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Potential of Kersen Leaf Extract (*Muntingia calabura* L) and Basil Leaf Extract (*Ocimum basilicum* L.) as Natural Antibacterial Candidates against *Salmonella typhi*

Mustika Weni[1], Sri Marfuati[2], Hikmah Fitriani[1]

[1]Departemen of Basic Medical Science, Faculty of Medicine, Swadaya Gunung Jati University, Indonesia
[2]Departemen of Pharmacology, Faculty of Medicine, Swadaya Gunung Jati University, Indonesia

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Corresponding Author:
Mustika Weni
mustikaweni261192@gmail.com

ABSTRACT

Salmonella typhi remains a global health issue with rising antibiotic resistance, necessitating alternative natural therapies. Kersen (*Muntingia calabura* L.) and basil (*Ocimum basilicum* L.) leaves contain bioactive compounds with antibacterial potential. The purpose of this research was to examine the antibacterial activity of kersen and basil leaf ethanol extracts against *Salmonella typhi* at various concentrations. The research applied an experimental Post-Test Only Control Group Design. Antibacterial activity assessment was performed using the well diffusion method with varying extract concentrations of 20%, 35%, 45%, 50%, and 75%. Data analysis was performed using the Shapiro–Wilk test was used to examine data normality, while group differences were evaluated with the Kruskal–Wallis non-parametric test. Kersen extract contained saponins, tannins, alkaloids, flavonoids; basil extract contained phenolics, saponins, tannins, alkaloids, steroids, flavonoids. Both showed concentration-dependent inhibition. At 75%, inhibition zones reached 13.15 mm (strong), while the lowest were 4.70 mm (kersen, weak) and 7.30 mm (basil, moderate). Negative control showed no activity. Statistical test $p=0.005$ confirmed significant differences between groups. Ethanol extracts of kersen and basil leaves possess significant antibacterial activity against S. typhi, supporting their potential as natural phytotherapeutics for typhoid.

1. Introduction

Typhus is an infectious disease caused by the bacteria *Salmonella Typhi*, classified as a Gram-negative microorganism, rod-shaped bacteria that has flagella and is equipped with a polysaccharide capsule (Khan & Shamim, 2022). This capsule structure increases the ability of bacteria to attack by protecting them from phagocytosis. These bacteria can attack humans, and if not treated properly, can cause serious complications that are potentially fatal. Transmission generally occurs through the oral-fecal route, with the main symptoms being high fever, abdominal pain and nausea, disturbances in bowel movements (Putu et al., 2019).

Salmonella Typhi is responsible for approximately 76.3% of global enteric fever cases, with mortality rates disproportionately higher among children and adults living in low-income regions, where access to healthcare and effective treatment is often limited. According to WHO data, the regions with the highest number of typhoid fever cases include Africa, Southeast Asia, and the Western Pacific region. The incidence of this disease is still very high, with an estimated 21 million cases each year, and more than 700 of them end in death (Maksura, 2021).

Despite advances in treatment and research, millions of people are still at risk of contracting enteric fever, which can lead to disability and death. The treatment of this disease faces a significant challenge due to the appearance of multidrug-resistant typhoid strains, including MDR and XDR (Khan & Shamim, 2022). Resistance to ampicillin, chloramphenicol and trimethoprim–sulfamethoxazole is commonly referred to as multidrug resistance (MDR). Meanwhile, XDR indicates resistance to five classes of antibiotics, including chloramphenicol, ampicillin, fluoroquinolones, co-trimoxazole, and third- generation cephalosporin antibiotics.

Commonly recommended antibiotics for the treatment of typhoid fever include chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, ceftriaxone, ciprofloxacin, tigecycline azithromycin and meropenem (Dyson et al., 2019). However, resistance to azithromycin has also been reported in a number of cases (Hooda et al., 2019). There is strong evidence to suggest a link between bacterial resistance and inappropriate antibiotic use, which is now a serious public health issue (Lee Ventola, 2015). In 2019, the death toll due to antibiotic resistance reached 1.27 million people and it is predicted that by 2050 the death toll due to antibiotic resistance will reach 10 million people per year (WHO, 2023). In addition to inappropriate use of antibiotics through irrational prescriptions, antibiotic misuse also includes the purchase of over-the-counter (OTC) drugs and patient non-compliance in following prescribed treatment (Babar, 2017). Indonesia is one of the developing countries in Southeast Asia that is experiencing rapid economic growth and development. This condition triggers urbanization and migration of workers between neighboring countries such as Malaysia, Thailand, and the Philippines. This movement of workers has the potential to facilitate the spread or cross-country transmission of Salmonella typhi strains in endemic areas.

