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Antibacterial Potential of *Gynura procumbens* Against UTI Pathogens

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ABSTRACT

Cases of urinary tract infections (UTIs) in Indonesia continue to increase. According to data from the Ministry of Health (2014), UTI cases reach 90–100 per 100,000 population annually. The incidence of UTI is mainly caused by bacterial infections, particularly *Escherichia coli* and *Klebsiella pneumoniae*. One of the medicinal plants with pharmacological potential is the connected leaf (*Gynura procumbens* (Lour.) Merr.). This research aims to determine the effectiveness and impact of *Gynura procumbens* leaf extract at concentrations of 25%, 50%, 75%, and 100% in inhibiting the growth of *E. coli* and *Klebsiella pneumoniae*. This research employed an experimental and completely randomized design (CRD). The independent variables were the concentrations of *Gynura procumbens* extract, and the dependent variables were the UTI-causing bacteria (*E. coli* and *Klebsiella pneumoniae*). Each concentration treatment (25%, 50%, 75%, and 100%) was repeated six times. The results showed that the average inhibition zone for *E. coli* was 6.33 mm at 25%, 6.91 mm at 50%, 9.39 mm at 75%, and 13.24 mm at 100% concentration. For *Klebsiella pneumoniae*, the average inhibition zone was 6.56 mm at 25%, 7.53 mm at 50%, 12.50 mm at 75%, and 15.54 mm at 100%. One-way ANOVA analysis resulted in a p-value of 0.000, indicating that *Gynura procumbens* leaf extract significantly inhibits the growth of UTI-causing bacteria (*Klebsiella pneumoniae* and *Escherichia coli*).

INTRODUCTION

Urinary tract infections (UTIs) are among the most common infectious diseases, with Escherichia coli and Klebsiella pneumoniae being the primary causative agents. UTIs account for approximately 40% of all infection cases annually, with E. coli identified as the most prevalent pathogen worldwide. Around 90% of UTI cases originate from the community, while the remaining 50% occur in hospital settings (Nguyen et al., 2022). *E. coli* is a bacterium commonly found in the human digestive tract. Although most are harmless, some pathogenic strains can lead to serious diseases such as UTIs, meningitis, diarrhea, and even sepsis. Uropathogenic strains of *Escherichia coli* (UPEC) belong to the group of Gram-negative bacteria capable of producing broad-spectrum β-lactamase enzymes, contributing to antibiotic resistance. UTIs are estimated to affect 150–250 million people worldwide yearly, with a lifetime prevalence of 40% in females and 12% in males (Decano et al., 2021; Onanuga et al., 2019). Inadequate treatment of UTIs may allow the infecting bacteria, particularly *E. coli*, to enter the bloodstream and cause bacteremia, increasing the risk of morbidity and mortality due to infectious diseases. The WHO and CDC recognize that antibiotic-resistant bacteria seriously threaten global health (Nishimura et al., 2021). In particular, *E. coli* strains producing extended-spectrum β-lactamase (ESBL) have demonstrated decreased treatment effectiveness, causing a significant economic burden, with losses exceeding six million dollars annually, affecting approximately 150 million people suffering from UTIs (Lishman et al., 2018).

The most dominant among other bacteria causing urinary tract infections (UTIs) is *K. pneumoniae,* characterized by the presence of a capsule. The morphology of *K. pneumoniae* is a gram-negative (-) bacterium, with a short rod shape and a size of 0.5 0.5 x 1.2 µ (Iien et al., 2020).

Klebsiella pneumoniae, a member of the Enterobacteriaceae bacterial group, is recognized as an opportunistic pathogen capable of causing various infectious diseases, particularly in individuals with compromised immune systems (Oh et al., 2021). According to Oliveira et al. (2022), the challenge persists with more than two generations of *K. pneumoniae* isolates exhibiting antibiotic resistance. This resistance is attributed to the production of β-lactamase by *K. pneumoniae*, which displays significant antibiotic resistance. The ongoing challenge lies in the inability of *K. pneumoniae* to attain optimal treatment. The heightened incidence of β-lactamase resistance serves as a severe warning regarding the issue of antimicrobial resistance (Oliveira et al., 2022). The findings of this study align with the research conducted by Hanna et al. (2020), which revealed the resistance of *E. coli* to several antibiotics, including sulfamethiazole, norfloxacin, ciprofloxacin, cefotaxime, co-trimoxazole, ceftazidime, meropenem, ampicillin, amikacin, metronidazole, tetracycline, and tigecycline (Hanna et al., 2020). As highlighted by Redhead et al. (2020), taking substantial measures for environmental health protection is crucial to prevent antibiotic resistance (Redhead et al., 2020).

