TRENDS IN SCIENCES 2026; 23(12): 14169
https://doi.org/10.48048/tis.2026.14169 RESEARCH ARTICLE

Synergistic Antibacterial Activity of Polyherbal Combination of *Orthosiphon aristatus* (Blume) Miq., *Sonchus arvensis* L., and *Curcuma longa* L. Extracts against *Pseudomonas aeruginosa* Associated with Urinary Tract Infections

Beni Lestari[1,2], Mae Sri Hartati Wahyuningsih[1,2,*], Shofiya Turrizqi Nur'azizah[3] and Arin Cahyulis Abidin[3]

[1]*Department of Pharmacology and Therapy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[2]*Herbal Medicine Center, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[3]*Bachelor Program, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*

([*]Corresponding author's e-mail: maeshw@ugm.ac.id)

Received: 23 April 2026, Revised: 4 June 2026, Accepted: 14 June 2026, Published: 30 July 2026

Abstract

Urinary tract infections (UTI) remain one of the most prevalent infectious diseases globally and are closely linked to nephrolithiasis (kidney stones). These infections are typically caused by bacteria, which results in urinary flow obstruction. This growing concern over antibiotic failure has intensified the search for safe and effective phytotherapeutic alternatives. Thus, this study aimed to explore the antimicrobial properties of the leaves of *Orthosiphon aristatus* (Blume) Miq. (kumis kucing; OA) and *Sonchus arvensis* L. (tempuyung; SA) and the rhizome of turmeric (*Curcuma longa* L.; CL) against 5 microorganisms commonly implicated in UTI. Plant materials were extracted using ethanol and characterized via thin-layer chromatography. Antibacterial activities were tested by using the disc diffusion method at varying concentrations and combinations. Chromatographic analysis identified predictive key bioactive constituents, namely, sinensetin in OA, luteolin in SA, and curcumin in CL. Antibacterial assays against *Pseudomonas aeruginosa* showed that single extracts OA, SA, and CL possessed low antibacterial activity. Interestingly, the addition of CL significantly enhanced antibacterial activity. The 1:1:1 combination yielded the highest synergistic zone of inhibition (3.81 ± 0.52); however, it remained lower than that of chloramphenicol as a positive control. No activity was observed against *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, and *Candida albicans*. The findings provide a scientific basis for the traditional use of OA and SA in treating urinary tract complications while highlighting the significant role of CL in enhancing antibacterial potency related to *P. aeruginosa* infection.

Keywords: Antibacterial, *Orthosiphon aristatus* (kumis kucing), *Sonchus arvensis* (tempuyung), C*urcuma longa* (turmeric), Urinary tract infections (UTI)

Introduction

Urinary tract infection (UTI) remains one of the most prevalent infectious diseases globally, posing a significant challenge to public health systems with approximately 3 million medical encounters annually. The pathogenesis of UTIs is often exacerbated by the presence of nephrolithiasis (kidney stones), which can develop at any point along the urinary tract, from the renal parenchyma and ureters to the bladder and urethra [1-3]. Although small calculi may pass spontaneously, larger stones often become lodged in the narrow lumen of the ureter, causing acute urinary obstruction and frequently develop an infection [4]. Epidemiologically,

the highest incidence rates of these infections are observed among women and older men [5].

Among the pathogens implicated in complicated UTIs, *Escherichia coli* remains the primary etiological agent responsible for UTIs. In addition, a diverse range of secondary pathogens also contribute to clinical cases including *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Furthermore, the complex etiology of UTIs can extend beyond bacteria to include fungal pathogens (predominantly *Candida albicans*) [6,7]. *P. aeruginosa* accounts for approximately 7% - 10% of nosocomial UTI and is the third most common cause of catheter-associated UTI. As a Gram-negative bacterium, it has a high intrinsic resistance to multiple antibiotic classes, primarily owing to its sophisticated efflux pump systems and low outer membrane permeability [8]. Antibiotics remain the primary line of treatment; thus, clinicians must weigh factors such as comorbidities, disease severity, and the escalating risk of multidrug resistance [9]. This growing concern over antibiotic failure has intensified the search for safe and effective phytotherapeutic alternatives. To this end, natural bioactive compounds offer a promising avenue to attenuate *P. aeruginosa* pathogenesis by actively impede core bacterial processes, including toxin secretion, enzymatic activity, and matrix synthesis [10,11].

Kumis kucing (*Orthosiphon aristatus* (Blume) Miq.; OA) is an Indonesian medicinal plant rich in bioactive compounds, such as methoxylated flavonoids, tannins, saponins, and polyphenols [12,13]. Beyond its established diuretic and anti-inflammatory properties [14,15], OA has demonstrated potent antibacterial activity against various pathogens, such as *P. aeruginosa* [16]. Flavonoids and tannins are hypothesized to disrupt bacterial membrane integrity, inhibit essential metabolic enzymes, and suppress biofilm maturation [17-19]. Similarly, the leaves of *Sonchus arvensis* L. (tempuyung; SA) have a long-standing history in Indonesian ethnomedicine as a primary treatment for nephrolithiasis (kidney stones) [20]. A previous study validated its antilithiatic capabilities by evaluating the dissolution of renal calculi via radiographic imaging and the microscopic examination of urinary crystals. Its high content of flavonoids, coumarins, phenolic acids, and essential minerals, such as potassium and magnesium, are key in the disintegration of kidney stones [21]. Pioneering research by Sardjito at Universitas Gadjah Mada provided radiographic and crystallographic evidence that SA extracts can successfully facilitate the breakdown of renal calculi [22]. Nevertheless, *Curcuma longa* L. (CL) primarily known as an anti-inflammatory agent with curcuminoids as a major constituent. Curcumin demonstrates potent broad-spectrum antimicrobial activity. The compound obstructs bacterial replication through its specific structural configuration and subsequent production of antioxidant metabolites [23,24].