In response to decreasing antibiotic effectiveness, attention has increasingly shifted toward natural products as alternative sources of antibacterial compounds. Many medicinal plants contain phytochemicals such as flavonoids, tannins, saponins, terpenoids, and phenolic acids, which are known to exhibit strong antibacterial activity (Ashraf et al., 2023). Several studies have demonstrated the antibacterial potential of kersen leaves (*Muntingia calabura* L). . Extracts of kersen leaves have been reported to inhibit pathogens including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, with the activity attributed largely to flavonoids, polyphenols, and alkaloids (Rahmawati et al., 2020; Chaudhary et al., 2019; Weni & Marfuati, 2024). Similarly, kemangi leaves (*Ocimum basilicum* L.) possess strong antibacterial and antioxidant properties due to the presence of linalool, eugenol, alkaloids, and phenolic compounds. Basil leaf extract has been shown to inhibit E. coli, Bacillus subtilis, S. aureus, and Salmonella species (Singh et al., 2020; Pratiwi et al., 2019; Weni & Marfuati, 2024).

Despite these findings, studies specifically evaluating the comparative antibacterial activity of kersen and basil leaf extracts against Salmonella Typhi—especially using varying extract concentrations—remain limited. This gap highlights the need for further investigation to determine their potential as natural antibacterial agents for typhoid management.

2. Methods

2.1. Tools and Materials

For this research, various laboratory tools were used, such as a blender, test tubes and racks, filter paper, petri dishes, glass slides, rotary vacuum evaporator, Bunsen burner, Erlenmeyer flasks, spatulas, microscope, a laminar air flow cabinet, digital scale, measuring cylinders, stirring rods, funnels, pipettes, an incubator, an autoclave, and a vortex mixer.

The main materials were kersen leaves (*Muntingia calabura* L.) and basil leaves (*Ocimum basilicum* L.), which were collected from the nearby environment. Other supporting materials included Nutrient Agar (NA), Mueller Hinton Agar (MHA), 96% ethanol, distilled water, DMSO, the McFarland standard, and the test bacterium Salmonella Typhi.

2.2. Sterilization of Tools

Instruments used in the research were first sterilized. Sterilization is generally performed by heating at high temperatures. Glassware and media were sterilized in an autoclave at 121°C and a pressure of 15 psi (approximately 1 atm) for 15-20 minutes. Loop needles were sterilized over a Bunsen burner. (Kartika et al., 2022).

2.3. Preparation of MHA Media (Anggraini et al., 2021)

MHA media in Petri dishes were inoculated with the bacterial suspension and spread uniformly with an L-shaped rod. Wells measuring 8 mm in diameter were prepared using a cork borer.

2.4. Plant Determination (Anggraini et al., 2021)

Morphological characteristics were examined to determine and verify the identity of kersen (Muntingia calabura L.) and basil (Ocimum basilicum L.) to ensure accurate species identification prior to extraction.

2.5. Preparation of Ethanol Extract of Kersen Leaves and Basil Leaves (*Ocimum basilicum* L.) (Anggraini et al., 2021)

Kersen (Muntingia calabura L.) and basil (Ocimum basilicum L.) leaves (10 kg) were washed, dried in sunlight, and milled into powder. In the maceration process, 96% ethanol was used as the extraction solvent. A total of 3 liters of ethanol was placed into a container, covered, and used to soak the leaves for three days. The resulting solutions of kersen and basil leaf extracts were the solution was filtered and evaporated at 40 °C to yield a thick extract.

2.6. Yield Method

Yield is calculated according to AOAC (1999) in Aristyanti et al. (2017) with the following formula:

$$\mathbf{Yield} = \frac{\textbf{Extract Wight}}{\textit{Raw material weight}} \text{ x } 100\%$$

2.7. Phytochemical Test of kersen leaves (*Muntingia calabura* L.) and basil leaves (*Ocimum basilicum* L.) (Sulistyarini et al., n.d.) :

2.7.1. Alkaloid Test (MMI)

Samples (0.5 g) were treated with 1 mL of 2N HCl and 9 mL of distilled water, cooled, filtered, and the filtrates were tested with Bouchardat, Dragendorff, Mayer, and Hager reagents.