As indicated in the study by Ashraf et al. (2020), *Gynura procumbens*, a plant, exhibits pharmacological properties that can potentially prevent and treat various diseases, including infectious and inflammatory conditions (Ashraf et al., 2020). According to Tristantini et al. (2021), *Gynura procumbens L*., a medicinal plant containing robust phenolic compounds, is a natural antioxidant that can reduce the likelihood of contracting chronic or severe diseases (Tristantini et al., 2021). The phytochemical content of the *Gynura procumbens* plant includes flavonoids, alkaloids, tannins, saponins, steroids, triterpenoids, and glycosides. The presence of flavonoids, tannins, saponins, triterpenoids, essential oils, polyphenols, and steroids in the composition exhibits antibacterial potential against both gram-positive and gram-negative bacteria (Ashraf et al., 2020).

Amin et al. (2021) conducted prior research, employing diverse concentrations of aqueous, ethanol, and n-hexane extracts, revealing remarkable antibacterial efficacy against four pathogenic bacterial strains, namely *Chromobacterium sp, Staphylococcus aureus, Enterococcus faecium,* and *Escherichia coli.* The aqueous extract of *Gynura procumbens* demonstrated potential antibacterial activity against *S. aureus* and *E. faecium* at 200µg/mL concentration, with mean inhibition zones (SD) of 15 (1.0) mm and 10 (0.55) mm, respectively. Additionally, at a concentration of 40 µg/mL, the aqueous extract of G. procumbens exhibited significant anti-inflammatory activity ($p < 0.01$) (Amin et al., 2021).

Several previous studies have explored the antibacterial effectiveness of natural ingredients. However, no research has specifically examined the antibacterial potential of *Gynura procumbens* (Lour.) Merr. Against two major bacterial species that cause urinary tract infections, namely *E. coli* and *Klebsiella pneumoniae*. This study is important because both bacteria exhibit strong resistance to antibacterial agents. This research aims to determine the effectiveness and impact of *Gynura procumbens* leaf extract at concentrations of 25%, 50%, 75%, and 100% in inhibiting the growth of *Klebsiella pneumoniae* and *Escherichia coli* bacteria.

METHOD

This experimental study employed a completely randomized design (CRD). The independent variable was the *Gynura procumbens (Lour.) Merr* leaves extract, while the dependent variables were the bacteria causing UTIs (*Klebsiella pneumoniae* and *E. coli*). The treatment consisted of concentrations of 25%, 50%, 75%, and 100% of *Gynura procumbens (Lour.) Merr* leaves extract, with each concentration replicated six times. The study was conducted from September 2022 to January 2023 at the Bacteriology Laboratory of the Medical Laboratory Technology Department, Tanjung Karang Health Polytechnic.

The equipment utilized included petri dishes, test tubes, a test tube rack, sterile swabs, tweezers, a vortex mixer, an incubator, an autoclave, an oven, an analytical balance, Erlenmeyer flasks, measuring pipettes, a vacuum pump, forceps, filter paper discs, glass beakers, calipers, a hot plate, a lighter, gloves, masks, and a spirit lamp. The materials used were *Gynura procumbens (Lour.) Merr* leaves extract, 96% ethanol, *Klebsiella pneumoniae* and *E. coli* bacteria, distilled water, Mueller Hinton Agar (MHA), Nutrient Broth media, Nutrient agar slants, and blank discs.