Based on those facts, extensive ethnopharmacological evidence supports the traditional use of *O. aristatus* and *S. arvensis* in managing urological problems, particularly due to their established diuretic and litholytic properties. Concurrently, curcumin has long been documented as a broad-spectrum anti-microbial agent. Although the potential of individual antibacterial properties of these plants are supported by their diverse secondary metabolite profiles, scientific data regarding their synergistic interactions with several microorganisms remain limited. Monotherapeutic antibacterial approaches often fail because a single plant matrix rarely possesses the diverse chemical architecture needed to simultaneously bypass the bacterium's outer membrane, disable its efflux pumps, and dismantle its biofilm shield. If only one plant is utilized, the pathogen can easily adapt via alternative resistance pathways, rendering the treatment ineffective. Therefore, this study aimed to evaluate the synergistic antibacterial properties of the leaves of OA and SA and the rhizome of CL individually and in various combinations. By characterizing the secondary metabolite profiles through thin-layer chromatography (TLC) and correlating them with the resulting zones of inhibition (ZOI), this study sought to establish a scientific foundation for safe, effective, and synergistic herbal therapy for patients with bacteria-related UTIs.

Materials and methods

Plant extract preparation

The plants used were the leaves of OA and SA and the rhizome of CL. Plants were identified by a plant taxonomist at the Pharmacology and Therapy Laboratory, Faculty of Medicine, Public Health, and Nursing, and the Pharmaceutical Biology Laboratory,

Gadjah Mada University, Indonesia. All dried plants were milled into fine powder using a grinding apparatus and subjected to extraction.

Each dried powder of the leaves of OA (30 g) and SA (27 g) and the rhizome of CL (67 g) was extracted by maceration with stirring for 24 h at room temperature using 1 L of 70% ethanol for each sample. The mixtures were filtered through a Buchner funnel, and the residues were re-macerated twice in the same conditions and then filtered. The filtrates were combined and evaporated using a rotatory evaporator to obtain a concentrated ethanol extract. The extraction yield was weighed to calculate the percentage yield. The crude extracts were stored in a dark vial for further analysis.

Microbial cultures

The microorganisms examined included *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 10145, *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Proteus mirabilis* (*P. mirabilis*; collection of the Faculty of Medicine, Universitas Gadjah Mada, isolated from patients with UTIs), *Escherichia coli* (*E. coli*) ATCC 25922, and *Candida albicans* (*C. albicans*) ATCC 10231. Stock cultures were prepared by cultivating the bacterium in Luria-Bertani (LB) broth at 37 °C for 18 - 24 h until reaching the mid-logarithmic phase.

Phytochemical screening

Preliminary phytochemical screening was conducted on the crude extract using TLC to identify the presence of major secondary metabolites. The extracts were dissolved in a mixture of chloroform and methanol ($CHCl_3$: MeOH, 1:1 v/v) to ensure complete solubilization of polar and nonpolar constituents. The Silica Gel GF_{254} served as a stationary phase, whereas the mobile phase consisted of a mixture of benzene and ethyl acetate (1:1 v/v). The developed TLC plates were visualized under UV lights at 254 and 366 nm. The reference markers were used as follow sinensetin for OA [25], luteolin for SA [26], and curcumin for CL [27]. The retention factor (Rf) value was calculated for each visible spot by the ratio of the distance traveled by the solute (compound) to the distance traveled by the solvent front.

***In vitro* antibacterial activity assessment**

Substances were screened for antibacterial activity using the disk diffusion method. Muller Hinton Agar (MHA) was used as the media. Briefly, 1 mL of bacterial inoculum was dispensed into a sterile 10-cm Petri dish and then added with autoclaved MHA. The plate was gently swirled to achieve uniform distribution of the bacteria within the medium and allowed to solidify under aseptic conditions. The samples were then incubated at 37 °C for 24 h. Plant extracts were prepared in dimethyl sulfoxide (DMSO). Sterile paper discs were placed onto the surface of the inoculated agar. Discs containing DMSO as a solvent were used as the negative control, whereas chloramphenicol (Sigma-Aldrich) served as the positive control. For the treated groups, sterile paper discs were impregnated with a predetermined volume of extract solution. The plates were subsequently incubated at 37 °C for 24 - 48 h before determining antibacterial activity. Antibacterial activity was evaluated by measuring the diameter of the clear zones from the edge of the well to the edge of the ZOI (in millimeters).

Statistical analysis

Data from the study of the inhibition zone diameter of *P. aeruginosa* are presented as mean ± SD. Data were tested using one-way analysis of variance, followed by Dunnett's multiple-comparison test using Graph Pad Prism software. Asterisks denote statistically significant at **** $p \leq 0.0001$, *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$.

Ethical approval

The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, under the Ethical Clearance Number: KE/FK/0595/EC. All procedures were performed in strict accordance with the established ethical guidelines for research.

Results and discussion

Yield rendement of extracts

The extraction of 500 g of dried plant materials from the leaves of OA and SA and rhizome of CL using 70% ethanol yielded 30, 27, and 67 g of concentrated crude extract after solvent evaporation, respectively. As shown in **Table 1**, this extraction process resulted in a yield of 6% (w/w) for OA, 5.4% (w/w) for SA, and 13.4% (w/w) for CL.