2.7.2. Tannin Test (MMI)

Extracts were treated with a few drops of 1% $FeCl_3$ solution; the formation of a blue-black or greenish precipitate indicated tannins.

2.7.3. Steroid and Triterpenoid Test (MMI)

Samples were dissolved in chloroform, followed by the addition of acetic anhydride and concentrated H_2SO_4 (Liebermann–Burchard test); a blue-green color indicated steroids, while a reddish-brown color indicated triterpenoids.

2.7.4. Saponin Test (MMI)

Samples were shaken vigorously with distilled water; stable froth persisting for 15 minutes indicated saponins.

2.7.5. Phenolic and flavonoid test

A 0.5-gram sample was dissolved in 2 ml of methanol and filtered, after which the filtrate was divided into two portions. To the first tube, NaOH was added, where the appearance of a red coloration indicated phenolic compounds. To the second tube, H_2SO_4 was added, and the development of a red color signified the presence of flavonoids.

2.8. Bacterial Inoculation on Slant Agar Media

Test bacteria were collected with a sterile loop needle and then placed on a slanted agar plate by scratching. They were then incubated in an aerobic incubator at 37°C for 24 hours. (Tatli Cankaya & Somuncuoglu, 2021)

2.9. Preparation of Bacterial Suspension

Cultures of the test bacteria from agar slants were diluted with physiological saline and vortexed. A 1 ml aliquot was then withdrawn using a syringe and transferred into a tube containing 9 ml of 0.9% NaCl solution. The suspension was adjusted until the turbidity matched the McFarland 0.5 standard, which is prepared from a mixture of 9.95 ml of 1% H_2SO_4 and 0.05 ml of 1% NaCl. (Wendersteyt et al., 2021) (Supartiningsih, Jon Kenedy Marpaung, 2020)

2.10. Preparation of Test Media the bacterial *(Alouw & Lebang, 2022.)*

SSA agar plates were inoculated with bacterial suspension and uniformly spread using an L-spreader. In the SSA media that has been inoculated with bacteria, holes are made using a cork borer with a size of 8 mm. 3 holes are made in each petri dish.

2.11. Preparation of Control Solution

A 10 μL aliquot of 10% DMSO was used as the negative control.

2.12. Antibacterial Activity Test (Rollando, 2019)

This study used 8 groups, namely 1 negative control group using 10% DMSO and 7 treatment groups namely single extract of kersen leaves (*Muntingia calabura* L.) and basil leaves (*Ocimum basilicum* L.) with extract concentrations of 20%, 35%, 45%, 50% and 75%

respectively as much as 50 µl to be tested. The inoculated plates were incubated at 37 °C for 24 hours. Inhibition zones were recorded with a caliper after 24 hours of incubation. Diameters were recorded across the center of the well, with 0 mm indicating no inhibition.

2.13. Data Analysis

Shapiro–Wilk was used to test data normality, and parametric analysis was performed with One-Way ANOVA at a significance threshold of $p < 0.05$.

3. Results and Discussion

3.1. Results

3.1.1. Yield

Yield is an indicator of the efficiency of the extraction process, describing how much active compound can be obtained from the raw material after dissolution and concentration. Based on the results obtained, the following results were obtained:

Tabel 1 Yield Result

Plant	Sample Weight (kg)	Simplicia Weight (gr)	Total Extract (gr)	Yield (%)
Kersen Leaves	10 kg	1101 gram	307 gram	27,89 %
Basil Leaves	10 kg	979 gram	112 gram	11,44 %

From the results obtained in the research table, the yield of Kersen leaf extract (*Muntingia calabura* L.) was 27.89% and the yield of ethanol extract of basil leaves (*Ocimum basilicum* L.) was 11.44%.

3.1.2. Phytochemical Content of Ethanol Extract of Kersen Leaves (Muntingia calabura L) and Kemangi Leaves (Ocimum basilicum L)

The secondary metabolites of basil (*Ocimum basilicum L.*) and kersen (*Muntingia calabura L.*). Phytochemical constituents of kersen (*Muntingia calabura* L.) and basil (*Ocimum basilicum* L.) leaves were qualitatively identified through phytochemical screening, and the outcomes for both ethanol extracts are shown in Table 2.