The *Gynura procumbens (Lour.) Merr* used for the extract preparation were fresh, green, intact leaves measuring 6.5 cm wide and 16.5 cm long, without any holes or tears. The leaves were thoroughly washed, air-dried, and then ground into a powder using a mixer and sieved through a 40-mesh sieve to obtain *Gynura procumbens (Lour.) Merr* leaves powder (Sinaga et al., 2017).

The concentrated leaves extract of *Gynura procumbens (Lour.) Merr* was prepared by adding 100 g of the dried *Gynura procumbens (Lour.) Merr* powder to a macerator. Subsequently, 1 liter of 95% ethanol was added to the macerator containing the *Gynura procumbens (Lour.) Merr* leaves powder. This mixture was soaked for 6 hours with continuous homogenization and left to stand for 24 hours to allow the extraction of active compounds from the leaves. The resulting macerate was separated from the leaf mixture and solvent through filtration. This separation process was repeated twice using the same solvent. All separated macerates were combined, and the concentrated extract was obtained by placing the macerates in a vacuum evaporator until the concentrated *Gynura procumbens (Lour.) Merr* leaves extract was obtained, as shown in Figure 1 and Figure 2 (Zulharmitta et al., 2017).

Figure 1. Maceration of *Gynura Procumbens (Lour.) Merr* leaves powder with 96% ethanol

Figure 2: The concentrated extract obtained from maceration

The obtained 100% concentration extract was diluted to 25%, 50%, 75%, and 100% (undiluted) as shown in Figure 3.

Figure 3. Preparing concentrations of 25%, 50%, and 75% from the concentrated extract (100%) of *Gynura Procumbens (Lour.) Merr* leaves

The bacteria-causing UTIs used in this study were pure strains of *Klebsiella pneumoniae* and *E. coli*. The positive control utilized was the antibiotic disk ciprofloxacin. The research procedure employed the Kirby-Bauer diffusion method to assess inhibitory activity. Sterile cotton swabs were immersed in bacterial suspensions of *Klebsiella pneumoniae* and *E. coli* in Nutrient Broth media with turbidity standardized to MacFarland 0.5. The swabs were left briefly to allow bacterial absorption. Subsequently, the swabs were removed, pressed against the inner wall of the test tubes while rotating, and then evenly streaked onto the entire surface of the Muller Hinton Agar media. The media was allowed to dry for 5 minutes to facilitate suspension absorption into the agar, as shown in Figure 4.

