Combination of Antibacterial Activity of Ethanol Extract of Meniran Leaves and Kenikir Leaves Against *Shigella dysenteriae*

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Abstract. Shigellosis is an acute inflammatory disease in the human digestive tract. One of the pathogenic bacteria that causes shigellosis is *Shigella dysenteriae*. Some natural ingredients believed to have good antibacterial activities are Meniran (*Phyllanthus niruri* L.) and Kenikir (*Cosmos caudatus* Kunth.). This study aimed to determine the compound groups and evaluate the antibacterial activity combination of Meniran (*Phyllanthus niruri* L.) and Kenikir (*Cosmos caudatus* Kunth.) leaf ethanolic extracts against *Shigella dysenteriae.* The ethanol extract of meniran leaves (M) and kenikir leaves (K) was made in five different combinations with three replications. The extraction was carried out by maceration method using a 96% ethanol. The concentration of the extract used was 50% with 3% DMSO dilution. The chemical compound groups were identified using various chemical reagents. The antibacterial activity test was carried out by the diffusion method. The antibacterial activity data of Meniran and Kenikir leaf ethanolic extracts were analyzed by the analysis of variance. The results showed that the ethanolic extracts of Meniran and Kenikir leaves contained several compound groups, such as saponins, tannins, alkaloids, flavonoids, and polyphenols. The ethanolic extract of Meniran leaves contains flavonoids, tannins and alkaloids with consentrations of 37.6 mgQE/g, 141 mgTAE/g, and 4.28 mgK/g, respectively. The ethanolic extract of Kenikir leaves contains flavonoids, tannins and alkaloids with consentrations of 33.8 mgQE/g, 76 mgTAE/g, and 4.603 mgK/g, respectively. The ethanolic extracts of Meniran and Kenikir leaves had antibacterial activity against *S. dysenteriae*. The most effective antibacterial composition was M 1:0 K, with an inhibition zone of 14.3 mm. The best antibacterial activity against *Shygella dysenteriae* is found in Meniran leaf ethanolic extract without combination with Kenikir.

Keywords: *Shigella dysenteriae* · meniran · kenikir · antibacterial activity

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1 Introduction

Shigella sp is a genus of bacteria, characterized as Gram negative, rod-shaped, immobile, non-spore-forming, and facultative-anaerobic bacteria. *Shigella* sp. is generally divided into four subgroups, namely *S.dysentriae* (subgroup A), *S. flexneri* (subgroup B), *S.boydii* (subgroup C), and *S.sonnei* (subgroup D) with multiple serotypes [1, 2]. *Shigella* sp. is a diarrheal disease causative pathogenic agent, that causes bacillary dysentery (shigellosis) throughout the world, especially affecting children under 5 years old [3–5]. The shigellosis cases are estimated to reach 165 million cases annually worldwide, as 99% of the cases occurs in developing countries, which are mostly reported in children (69%). *Shigella* sp. has also recently been found causes an increased mortality level in children due to diarrhea, reaching at 1.1 million worldwide. *S. boydii* and *S. sonnei* cause mild diarrheal disease (watery diarrhea or bloody diarrhea), while *S. dysentriae* and *S. flexneri* cause endemic and epidemic shigellosis cases in the developing countries with high transmission rates. *S. dysenteriae* (sub group A, also known as Shiga bacillus) can cause more severe and prolonged illness, leading to death [6].

Previous studies showed that *Shigella* was resistant to several antibiotics, including ampicillin (83.1%), amoxicillin (84.1%), erythromycin (86.5%) [5], tetracycline (88.4%), and trimethoprim-sulfamethoxazole (82.9%) [7]. Antibiotic resistance can occur due to several mechanisms, such as decreasing the cellular membrane permeability, extrusion by active efflux pumps, overexpression of drug-modifying enzymes, and inactivating the target enzyme modifications by mutation [1]. The use of antibiotics is challenging, because it results in an increased multi-resistant microbial strains. Thus, an innovation about alternative medicines from plants is necessary, which have antimicrobial potentials [8]. The medicinal potential plants have advantages, such as low toxicity, environmentally safe, and residual absence [9].

Indonesia is biodiversity rich tropical country, especially plants with medicine potential. Many plants have antibacterial potentials, containing bactericidal compounds (bacterial killer) and bacteriostatic compounds (bacterial growth inhibitors) [9]. Several plants which have medicinal and antibacterial potentials are *Meniran* (*Phyllanthus niruri* L.) [10] and *Kenikir* (*Cosmos caudatus* Kunth) [11]. *Meniran* plants can grow wild in the tropical climate and have been used as an antibacterial agent [12]. Also, Meniran has been widely used as an antiviral, an anti-tumor, an anti-carcinogenic [13], an anti-inflammatory, an antioxidant, a hepatoprotection agent, an immunomodulator, and an antifungal [14]. The results showed that the active compounds of *Meniran* leaves include terpenoids, phenols, alkaloids, flavonoids, saponins, tannins [15], lignin, fatty acids (ricinoleic acid, linoleic acid, linolenic acid), vitamin C, potassium, resin, and geranin [16].