Table 1 Yield percentage of ethanolic extract of plants used in the study.

Plants name	Solvent	Weight of extract (g)	Extract yield (%)
Orthosiphon aristatus (the leaves)	Ethanol	30	6
Sonchus arvensis (the leaves)	Ethanol	27	5.4
Curcuma longa (the rhizomes)	Ethanol	67	13.4

Phytochemical component of extracts

To validate the chemical baseline of the crude ethanolic extracts, qualitative colorimetric screening was conducted alongside TLC profiling. The screening confirmed that the presence of diverse secondary metabolites in the 3 extracts, displaying several colorful fluorescence spots under UV 366 nm and quenching spots under UV 254 nm at different Rf values as shown in **Figure 1** and **Table 2**. The target bioactive markers were identified clearly, specifically sinensetin for *O. aristatus*, luteolin for *S. arvensis*, and curcumin for *C. longa*. Chromatographic analysis of the OA leaf extract revealed 9 different spots characterized by bright-blue to pink fluorescence under UV 366 (**Figure 1, left panel**). The bright-blue fluorescence is a hallmark of sinensetin or polymethoxyflavonoid derivatives which appeared as a clear quenching spot and transforms into vibrant blue fluorescent at Rf 0.36. These observations are consistent with standard chromatography profiles demonstrating blue fluorescence at UV 366 nm, as reported by several previous studies [28-30]. Analysis of SA extract yielded 6 distinct spots under UV 366 nm characterized primarily by pink, green, and bluish fluorescence (**Figure 1, center panel**). The reference flavonoid luteolin exhibited yellow-green fluorescent under UV 366 nm at Rf 0.26, which aligns with established chromatographic standards in Farmakope Herbal Indonesia and other studies [26,31]. A prominent yellow spot was found in CL under UV 366 nm, with the most intense fluorescence appearing at an Rf value of 0.81 (**Figure 1, right panel**). This yellow fluorescence confirms the presence of major active constituents, curcumin, which serve as a vital bioactive marker for this species [27,32].

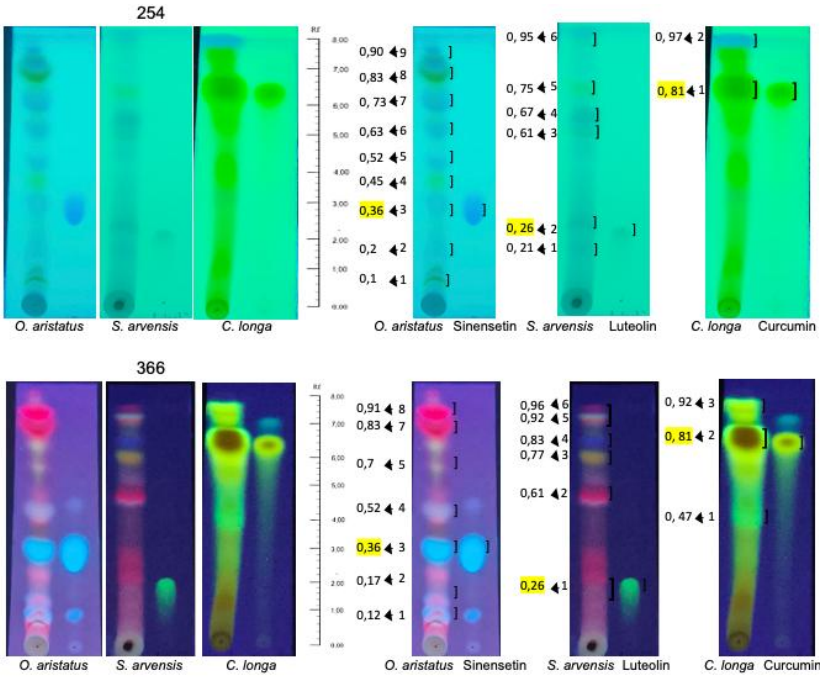


Figure 1 Thin Layer Chromatography (TLC) Profile of ethanolic extract of *O. aristatus* (OA), *S. arvensis* (SA), and *C. longa* (CL) extracts. The chromatogram displays the separation of secondary metabolites from 3 extracts with stationary phase: Silica gel GF_{254} and mobile phase consisted of a mixture of benzene and ethyl acetate (1:1 v/v) under UV 254 and 366 nm. The reference markers were used as follow sinensetin for OA, luteolin for SA, and curcumin for CL.

Table 2 Thin Layer Chromatography (TLC) Profile of ethanolic extract of *O. aristatus* (OA), *S. arvensis* (SA), and *C. longa* (CL) extracts with Retention Factor (Rf) values.

