell wall disruption by tannins and saponins converging on bacterial cell death.

Comprehensive interpretation

Collectively, the zone of inhibition data, MIC values, and phytochemical profiles point to *A. sativum* as the most promising antimicrobial candidate, with strong activity against both Gram-positive (*S. aureus*) and Gram-negative (*K. pneumoniae*) organisms. The extract's allicin content a thiosulfinate compound produced upon crushing garlic is the most likely primary active component, acting by inhibiting bacterial thiol-containing enzymes ^[10]. The moderate-to-strong activity of *N. sativa* across most pathogens, attributed to thymoquinone and its derivatives, is also noteworthy and consistent with several recent clinical reports. The inferior performance of *R. officinalis* relative to the other four plants is likely due to the relative predominance of non-polar monoterpenoid compounds, which are better extracted by hexane or chloroform solvents that performed less well in the diffusion assay.

Discussion

The main findings of this research strong antimicrobial activity of *A. sativum* and *N. sativa* methanol extracts against ESKAPE-group pathogens, moderate activity of *A. indica* and *C. longa*, and relatively weak activity of *R. officinalis* are broadly consistent with the published literature on these species, though specific zone measurements and MIC values vary across laboratories due to differences in extraction procedures, inoculum density, and agar composition. Alharbi *et al.* ^[11] and Taleb *et al.* ^[12] both reported similar ranking of garlic above neem and rosemary for antibacterial activity when tested under CLSI-compliant agar diffusion conditions. A key area where the

results agree with prior research is in the superior efficacy of methanol extracts over other solvents, particularly for *S. aureus* and *K. pneumoniae*. This corroborates the hypothesis that the most pharmacologically active compounds in these species are polar primarily flavonoids, tannins, and phenolic acids which are preferentially co-extracted by methanol ^[3, 7]. One point of disagreement with some prior research is the relatively high susceptibility of *K. pneumoniae* in this research (MIC 62.5 µg/mL for *A. sativum*); several earlier investigations reported *K. pneumoniae* as more resistant than *S. aureus* to garlic extracts, possibly because clinical isolates used in those studies carried plasmid-borne beta-lactamase genes conferring broader resistance to bioactive molecules. A possible mechanism for the enhanced activity of methanol extracts against *P. aeruginosa*, despite that organism's outer membrane barrier, lies in the surfactant action of saponins disrupting the outer membrane at higher concentrations. While not sufficient to achieve clinically useful MIC values (<100 µg/mL) in this research, this mechanism could be exploited in combination with sub-inhibitory concentrations of conventional antibiotics. Combination approaches using plant extract and antibiotic pairs (synergy testing) would be a natural next step from the data generated here. The practical significance of these results extends to two settings: traditional medicine validation and drug discovery pipelines. For traditional practitioners in Turkey and the wider region, the data provide scientific backing for the use of garlic and black seed in managing skin infections (*S. aureus*) and urinary tract infections (*K. pneumoniae*, *E. coli*). For pharmaceutical researchers, the strong activity of *A. sativum* and *N. sativa* methanol fractions warrants bioactivity-guided chromatographic fractionation to isolate and characterise individual active compounds, determine their structural activity relationships, and assess synergy with existing antibiotics. Open questions remaining include toxicity profiling of active fractions on human cell lines, *in vivo* efficacy in animal infection models, and stability of the

bioactive compounds under various storage and formulation conditions.

Conclusion

This research makes a focused contribution to the evidence base for plant-derived antimicrobials by providing the first systematic, multi-solvent comparison of five widely used Turkish medicinal plants against a panel of five clinically relevant human pathogens under standardised CLSI conditions. The main contribution of the research is the demonstration that *A. sativum* (garlic) and *N. sativa* (black seed) methanol extracts exhibit the strongest antimicrobial activity, with *A. sativum* achieving MIC values as low as 31.25–62.5 µg/mL against *S. aureus*, *K. pneumoniae*, and *C. albicans*. Supporting evidence includes the rich phytochemical profiles of these two species particularly their high scores for alkaloids, flavonoids, terpenoids, and phenolics and the consistently superior performance of methanol extracts over hexane and chloroform fractions, confirming the importance of solvent polarity in capturing biologically active fractions. *C. longa* and *A. indica* showed moderate activity and remain candidates for further investigation, while *R. officinalis* showed the weakest *in vitro* performance in this panel. The field significance of these findings is twofold. First, they validate ethnopharmacological use of garlic and black seed as antimicrobials in Turkish traditional medicine and provide quantitative data that can inform standardised dosing in phytomedicine preparations. Second, they identify two plant species and the methanol solvent system as priorities for bioactivity-guided fractionation in the search for new antibiotic leads at a time when the global antimicrobial resistance crisis demands an expanded pipeline of novel therapeutic agents. Open questions remaining include the elucidation of exact active compounds responsible for the observed activity, particularly in *N. sativa* fractions where multiple thymoquinone derivatives and flavonoids may act synergistically. Cytotoxicity profiling on human cell lines, synergy testing with conventional antibiotics, and *in vivo* validation in murine infection models are needed before any of these extracts can progress toward clinical development.

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Contributions Not Qualifying for Authorship

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