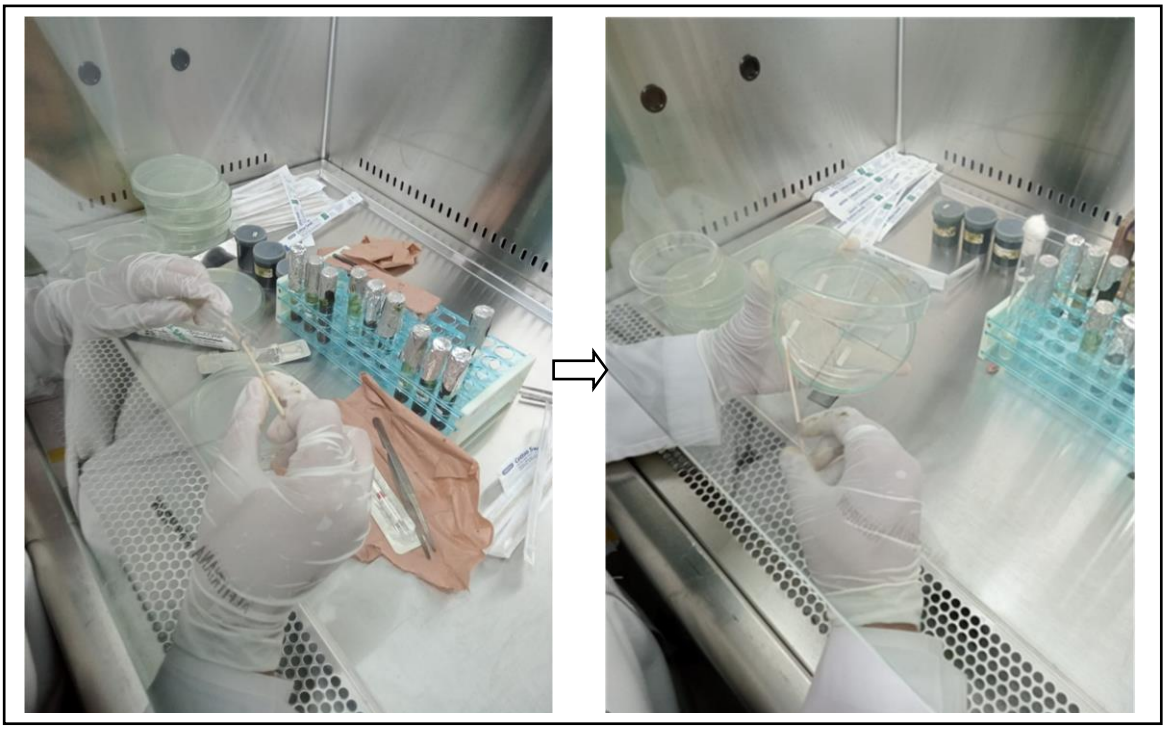

Figure 4. Taking bacterial suspension with a sterile cotton swab and streaking it across the entire surface of the Mueller Hinton agar plate

Disc blanks were soaked in each concentration of *Gynura Procumbens (Lour.) Merr* leaves extract for 15 minutes, removed with sterile forceps, and placed on the Muller Hinton Agar media surface previously streaked and spaced 15 mm apart. The discs were pressed using sterile forceps and then incubated at 37°C for 24 hours as shown in Figure 5.

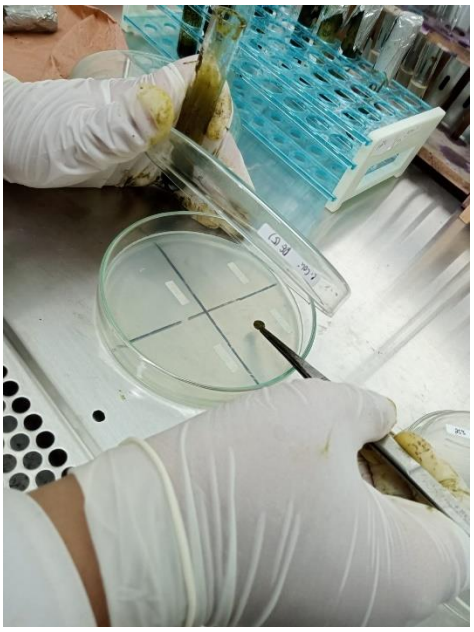

Figure 5. Placing sterile disks with concentrated *Gynura Procumbens (Lour.) Merr* leaves extract

RESULTS

The effectiveness test of *Gynura procumbens (Lour.) Merr* leaves extract at concentrations of 25%, 50%, 75%, and 100% with six repetitions obtained results that inhibited the growth of bacteria that cause UTIs in this study using *E. coli* and *Klebsiella pneumoniae* bacteria at each concentration tested, as shown in Table 1.

Table 1. Diameter of the inhibition zone of *Gynura procumbens (Lour.)* Merr leaves extract is used against the growth of bacteria causing UTI (*E. coli*)

Concentration (%)	Diameter of zone of inhibition (mm) on each repetition						Total (mm)	Average (mm)
	I	II	III	IV	V	VI		
25	6.25	6.32	6.20	7.15	6.04	6.05	38.01	6.33
50	7.53	7.24	7.08	7.32	6.24	6.10	41.51	6.91
75	8.28	13.05	11.45	9.22	7.18	7.14	56.32	9.39
100	11.44	15.22	15.10	12.18	13.00	12.50	79.44	13.24

Control (+): Ciprofloxacin diameter zone of inhibition 25.67 mm
Control (-): Sterile aquadest diameter zone of inhibition (0 mm)

E. coli bacteria were examined at a 25% concentration, resulting in an average inhibition zone diameter of 6.33 mm. Meanwhile, at a concentration of 100%, the average diameter of the formed inhibition zone increased to 13.24 mm. The test results of *Gynura Procumbens (Lour.) Merr* leaves extract effectively inhibits the growth of *K. pneumoniae* with average inhibition zone diameters formed at concentrations of 25% (6.65mm), 50% (7.35mm), 75% (12.50mm), and 100% (15.04mm) as shown in Table 2.