Table 2 Phytochemical test results of kersen leaves (*Muntingia calabura* L) and basil leaves (*Ocimum basilicum* L)

Secondary Metabolite Compounds	kersen leaves (*Muntingia calabura* L)	basil leaves (*Ocimum basilicum* L)
Alkaloids	+	+
Flavonoids	+	+
Phenolic	-	+
Saponins	+	+
Tannins	+	+
Steroid	-	+

Phytochemical screening revealed the presence of alkaloids, tannins, saponins, and flavonoids in the ethanol extract of kersen leaves, while the ethanol extract of basil leaves showed the presence of phenolics, alkaloids, saponins, steroids, flavonoids and tannins.

3.1.3. Activity of the Ethanol Extract of Kersen Leaves (Muntingia calabura L) as an Antibacterial Salmonella Typhi

The research findings revealed that ethanol extract of kersen leaves possessed antibacterial activity against Salmonella Typhi, which was evaluated using the inhibition zone assay via the well diffusion method (Table 3).

The antibacterial activity test of kersen leaf extract showed a concentration-dependent effect, where higher concentrations produced larger inhibition zones. The widest inhibition zone was observed at 75% concentration, measuring 13.15 mm and classified as strong. At 20% concentration, the mean diameter was 4.70 mm (weak category); at 35% it was 7.00 mm (moderate); at 45% it reached 8.96 mm (moderate); and at 50% it measured 10.05 mm (strong). In contrast, the negative control (DMSO) showed no inhibition zone. Statistical analysis using the Kruskal–Wallis test yielded a p-value of <0.05, confirming that the differences among concentrations were significant. Normality testing with Shapiro–Wilk showed non-normal distribution ($p < 0.05$), leading to non-parametric analysis. The Kruskal–Wallis test returned a p-value of 0.005 ($p < 0.05$), indicating significant variation across extract concentrations.

Tabel 3 Inhibition zone of well diffusion method of ethanol extract of kersen leaves (*Muntingia calabura* L) against *Salmonella Typhi*

Consentration	Inhibition Zone Diameter (mm)	Category
Negative control	0^{a}	no inhibition zone
20%	4.70 ± 0.34^{b}	Week
35%	7.00 ± 0.08^{c}	Currently
45%	8.96 ± 0.12^{d}	Currently
50%	10.5 ± 0.04^{e}	Strong
75%	13.15 ± 0.32^{f}	Strong

3.1.4. Activity of the Ethanol Extract of Basil Leaves (Ocimum basilicum L) as an Antibacterial Salmonella Typhi

The research findings demonstrated that the ethanol extract of basil leaves exhibited antibacterial activity against Salmonella Typhi. This activity was evaluated using the inhibition zone assay through the well diffusion method, as presented in Table 4.

Tabel 4 Inhibition zone of well diffusion method of Ethanol Extract of Basil leaves (*Ocimum basilicum* L) against *Salmonella Typhi*

Consentration	Inhibition Zone Diameter (mm)	Category
Negative control	0^{g}	no inhibition zone
20%	7.30 ± 0.30^{h}	Currently
35%	9.93 ± 0.08^{d}	Currently
45%	$11.63 \pm 0.^{12e}$	Strong
50%	12.76 ± 0.04^{i}	Strong
75%	14.85 ± 0.32^{j}	Strong

The antibacterial activity test of basil leaf extract revealed a concentration-dependent effect, where higher concentrations produced larger inhibition zones. The widest zone was observed at 75% concentration with a diameter of 13.15 mm, classified as strong. At 20% concentration, the mean diameter was 7.30 mm (moderate); at 35% it was 9.93 mm (moderate); at 45% it reached 11.63 mm (strong); and at 50% it measured 10.05 mm (strong). In contrast, the negative control (DMSO) showed no inhibition zone.

A p-value of <0.05 from the Kruskal–Wallis analysis demonstrated significant variation across concentrations. However, the Shapiro–Wilk test indicated a lack of normality in the data ($p < 0.05$), thus non-parametric testing was applied for further evaluationA p-value of 0.005 ($p < 0.05$) obtained from the Kruskal–Wallis test demonstrated that the antibacterial effects varied significantly among the different extract concentrations. To see the significance between the two groups, the Post Hoc Mann-Whitney test was continued.