No	Plants name	Observation	Colour	Calculated Rf value (sample)	Standard	Calculated Rf value (standard)
1.	*Orthosiphon aristatus* (the leaves)	254 nm	Yellowish green	0.1	**Sinensetin**	**0.36**
			Pale Blue	0.2		
			Pale Blue	**0.36**		
			Yellowish green	0.45		
			Pale Blue	0.52		
			Pale Blue	0.63		
			Pale Blue	0.73		
			Yellowish green	0.83		
			Pale Blue	0.90		
		366 nm	Bright blue	0.12	**Sinenstin**	**0.36**
			Bright blue	0.17		
			Bright blue	**0.36**		
			Bright blue	0.52		
			Pink	0.7		
			Pink	0.83		
			Pink	0.91		
2.	*Sonchus arvensis* (the leaves)	254 nm	Faint dark	0.21	**Luteolin**	**0.26**
			Faint dark	**0.26**		
			Faint dark	0.61		
			Pale blue	0.67		
			Yellowish green	0.75		
			Faint dark	0.95		
		366 nm	**Green**	**0.26**	**Luteolin**	**0.26**
			Pink	0.61		
			Yellow	0.77		
			Blue	0.83		
			Orange	0.92		
			Pink	0.96		
3.	*Curcuma longa* (the rhizomes)	254 nm	**Deep dark**	**0.81**	**Curcumin**	**0.81**
			Bluish dark	0.97		
		366 nm	Yellowish green	0.47	**Curcumin**	**0.81**
			Bright yellow	**0.81**		
			Yellowish green	0.92		

Antibacterial activity of single extract

The individual antibacterial activities of these single extracts were measured by the diameter of the ZOI against 5 microorganisms commonly implicated in UTI: *P. aeruginosa*, *S. aureus*, *P. mirabilis*, *E. coli*, and *C. albicans*. As shown in **Figure 2**, all extracts were unable to inhibit the growth of *S. aureus* (**Figure 2(A)**), *P. mirabilis* (**Figure 2(B)**), *E. coli* (**Figure 2(C)**), and *C. albicans* (**Figure 2(D)**). Interestingly, against *P. aeruginosa*, the extracts revealed measurable inhibition (**Figure 3**). This method utilized DMSO as the negative control and chloramphenicol as the positive control, with a total incubation time of 48 h. No antimicrobial activity was shown with DMSO. The positive control (chloramphenicol) demonstrated an ZOI of 9.07 ± 1.81 mm, which was significant when compared with other treatments. Among the single extracts at a concentration of 75% (w/v), CL and SA exhibited similar potency with average ZOIs of 1.49 ± 1.65 and 1.61 ± 1.05, respectively (**Table 3**), whereas 75% (w/v) OA showed lower ZOI values of 0.75 ± 1.03.

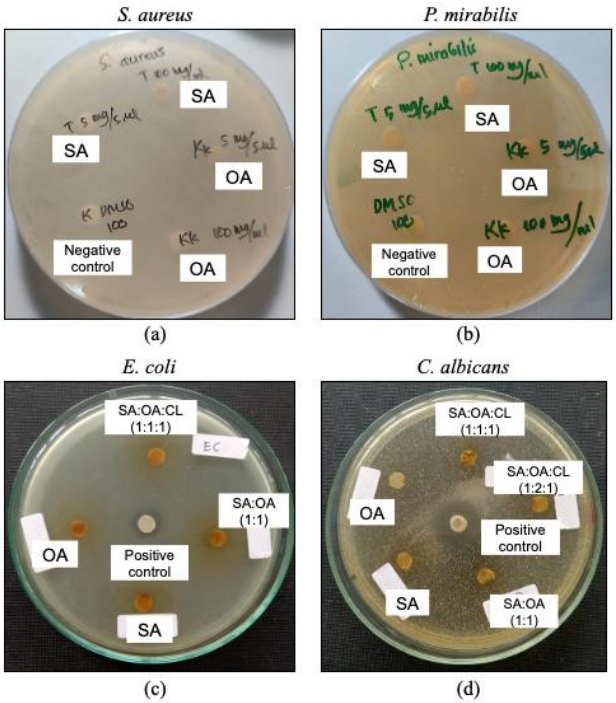


Figure 2 The representative images of disc diffusion assay of *O. aristatus* (OA), *S. arvensis* (SA), and *C. longa* (CL) extracts against *S. aureus* (A), *P. mirabilis* (B), *E. coli* (C), and the fungus *C. albicans* (D). The images display the zones of inhibitions (ZOI).

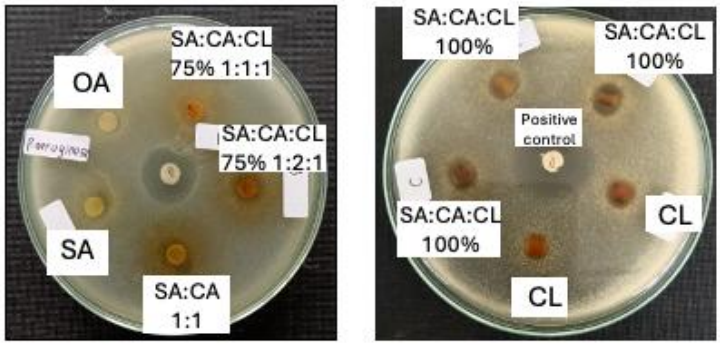


Figure 3 The representative images of disc diffusion assay evaluating antibacterial activity of *O. aristatus* (OA), *S. arvensis* (SA), and *C. longa* (CL) extracts against *P. aeruginosa* in different concentration, combination, and ratio. The images display the zones of inhibitions (ZOI).

Table 3 The Zone of Inhibitions (ZOI) of different plant extracts against *P. aeruginosa* tested strain by disc diffusion method.