Table 2. Diameter of the inhibition zone of *Gynura procumbens (Lour.)* Merr leaves extract is used against the growth of bacteria causing UTI (*Klebsiella pneumoniae*)

Concentration (%)	Diameter of zone of inhibition (mm) on each repetition						Total (mm)	Average (mm)
	I	II	III	IV	V	VI		
25	6.08	7.10	7.32	6.44	6.26	6.16	39.36	6.56
50	6.10	8.25	8.48	6.50	7.35	7.41	44.09	7.35
75	11.24	15.40	14.02	12.07	10.10	12.14	74.97	12.50
100	14.08	17.00	16.56	13.38	15.20	14.00	90.22	15.04

Control (+): Ciprofloxacin diameter zone of inhibition 25.67 mm
Control (-): Sterile aquadest diameter zone of inhibition (0 mm)

The average inhibition zone diameter formed from each tested concentration against the growth of *K. pneumoniae* was further subjected to an Anova test. For all concentrations of (25%, 50%, 75%, and 100%), a p-value<0.001 was obtained. The interpretation of the results is that the null hypothesis (H0) is rejected, indicating an influence of *Gynura Procumbens (Lour.) Merr* leaves extract in inhibiting the growth of *K. pneumoniae* as presented in Table 3.

Table 3. Zone of inhibition of *Gynura procumbens (Lour.) Merr* leaves ethanol extract against *Klebsiella pneumoniae* and *Escherichia coli* bacteria

Concentration (%)	Mean diameter of inhibition zone (mm) on Test Bacteria	
	Klebsiella pneumonia	*Escherichia coli*
25	6.56	6.33
50	7.35	6.91
75	12.50	9.39
100	15.04	13.24
Control (+)	20.70	23.30
Control (-)	0.00	0.00

The mean diameter of the inhibition zones formed by each concentration tested against *K. pneumoniae* growth was subjected to an Anova test. Across all concentrations (25%, 50%, 75%, and 100%), a p-value of < 0.001 was obtained. The interpretation of these results is the rejection of the null hypothesis (H0), suggesting that *Gynura Procumbens (Lour.) Merr* leaves extract significantly inhibits the growth of *E. coli*, as shown in Table 4.

Table 4. Anova test of *Gynura procumbens (Lour.) Merr.* leaves extract on the growth of *Klebsiella pneumonia*e bacteria

Concentration (%)	Mean diameter of inhibition zone (mm) on *Klebsiella pneumonia*	
	Klebsiella pneumonia	*p-value*
25	6.56	
50	7.35	0.000
75	12.50	
100	15.04	

Following the Anova test, a Post hoc test was conducted to determine the differences in the mean diameter of the inhibition zones formed by each concentration group of *Gynura Procumbens (Lour.) Merr* leaves extract was tested against the two bacteria. Table 5 presents the Anova test results, while Table 6 displays the Post hoc test results. The presence of asterisks indicates that all concentration groups tested exhibit significant differences.

Table 5. Anova test of *Gynura procumbens (Lour.) Merr.* leaves extract affects the growth of *Escherichia coli* bacteria

Concentration (%)	Mean diameter of inhibition zone (mm) on *Escherichia coli*	
	Escherichia coli	*p-value*
25	6.33	
50	6.91	0.000
75	9.39	
100	13.24	

Table 6. Post hoc test *of Gynura procumbens* extract *(Lour.) Merr* leaves*.* Against the growth of *Klebsiella pneumonia*e and *Escherichia coli* bacteria

Concentration (%)	Mean diameter of inhibition zone (mm) on Test Bacteria	
	Klebsiella pneumonia	***Escherichia coli***
25	6.56**	6.33*[3]
50	7.35**	6.91*[3]
75	12.50*	9.39[3]
100	15.04*	13.24[3]