3.1.5. Differences Between Concentration Groups

To see the significance between the two groups, a post hoc test was conducted. The test results can be seen in Table 5.

Tabel 5 Difference Test Between Concentration Groups

Consentration	K(-)	Kr 20%	Kr 35%	Kr 45%	Kr 50%	Kr 75%
K(-)	-	0.000	0.000	0.000	0.000	0.000
Ke 20%	0.000	-	0.005	0.000	0.000	0.000
Ke 35%	0.000	0.005	-	0.039	0.001	0.000
Ke 45%	0.000	0.000	0.039	-	0.091	0.000
Ke 50%	0.000	0.000	0.001	0.091	-	0.007
Ke 75%	0.000	0.000	0.000	0.000	0.007	-

Ket : K(-) : Negative Control 10%, Ke : Basil Leaves, Kr : Kersen Leaves

Based on table 5, in this study there were 4 groups that were not significant ($p>0.05$), 45% concentration of kersen leaves extract was not significant with 35% basil leaves extract and 50% concentration of kersen leaves extract with 45% basil leaves extract and there were 26 significant groups ($p<0.05$).

3.2. Discussion

3.2.1 Extraction Method and Solvent Selection

Basil and kersen leaves that have undergone the determination process are then prepared by maceration with ethanol serving as the solvent. Variations in the resulting yield values are influenced by the duration and volume of the solvent used, as increasing the intensity of contact between the solvent and the material can optimize the extraction of secondary metabolites such as tannins, flavonoids, polyphenols, and terpenoids. Furthermore, selecting a solvent with a polarity level that is appropriate or close to the polarity of the target compound also plays a role in increasing the efficiency of the extraction process for these compounds (Prasetya 2020). Ethanol 96% It was selected due to its selectivity, non-toxic, well absorbed, and highly efficient in extracting non-polar, semi-polar, and polar compounds. Compared to lower ethanol concentrations, 96% ethanol penetrates plant cell walls more effectively, producing a more concentrated extract. Ninety-six percent ethanol was selected as the solvent due to its universality, non-toxicity, high extraction capacity, and ability to extract compounds of varying polarity while penetrating cell walls more effectively than lower ethanol concentrations (Vita Wendersteyt et al., 2021).

3.2.2 Phytochemical Profile of Kersen and Basil Leaf Extracts

This is proven by the content of secondary metabolite compounds obtained through identification results in ethanol extracts of basil leaves and kersen leaves, which show positive content of flavonoids, tannins, phenolics, saponins, alkaloids and tannins for kersen leaves, while the ethanol extract of basil leaves contains flavonoids, phenolics, saponins, tannins, alkaloids and steroids. This result is consistent with the findings of Sri Marfuati, who reported that the ethanol extract of kersen leaves contains several bioactive compounds, including flavonoids, steroid, tannins, and triterpenoids. (Marfuati et al., 2024). Alkaloids have 2 compound components, namely alkaloid salts which are polar and free alkaloids which are semi-polar, so the use of semi-polar organic solvents is adjusted to the properties of the free alkaloid compounds (Fadhly (2015)). Flavonoids bound to aglycones make them semi-polar, making them soluble in semi-polar solvents. Polyphenols are compounds containing –OH groups that can also dissolve in semi-polar solvents (Gazali et al., 2025).

3.2.3 Yield Differences Between Kersen and Basil Leaf Extracts

The extract yield value is calculated by comparing the weight percentage of the final product (extract, oil, etc.) to the weight of the initial raw material after the extraction or processing process. Yield data is closely related to the active compounds in a sample. A higher yield was obtained from kersen leaves (Muntingia calabura L.) at 27.89%, compared to basil leaves (Ocimum basilicum L.), which yielded 11.44%. This difference in yield is influenced by the variation in the type of leaves used, because each leaves contains different compounds. Other factors that also affect the yield include the natural characteristics of the compound, the method and duration of extraction, the size of the material particles, and the ratio between the solvent and the sample (Harborne, 1987). The kersen leaf ethanol extract showed the highest yield (27.89%), suggesting a higher content of polar bioactive compounds than basil leaves (Ocimum basilicum L.).