Code	Samples Group	Zone of Inhibition (ZOI) Diameter (mm) ± SD
	Positive control (chloramphenicol)	9.07 ± 1.81
A	Negative control (DMSO)	0
B	SA 75%	1.61 ± 1.05
C	OA 75%	0.75 ± 1.03
D	CL 75%	1.49 ± 1.45
E	SA 75%: OA 75% (1:1)	1.91 ± 0.93
F	SA 100%: OA 100% (1:1)	1.7 ± 1.57
G	SA 75%: OA 75%: CL 75% (1:1:1)	3.81 ± 0.52
H	SA 100%: OA 100%: CL 100% (1:1:1)	2.85 ± 1.22
I	SA 75%: OA 75%: CL 75% (1:2:1)	3.19 ± 0.48
J	SA 100%: OA 100%: CL 100% (1:2:1)	2.09 ± 0.26

Antibacterial activity of combined extracts

Based on **Figure 3** and **Table 3**, an increase in antibacterial activity was observed when the 3 extracts were combined. Interestingly, increasing the ratio of OA (**Figure 4**, groups I and J) or using 100% concentrations (**Figure 4**, group H) did not result in a higher ZOI than using 75%. The most effective herbal formulation was the triple combination (75% SA - 75% OA - 75% CL, ratio 1:1:1) in group G, which reached a ZOI of 3.81 ± 0.52 (**Figure 4**, group G). OA + SA showed an interesting additive effect (**Figure 4**, groups E and F), though it is not potent as the triple combination that includes CL. Collectively, these results were consistent with the data from a single extract, where the addition of turmeric is essential to maximize the inhibitory response.

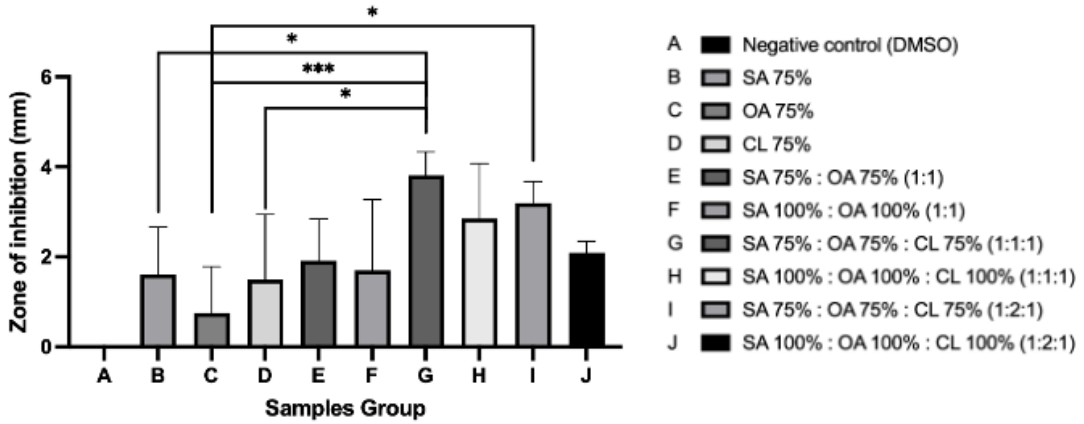


Figure 4 The antibacterial activity of single and combined extracts against *P. aeruginosa*. The bar graph represents the mean of Zone of Inhibitions (ZOI) (mm) ± standard deviation (SD) for each treatment group after 48 h of incubation (n = 5). Asterisks denote statistically significant at *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

Discussion

This study evaluated the antibacterial effect of a single extract of OA, SA, and CL and the combination against microorganisms commonly implicated in UTI. *P. aeruginosa* is the third most common cause of catheter-associated UTIs and is clinically serious with high rates of biofilm formation, multidrug resistance, and intracellular persistence.

Generally, the single treatment of 3 extracts: The leaves of OA and SA and the rhizome of CL successfully produced ZOI against *P. aeruginosa*. The antibacterial data obtained from the double and triple treatments showed better effects than single treatment, in line with the results of previous study showing that this synergism between bioactive compounds derived from diverse herbal sources significantly amplifies antimicrobial efficacy [33]. Despite the observed synergy, all herbal treatments remained significantly less potent than chloramphenicol (9.07 ± 1.81 mm). This disparity is likely due to the complex defense mechanisms of *P. aeruginosa*. As a Gram-negative bacterium, it possesses a highly selective outer membrane and active efflux pump systems that expel foreign compounds before they reach toxic levels [34]. Furthermore, the total lack of activity against *S. aureus*, *E. coli*, *P. mirabilis*, and *C. albicans* suggests that the current extract concentrations (75% - 100%) are below the minimum inhibitory concentration for these strains.

Several studies have attributed the antimicrobial potency of botanical extracts to a high concentration of phenolic compounds [18,35-38]. Specifically, flavonoids exhibit diverse and extensive antibacterial activity, although their efficacy varies depending on the specific microorganism and its ecological niche [39,40]. The TLC analysis (**Figure 1**) successfully identified key metabolic "fingerprints" that correlate with the antibacterial results. The intense red fluorescent spots (Rf 0.36) in OA indicate high concentrations of sinensetin. A previous study demonstrated that sinensetin, a polymethoxylated flavone, exhibited significant antimicrobial potential against Gram-negative bacteria, including *E. coli*, *P. aeruginosa*, *P. vulgaris*, and *S. aureus* [34]. Similarly, the TLC baseline for *S. arvensis* confirmed the presence of luteolin at Rf 0.26. Prior literature establishes that *Sonchus* family are exceptionally rich in luteolin and apigenin derivatives which correlate with antimicrobial properties [41]. *S. oleraceus* and *S. arvensis* often show stronger activity among *Sonchus* family [42]. Interestingly, the addition of CL resulted in a significant increase in antibacterial activity both in single and combination treatments. Based on the results of the TLC analysis, the prominent yellow spot in CL confirms the presence of curcuminoids. Prior studies have documented that curcumin is highly potent against *P. aeruginosa* with MICs ranging from 2.5 mg/mL to 5 mg/mL. Sethupathy *et al.* [43] demonstrated that curcumin downregulates key antioxidant enzymes, induces oxidative stress, and blocks the production of quorum sensing (QS) communication in *P. aeruginosa*. Supporting this data, several studies classified curcumin as a competitive QS antagonist and inhibit biofilm development [44-47].