The effectiveness test results of *Gynura procumbens (Lour.) Merr* leaves extract against pathogenic bacteria causing urinary tract infections, specifically *Klebsiella pneumoniae* and *Escherichia coli*, revealed the development of growth inhibition zones for both bacterial strains. These findings suggest that the ethanol extract of *Gynura procumbens (Lour.) Merr* leaves contain antibacterial properties capable of impeding the growth of *Klebsiella pneumoniae* and *Escherichia coli*. Moreover, the diameter of the inhibition zone increased proportionally with the concentration tested, indicating a correlation between extract concentration and inhibitory effect on the growth of *Klebsiella pneumoniae* and *Escherichia coli.*

In their study, Maharani et al. (2018) noted a correlation between extract concentration and antibacterial effectiveness. The application of higher extract concentrations resulted in larger inhibition areas, signifying an augmentation in antibacterial strength (Maharani et al., 2018). The increase in the inhibition zone size and the higher concentration of *Gynura procumbens (Lour.) Merr* leaves extract clearly indicate a significant improvement in its antibacterial effectiveness. This strong evidence supports the claim that higher concentrations of ethanol extract from *Gynura procumbens (Lour.) Merr*. possess increased and robust antibacterial potential. The size of the inhibition zone, a direct indicator of the extract's inhibitory effects on both bacterial strains, unmistakably highlights the superior antibacterial power of the ethanol extract of *Gynura procumbens (Lour.) Merr* leaves at higher concentrations.

DISCUSSION

The extract of *Gynura procumbens (Lour.) Merr* leaves comprise triterpenoids, polyphenols, saponins, steroids, chlorogenic acid, caffeic acid, vanillic acid, coumaric acid, para-hydroxybenzoate acid, flavonoids, and essential oils. Among these, flavonoids and tannins are active plant compounds with potential antibacterial properties (Kim et al., 2021). These compounds have antimicrobial properties that can inhibit bacterial growth and reproduction. The potential inhibitory effects of *Gynura procumbens (Lour.) Merr* ethanol leaves extract on *Klebsiella pneumoniae* and *Escherichia coli* bacteria can vary, influenced by factors like the extraction potency, extract concentration, and the resistance of the bacteria to these active compounds. The capacity of antibacterial agents to impede the proliferation of tested *E. coli* and *Klebsiella pneumoniae* bacteria varies at concentrations of 25%, 50%, 75%, and 100%, resulting in distinct inhibition zones contingent on the specific bacteria under examination.

The average inhibition zones ranged from 6.33 mm to 13.24 mm for *E. coli* and from 6.56 mm to 15.04 mm for *Klebsiella pneumoniae*. Subsequent application of the One-Way Anova test resulted in a p-value of 0.000 for both bacteria, indicating a significant effect of the tested concentrations (25%, 50%, 75%, and 100%) in inhibiting the growth of *E. coli* and *Klebsiella pneumoniae* bacteria. The p-value <0.05 signifies the concentration's efficacy in hindering bacterial growth. As the concentration increases, the active substances (triterpenoids, polyphenols, saponins, steroids, chlorogenic acid, caffeic acid, and vanillic acid) demonstrate an enhanced ability to inhibit bacterial growth. This trend is consistent with the inhibitory effect of concentrations on the growth of *E. coli* and *Klebsiella pneumoniae*, the predominant bacteria causing urinary tract infections (UTIs).

A significant result was obtained with a sig after conducting additional analysis with linear regression testing. F Change of 0.000 is smaller than the significance value of <0.05. This implies a linear effect or an impact of *Gynura procumbens (Lour.) Merr* leaves extract inhibits the growth of *E. coli* and *Klebsiella pneumoniae* bacteria, the causative agents of UTI. In both tested bacteria,

a capsule in the cell structure shields bacterial cells from adverse effects, including antimicrobials. Bacteria that have capsules are generally more resistant than bacteria that do not have capsules. However, the *Gynura procumbens (Lour.) Merr* leaves extract inhibited the growth of both bacteria, a novelty from other similar studies.