3.2.4 Antibacterial Activity Based on Concentration Variations

Antibacterial activity of kersen and basil leaves extracts was tested using the well diffusion method. The disc diffusion method offers advantages requires no special equipment and is more flexible, making it easier to perform. It also has a conformity rate of 82%-100% (Yolla Arinda Nur Fitriana et al., 2019). The media used in this test is Mueller Hinton Agar (MHA) because it has neutral properties so it does not affect the test procedure and has good nutritional content for bacterial culture (Utomo et al., 2018). The 96% ethanol concentrations of Kersen leaves and Basil leaves used in this test were 20%, 35%, 45%, 50%, and 75%. The purpose of varying the extract concentrations was to determine which concentration had good antibacterial activity. According to the mean inhibition zone data in Table 3 and Table 5, the 96% ethanol extracts of kersen leaves and basil leaves with concentrations of 20%, 35%, 45%, 50%, and 75% could inhibit Salmonella Typhi as indicated by the formation of a clear zone in the test. Several factors can influence the formation of the inhibition zone, such as the turbidity of the bacterial suspension. If the bacterial suspension is more turbid than the standard, the resulting inhibition zone diameter will be larger, and vice versa (Zeniusa et al., 2019). In this study, it was conducted visually using a 0.5 McFarland standard as a comparison. Incubation temperature can also be a factor in the formation of inhibition zones. Incubation temperatures below 35ºC will result in a larger inhibition zone diameter. Optimal incubation temperatures will also achieve optimal growth (Zeniusa et al., 2019). In this study, the incubation temperature was 37ºC. Furthermore, the thickness of the agar medium can also be a factor in the formation of inhibition zones.

As presented in Table 3 and Table 4, kersen and basil leaf extracts demonstrated inhibition of Salmonella Typhi. The inhibitory effect on Salmonella Typhi was concentration-dependent, with higher concentrations showing stronger activity. The results of the test of kersen leaves against Salmonella thypi bacteria (Table 3) with concentrations of 20%, 35%, 45%, 50%, and 75% showed inhibition zones of 4.70 ± 0.34 with a weak category, 7.00 ± 0.08 with a moderate category, 8.96 ± 0.12 with a moderate category, 10.5 ± 0.04 with a strong category, 13.15 ± 0.32 with a strong category. The results of the basil leaves test against Salmonella thypi bacteria (Table 4), 7.30 ± 0.30 with a moderate category, 9.93 ± 0.08 with a moderate category, 11.63 ± 0.12 with a strong category, 12.76 ± 0.04 with a strong category, 14.85 ± 0.32 with a strong category. Based on Table 3 and Table 4, the inhibitory capacity of basil leaves is higher than that of kersen leaves.

The average inhibition zone diameter of Salmonella Typhi increased in line with the rising concentrations of both kersen and basil leaf extracts. These findings are in agreement with previous studies reporting that higher extract concentrations result in larger inhibition zones. Research by Mustika Weni (2024) demonstrated that the ethyl acetate fraction of kersen leaves was capable of inhibiting Staphylococcus aureus and Escherichia coli, with greater inhibition observed at higher concentrations. Similarly, Sri Marfuati (2024) reported that the ethyl acetate fraction of basil leaves exhibited inhibitory activity against Escherichia coli, showing variations in inhibition zone diameters across different concentrations.

This is in accordance with the classification according to Davis and Stout antibacterial strength is divided into four categories, namely weak inhibition power (20 mm) (Davis & Stout, 1971). Basil leaf ethanol extract showed stronger antibacterial activity against S. Typhi than kersen leaf ethanol extract. At 75% concentration, basil leaf extract achieved its highest antibacterial effect, with a mean inhibition zone of 14.85 mm (strong category). For kersen leaf extract, the optimal concentration was 75%, producing a mean inhibition zone of 13.15 mm (strong category). Based on the results of previous studies, it was stated that the kersen leaves fraction has antibacterial activity against other bacteria, namely Escherichia coli and Staphylococcus aureus at a concentration of 20% having an inhibitory power of 8,817 mm and 7,987 mm in the moderate category (Marfuati et al., 2024). The difference in inhibitory power between the extract and fraction of kersen leaves occurs because the crude extract contains more complex secondary metabolite compounds than the fraction. The contributing factor is due to the antagonistic activity between the secondary metabolites in the crude ethanol extract of kersen leaves, so that their work is not synergistic in inhibiting bacteria. The negative control, 100% DMSO used as the solvent for the ethanol extracts of kersen and basil leaves, showed no inhibition zone, indicating that the solvent itself had no effect on the antibacterial activity. This is influenced by the level of concentration of the extract, the type of bacteria used, the type of solvent used, and the different sensitivity of each bacteria (Sudarwati & Sumarni, 2016).