While these 2 herbs, *O. aristatus* and *S. arvensis*, possess long-standing clinical evidence for treating nephrological conditions especially managing kidney stones and urinary flow, their direct bactericidal activity against resistant strains is often limited. The addition of curcumin serves as a crucial pharmacological upgrade with antibacterial properties. By combining these 3 agents, the therapeutic objective becomes multifaceted: it simultaneously promotes diuresis, aids in stone dissolution, and prevents pathogen colonization, thereby offering a superior polyherbal strategy for complicated, stone-associated urinary tract infections. Although these formulations are not intended to replace for acute antibiotic therapy, such as chloramphenicol, they mays serve as promising supportive or preventive options for UTIs complicated by nephrolithiasis. Future studies should determine the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and antibiofilm capacity to further validate their clinical application against resistant uropathogens.

Conclusions

This study confirms that the ethanolic extracts of OA, SA, and CL exhibit selective antibacterial activity against *P. aeruginosa*. These findings provide a scientific basis for their traditional use and highlight the role of CL as a potent antibacterial enhancer. The results validate the superiority of a polyherbal approach, with the 1:1:1 combination of OA:SA:CL demonstrating significant synergism. This enhanced efficacy is attributed to the presence of sinensetin, luteolin, and curcumin, which likely target bacterial defenses more effectively than single extracts.

Acknowledgements

The authors would like to express their sincere gratitude to Mrs. Minarni and Mrs. Widyati Prihatiningtyas for their invaluable technical assistance in the preparation of the plant extracts and the management of the bacterial cultures. The authors

would like to thank the scientific publication and journal section of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Indonesia for their assistance in English language editing through Enago (https://www.enago.com).

This research was funded by The DAMAS Research Grant of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Indonesia with contract number 1464/UN1/FKKMK/PPKE/PT/2024.

Declaration of generative AI in scientific writing

During the preparation of this manuscript, artificial intelligence tools (ChatGPT and Gemini AI) were used for translation and grammar improvement purposes. The manuscript has undergone professional language editing by Enago proofreading services, and no content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

CRediT author statement

Beni Lestari: Writing-original draft, Methodology, Investigation, Data curation, Visualization, Formal analysis, Conceptualization. **Mae Sri Hartati Wahyuningsih:** Methodology, Investigation, Resources, Supervison, Funding acquisition, Conceptualization. **Shofiya Turrizqi Nur'azizah:** Investigation. **Arin Cahyulis Abidin**: Investigation.

References

[1] E Rando, F Giovannenze, R Murri and E Sacco. A review of recent advances in the treatment of adults with complicated urinary tract infection. *Expert Review of Clinical Pharmacology* 2022; **15(9)**, 1053-1066.

[2] A Razi, A Ghiaei, FK Dolatabadi and R Higuchi. Unraveling the association of bacteria and urinary stones in patients with urolithiasis: An update review article. *Frontiers in Medicine* 2024; **11**, 1401808.

[3] J Josephs-Spaulding, TJ Krogh, HC Rettig, M Lyng, M Chkonia, S Waschina, S Graspeuntner, J Rupp, J Møller-Jensen and C Kaleta. Recurrent urinary tract infections: Unraveling the complicated environment of uncomplicated rUTIs. *Frontiers in Cellular and Infection Microbiology* 2021; **11**, 562525.

[4] JC Nickel. Management of urinary tract infections: Historical perspective and current strategies: Part 2--Modern management. *The Journal of Urology* 2005; **173(1)**, 27-32.

[5] Y He, J Zhao, L Wang, C Han, R Yan, P Zhu, T Qian, S Yu, X Zhu and W He. Epidemiological trends and predictions of urinary tract infections in the Global Burden of Disease Study 2021. *Scientific Reports* 2025; **15(1)**, 4702.

[6] N Sathe, P Beech, L Croft, C Suphioglu, A Kapat and E Athan. *Pseudomonas aeruginosa*: Infections and novel approaches to treatment. *Infectious Medicine* 2023; **2(3)**, 178-194.

[7] G Mancuso, A Midiri, E Gerace, M Marra, S Zummo and C Biondo. Urinary tract infections: The current scenario and future prospects. *Pathogens* 2023; **12(4)**, 623.

[8] LM Nolan and LP Allsopp. Antimicrobial weapons of *Pseudomonas aeruginosa*. *Advances in Experimental Medicine and Biology* 2022; **1386**, 223-256.

[9] C Hickey, B Schaible, S Nguyen, D Hurley, S Srikumar, S Fanning, E Brown, B Crifo, D Matallanas, S McClean, CT Taylor and K Schaffer. Increased virulence of bloodstream over peripheral isolates of *P. aeruginosa* identified through post-transcriptional regulation of virulence factors. *Frontiers in Cellular and Infection Microbiology* 2018; **8**, 357.

[10] D Reynolds and M Kollef. The epidemiology and pathogenesis and treatment of *Pseudomonas aeruginosa* infections: An update. *Drugs* 2021; **81(18)**, 2117-2131.