The findings align with the research conducted by Amin et al. (2021), concluded that *G. procumbens* leaves extract holds promise as a potential treatment for diverse infections (Amin et al., 2021). Additionally, Ashraf's research (2020) revealed that only the methanol extract of *Gynura procumbens* exhibited antimicrobial activity against the tested *Staphylococcus aureus* (Ashraf et al., 2020). In a parallel context, Bakhtra's 2018 research examined various fractions of *Gynura procumbens (Lour.) Merr* extract using *Shigella dysenteriae* as the test bacteria. The outcomes revealed that the ethyl acetate fraction, at a 30% concentration, emerged as the most effective antibacterial agent, demonstrating an inhibition zone of 10.5 mm. Conversely, the hexane and butanol fractions, also at a 30% concentration, exhibited lower inhibitions of 7 mm each (Bakhtra, 2018).

The results of other similar studies, by Nova Suryati, et al (2017). The results of testing *Alue vera* extract against *E. coli* growth, with concentrations of 6.25%, 12.5%, 25%, 50%, and 100%, showed that *Alue vera* extract at all concentrations tested could not inhibit *E. coli growth* (Suryati et al., 2018). In a different investigation conducted by Huurun Lien et al. (2020), examining the impact of Turi Leaves methanol extract on the growth of *Klebsiella pneumoniae*, concentrations of 10%, 25%, 40%, and 55% were tested. The findings indicate that the concentration of 55% proves to be effective in inhibiting the growth of *Klebsiella pneumoniae* bacteria (Iien et al., 2020).

The results of research by Prasetyorini et al. (2019) show that the *G. procumbens* leaves extract, with a 30% LDH value, reaches a peak of 4.5 mm at a concentration of 30%, while in *E. scaber* leaves extract, the highest LDH value is 4.3 mm, and at a concentration of 50% extract gives an inhibitory effect on the growth of *S. thypi* bacteria, the level of inhibition is a weak category (Prasetyorini et al., 2019). The results of the study by Nasirudin and Sinha (2020) confirmed that the analyzed samples showed high levels of antioxidant efficacy. In addition, the antibacterial activity was verified through the negative impact on all types of bacteria tested. These findings imply that *Gynura procumbens (Lour.) Merr* extract can be considered an herbal source containing a wide array of bioactive compounds (Nasiruddin & Sinha, 2020).

With the known antibacterial potential of *Gynura procumbens (Lour.)Merr* leaves ethanol extract against *Klebsiella pneumoniae* and *Escherichia coli* from this study, further comprehensive research is needed to broaden our horizons on the potential use of *Gynura procumbens (Lour.)Merr* leaves are a natural antibacterial source. The findings from this research are anticipated to lay a strong scientific groundwork for the formulation of antimicrobial products derived from Gynura procumbens (Lour.) Merr leaves. These products hold the potential for future application in health and pharmaceuticals. Thus, this effort can positively contribute to developing innovative solutions for overcoming the increasingly complex challenges of bacterial infections that cause UTIs.

CONCLUSION

Gynura procumbens (Lour.) Merr leaves extract has the potential as an antibacterial, at concentrations tested 25% - 100%, with the formation of an inhibition zone diameter. The zone of inhibition formed against each test bacterium causing a urinary tract infection (UTI) is different. *Klebsiella pneumoniae* test bacteria mean inhibition zone 6.56 mm - 15.04 mm. The inhibitory effects of *Gynura procumbens (Lour.) Merr* leaves extract on test bacteria, specifically *Escherichia coli*, manifested with an average inhibition zone ranging from 6.33 mm to 13.34 mm. Crucially, the statistical analysis yielded a p-value of 0.000 for both test bacteria, underscoring the extract's remarkable efficacy in hindering the growth of *Klebsiella pneumoniae* and *Escherichia coli* bacteria— two primary causes of urinary tract infections (UTIs).

AUTHOR'S DECLARATION

Authors' contributions and responsibilities

SA: Conceptualization, methodology, investigation, data analysis, writing original draft; **MT:** Conceptualization, formal analysis, review & editing; **All authors:** Review and approval of final manuscript.

Availability of data and materials

All data are available from the authors.

Competing interests

The authors declare no competing interests.

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