3.2.5 Mechanistic Insights: Role of Phytochemicals in Antibacterial Activity

Basil leaves extract has higher activity in inhibiting bacterial growth compared to kersen leaf extract, this is influenced by secondary metabolite compounds such as tannins, alkaloids, saponins and flavonoids. while basil leaves contain alkaloids, flavonoids, phenolics, steroids and saponins which have an important role in the difference in effectiveness in inhibiting bacterial growth (Andri Priono, n.d.). The most active secondary metabolites are flavonoids. This is due to the polar nature of flavonoids, which more easily penetrate the peptidoglycan layer of bacterial cell membranes (Sudarwati & Sumarni, 2016). Basil leaves contain phenolics and steroids that can inhibit bacterial growth. Phenolics can interfere with enzymes in bacteria that precipitate proteins into toxins. Steroids work by causing bacterial cells to leak, specifically steroid-sensitive lysosomes. The steroids interact with bacterial cells to make them permeable, causing changes in the morphological components that make up bacterial cells. This makes the cells fragile and causes cell lysis.

Phytochemically, kersen leaves are known to contain flavonoids (quercetin, kaempferol) saponins and polyphenolic compounds possessing antioxidant. (Setiabudi et al.,2020), anti-inflammatory, as well as antibacterial potential (Marfuati et al., 2024). Basil leaves are also rich in flavonoids (apigenin, orientin, vicenin), essential oils (eugenol, linalool), and tannins. These compounds work through mechanisms such as free radical scavenging, modulating inflammatory enzymes, and disrupting microbial membranes. At sufficiently high concentrations, the number of active molecules available is sufficient to cover all target areas, so increasing the dose does not significantly increase the effect.

3.2.6 Interpretation of Statistical Differences (Post Hoc Analysis)

Post hoc test was conducted after ANOVA analysis showed significant differences between treatment groups, aimed to specifically identify which pairs of groups were significantly different. In this study, comparisons were made on various concentrations of Kersen (Muntingia calabura L.) and basil (Ocimum basilicum L.) ethanol extracts tested at multiple concentrations of 20%, 35%, 45%, 50%, and 75% with negative control (DMSO). Based on Table 6, the results of the post hoc test showed that there were four pairs of groups that were not significant (p>0.05), namely: 45% concentration of kersen leaves extract was not significant with 35% basil leaves extract and 50% concentration of kersen leaves extract with 45% basil leaves extract and there were 26 significant groups (p<0.05). This insignificance indicates that at this mid-concentration range, the dissolved active compound has reached its maximum biological effect threshold, so that increasing the concentration no longer results in a significant difference in response. This phenomenon can be explained by the theory of receptor saturation, or the optimum point of bioactive compound activity, where, after a certain concentration, the compound's binding to the biological target reaches its maximum capacity. In contrast, the other 26 group pairs showed significant differences (p<0.05). This suggests that changes in concentration at lower or significantly different levels can affect the levels of active compounds interacting with biological targets, resulting in significant differences in response. At low concentrations, the amount of active compound available may not be sufficient to elicit maximum effect, so increasing the concentration proportionally increases the measurable biological effect.

Overall, the results of this post hoc test indicate that varying the concentration of ethanol extracts of cherry and basil leaves affects the biological responses, with most differences being significant. However, at a certain intermediate concentration, the effect tends to stabilize due to the saturation point of phytochemical action being reached. This finding is important for determining the optimal concentration in pharmacological applications or functional foods, thus maximizing biological benefits without wasting raw materials.

4. Conclusion

This research confirmed that kersen and basil leaf ethanol extracts exhibit antibacterial activity against Salmonella typhi across various concentrations. Higher concentrations produced stronger inhibition, indicating that both extracts have meaningful potential as natural antibacterial agents for typhoid.

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