[11] F Khan. Multifaceted strategies for alleviating *Pseudomonas aeruginosa* infection by targeting protease activity: Natural and synthetic molecules. *International Journal of Biological Macromolecules* 2024; **278**, 134533.

[12] K Ashraf, S Sultan and A Adam. *Orthosiphon stamineus* Benth. is an outstanding food medicine: Review of phytochemical and pharmacological activities. *Journal of Pharmacy and Bioallied Sciences* 2018; **10(3)**, 109-118.

[13] WH Xu, HT Wang, Y Sun, ZC Xue, ML Liang and WK Su. Antihyperuricemic and nephroprotective

effects of extracts from *Orthosiphon stamineus* in hyperuricemic mice. *Journal of Pharmacy and Pharmacology* 2020; **72(4)**, 551-560.

[14] NA Abd Aziz, R Hasham, MR Sarmidi, SH Suhaimi and MKH Idris. A review on extraction techniques and therapeutic value of polar bioactives from Asian medicinal herbs: Case study on *Orthosiphon aristatus*, *Eurycoma longifolia* and *Andrographis paniculata*. *Saudi Pharmaceutical Journal* 2021; **29(2)**, 143-165.

[15] MB Ambursa, MNG Rahman, SA Sulaiman, AD Zakaria, MAD Daud, Z Zakaria, Z Zahari and MPK Wong. An *in vitro* study of *Orthosiphon stamineus* (Misai Kucing) standardized water extract as a chemolytic agent in urolithiasis. *Journal of Pharmacy and Bioallied Sciences* 2021; **13(4)**, 373-379.

[16] MA Alshawsh, MA Abdulla, S Ismail, ZA Amin, SW Qader, HA Hadi and NS Harmal. Free radical scavenging, antimicrobial and immunomodulatory activities of *Orthosiphon stamineus*. *Molecules* 2012; **17(5)**, 5385-5395.

[17] MA Hossain and SMM Rahman. Isolation and characterisation of flavonoids from the leaves of medicinal plant *Orthosiphon stamineus*. *Arabian Journal of Chemistry* 2015; **8(2)**, 218-221.

[18] JB do Nascimento and JGM da Costa. Flavonoids: A review of antibacterial activity against Gram-negative bacteria. *International Journal of Microbiology* 2025; **2025**, 9961121.

[19] NF Shamsudin, QU Ahmed, S Mahmood, SAA Ali Shah, A Khatib, S Mukhtar, MA Alsharif, H Parveen and ZA Zakaria. Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation. *Molecules* 2022; **27(4)**, 1149.

[20] N Suliska, N Kurniati and EY Sukandar. *Anredera cordifolia* (Ten.) Steenis and *Sonchus arvensis* L. inhibit gentamicin-induced nephrotoxicity: The role of urinary N-acetyl beta-D-glucosaminidase. *Journal of Reports in Pharmaceutical Sciences* 2021; **10**, 256-260.

[21] N Parisa, R Hidayat, Z Maritska and BA Prananjaya. Evaluation of the anti-gout effect of *Sonchus arvensis* on monosodium urate crystal-induced gout arthritis via anti-inflammatory action - an *in vivo* study. *Medicine and Pharmacy Reports* 2021; **94(3)**, 358-365.

[22] A Rosyidah, S Widyarti and S Rahayu. The effect of Calcusol™ to the plasma free radical and serum creatinin in *Mus musculus* nephrolithiasis model. *Journal of Tropical Life Science* 2013; **3(3)**, 143-148.

[23] D Zheng, C Huang, H Huang, Y Zhao, MRU Khan, H Zhao and L Huang. Antibacterial mechanism of curcumin: A review. *Chemistry and Biodiversity* 2020; **17(8)**, e2000171.

[24] C Dai, J Lin, H Li, Z Shen, Y Wang, T Velkov and J Shen. The natural product curcumin as an antibacterial agent: Current achievements and problems. *Antioxidants* 2022; **11(3)**, 459.

[25] Z Li, B Qu, L Zhou, Chen H, J Wang, W Zhang and C Chen. A new strategy to investigate the efficacy markers underlying the medicinal potentials of *Orthosiphon stamineus* Benth. *Frontiers in Pharmacology* 2021; **12**, 748684.

[26] K Sheng, J You, S Tian, Y Lu, J Wu and J Zhang. Optimization of extraction process for flavonoids from *Sonchus oleraceus* L. and evaluation of anti-inflammatory activity of luteoloside. *Molecules* 2026; **31(7)**, 1105.

[27] L Zeng, T Yang, K Yang, G Yu, J Li, W Xiang and H Chen. Efficacy and safety of curcumin and *Curcuma longa* extract in the treatment of arthritis: A systematic review and meta-analysis of randomized controlled trial. *Frontiers in Immunology* 2022; **13**, 891822.

[28] RH Andira, W Yuniati and A Tjitraresmi. Review: Isolasi, analisis, aktivitas farmakologi dan toksisitas sinensetin (*in Indonesian*). *Indonesian Journal of Biological Pharmacy* 2024; **4(1)**, 53-61.

[29] K Kartini, RE Putri and R Budiono. Quantification of sinensetin in *Orthosiphon stamineus* from various phytogeographical zones in Indonesia. *Journal of Applied Pharmaceutical Science* 2023; **13(03)**, 183-191.

[30] NP Ramadhani, YB Murti and AP Gani. Profiling rosmarinic acid and sinensetin content of *Orthosiphon aristatus* from 3 different locations with variety ethanol concentration. *Journal of Food and Pharmaceutical Sciences* 2024; **12(2)**, 116-124.

[31] S Fauziah, P Melati, AH Mulyati, M Rafi and E Rohaeti. Thin layer chromatography fingerprint analysis of tempuyung (*Sonchus arvensis* L.). *Jurnal Kimia Sains dan Aplikasi* 2023; **26(5)**, 187-193.

[32] AP Aswathi, SB Raghav and D Prasath. Assessment of genetic variation in turmeric (*Curcuma longa* L.) varieties based on morphological and molecular characterization. *Genetic Resources and Crop Evolution* 2023; **70(1)**, 147-158.

[33] NAM Helmy, AF Basyony, STK Tohamy and SA Zaki. Effective therapeutic strategies against *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex infections. *Scientific Reports* 2025; **15(1)**, 41462.

[34] A Elfadadny, RF Ragab, M AlHarbi, F Badshah, E Ibáñez-Arancibia, A Farag, AO Hendawy, PR De los Ríos-Escalante, M Aboubakr, SA Zakai and WM Nageeb. Antimicrobial resistance of *Pseudomonas aeruginosa*: Navigating clinical impacts, current resistance trends, and innovations in breaking therapies. *Frontiers in Microbiology* 2024; **15**, 1374466.

[35] MM Hasan, P Roy, M Alam, MdM Hoque and W Zzaman. Antimicrobial activity of peels and physicochemical properties of juice prepared from indigenous citrus fruits of Sylhet region, Bangladesh. *Heliyon* 2022; **8(7)**, e09948.

[36] YOD Nganso, EMS Tchana, AD Kahouo, AG à N Amang, K Abah, H Fomena and H Mamoudou. Inhibitory effect and antimicrobial activity of secondary metabolites of *Khaya senegalensis* (Desr.) A. Juss. (Meliaceae). *Science Journal of Chemistry* 2020; **8(4)**, 81-94.

[37] SS Patel, A Acharya, RS Ray, R Agrawal, R Raghuwanshi and P Jain. Cellular and molecular mechanisms of curcumin in prevention and treatment of disease. *Critical Reviews in Food Science and Nutrition* 2020; **60(6)**, 887-939.

[38] HB De Silva, Y Dai, S Homer-Vanniasinkam and M Edirisinghe. Antimicrobial effect of spices and their phytochemicals: A novel approach to overcoming antibiotic resistance. *MedComm* 2026; **7(4)**, e70680.

[39] ME Abd El-Hack, MT El-Saadony, AA Swelum, M Arif, MM Abo Ghanima, M Shukry, A Noreldin, AE Taha and KA El-Tarabily. Curcumin, the active substance of turmeric: Its effects on health and ways to improve its bioavailability. *Journal of the Science of Food and Agriculture* 2021; **101(14)**, 5747-5762.

[40] F Apriliani, Suwendar and BTU Laksono. Uji aktivitas antibakteri ekstrak etanol dan fraksi ekstrak daun kumis kucing (*Orthosiphon stamineus*) terhadap *Escherichia coli* secara *in vitro* menggunakan metode mikrodilusi (*in Indonesian*). *Bandung Conference Series: Pharmacy* 2024; **4(2)**, 305-312.

[41] [86] MAE Mohammed, YA Ali, TE Hagr, SY Altay, RA Musa and HM Hamadnalla. Phytochemical screening, antimicrobial and antioxidant activities of *Sonchus oleraceus* L. (leaves) extracts. *Journal of Pharmaceutical Research & Reports* 2020; **1(1)**, 1-4.

[42] NN Zulkefle, N Zainal, DK Wahyuni, S Mahmood, KDR Ramarao and KL Chin. Antimicrobial properties of *Sonchus* species: a review. *Asian Pacific Journal of Tropical Biomedicine* 2025; **15(5)**, 177.

[43] S Sethupathy, KG Prasath, S Ananthi, S Mahalingam, SY Balan and SK Pandian. Proteomic analysis reveals modulation of iron homeostasis and oxidative stress response in *Pseudomonas aeruginosa* PAO1 by curcumin inhibiting quorum sensing regulated virulence factors and biofilm production. *Journal of Proteomics* 2016; **145**, 112-126.

[44] A Shariati, E Asadian, F Fallah, T Azimi, A Hashemi, J Yasbolaghi Sharahi and M Taati Moghadam. Evaluation of nano-curcumin effects on expression levels of virulence genes and biofilm production of multidrug-resistant *Pseudomonas aeruginosa* isolated from burn wound infection in Tehran, Iran. *Infection and Drug Resistance* 2019; **12**, 2223-2235.

[45] A Shukla, G Shukla, P Parmar, B Patel, D Goswami and M Saraf. Exemplifying the next generation of antibiotic susceptibility intensifiers of phytochemicals by LasR-mediated quorum sensing inhibition. *Scientific Reports* 2021; **11(1)**, 22421.

[46] S Fernandes, A Borges, IB Gomes, SF Sousa and M Simões. Curcumin and 10-undecenoic acid as

natural quorum sensing inhibitors of LuxS/AI-2 of *Bacillus subtilis* and LasI/LasR of *Pseudomonas aeruginosa*. *Food Research International* 2023; **165**, 112519.

[47] A Shariati, M Noei, M Askarinia, A Khoshbayan, A Farahani and Z Chegini. Inhibitory effect of natural compounds on quorum sensing system in *Pseudomonas aeruginosa*: A helpful promise for managing biofilm community. *Frontiers in Pharmacology* 2024; **15**, 1350391.