Kenikir plants (*Cosmos caudatus* Kunth) are found in tropical areas, such as Indonesia, Malaysia, Thailand, South America, Mexico, and the United States. This plant is classified in the Asteraceae family, *Cosmos* genus, and *C. caudatus* Kunth species [17]. *Kenikir* leaves are usually used by the community as salads, fresh vegetables, or as appetizers due to distinctive taste and aroma. *Kenikir* leaves have been used for blood circulation improvement, body heat reduction, bone marrow strengthening (due to high calcium content), anti-aging agent, to fresh breath promoter, and infection treatment associated with pathogenic microorganisms [18]. The results showed that *kenikir*

leaves contain active compounds, including flavonoids, phenolic acids, anthocyanins, phenolics, flavonols, flavones [19], ascorbic acid, quercetin, chlorogenic acid, and catechin [20]. According to previous studies, the ethanolic extract of meniran leaves had antibacterial activity against Staphylococcus aureus (Dr. Moewardi Hospital isolate) and Staphylococcus aureus (laboratory isolate) with inhibitory zones of 20 mm and 18 mm, respectively [16]. *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtillis,* and *Escherichia coli* can be inhibited by kenikir leaves extracted with n-hexane, diethyl ether and ethanol as solvents [18]. *Meniran* and *Kenikir* leaves both have antibacterial activity, which requires a further research on the combination of those species. The combination of *Meniran* and *Kenikir* leaves is expected as an alternative antibacterial drug against *S. dysentriae.*

2 Materials and Methods

2.1 Materials

Meniran and *Kenikir* leaves were obtained from the Center for Research and Development of Medicinal Plants and Traditional Medicines, *Tawangmangu*, *Karanganyar*, Central Java, Indonesia. *S. dysentriae* were obtained from diarrheal patient and isolated from the Microbiology laboratory, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia. *S. dysentriae* bacteria were characterized by biochemical assays and culture. The Ethanol 96%, potassium acetate, chloroquine, bromocresol green, chloroform, phosphate buffer were obtained from *Merck*, *Darmstadt*, *Germany*. The study used agar media for bacterial growth and antibacterial test: Brain-heart infusion agar media, Muller-Hilton agar media, Salmonella-Shigella agar media, Kligler's iron agar media, lysine iron agar media, sulfide indo motility (SIM) media, and Citrate media obtained from *Merck*, *Darmstadt*, *Germany*. The compound groups for identification materials: HCl 2N, FeCl3, Dragendroff's reagent, Quercetin, AlCl3, tannic acid, foline reagent, Na2CO3, concentrated HCl, Ethanol 96%, FeCl3 5%, were purchased from *Sigma Aldrich*, *Singapore.* Materials for Gram staining (Crystal violet, iodine, alcohol-acetone, and safranin), immersion oil, aquadest, ethanol, DMSO 2% as negative control were obtained from *Sigma Aldrich, Singapore*. Antibacterial test was carried out using Ciprofloxacin (5 μg/ml) as positive control.

2.2 Moisture Content Determination of *Meniran* and *Kenikir* Leaf Powders

The moisture contents were determined using a Bidwel-Sterling tool with a xylene solvent. The powder was weighed at 20 g, then added with 20 ml of xylene solvent in a rounded bottom flask. The condenser was mounted on a rounded bottom flask, containing the solution. This method was carried out three times in circulation, with no water dripping on the scale tube, occurred for 15 min [12].

2.3 Extraction of *Meniran* and *Kenikir* Leaves

Meniran and *Kenikir* leaf powders were weighed and mixed according to the ratio in Table 1. Mixing kenikir leaf powder and meniran leaf powder was based on previous

Table 1. Ratio comparison of Meniran and Kenikir leaf powders

Ratio of *Meniran* leaf powder: *Kenikir* leaf powder	*Meniran* leaf powder (*P. niruri* L.) (g)	*Kenikir* leaf powder (*C. caudatus* Kunth.) (g)
1:0	100	0
2:1	67	33
1:1	50	50
1:2	33	67
0:1	0	100

Table 2. Procedures for identification of compound groups in Meniran and Kenikir [24]

No	Group of compounds	Procedure of identification
1	Flavonoids	Extract 2 ml + 2 ml 96% ethanol heated + 0.05 g zinc powder + 2 ml 2N HCl (let stand) + 2 ml concentrated HCl
2	Tannins	Extract 2 ml + 10 ml hot distilled water (dissolved) + 3 drops $FeCl_3$ 1%
3	Alkaloids	Extract 2 ml + 2 ml HCl heated + 3 drops Dragendroff's reagent
4	Saponins	Extract 2 ml + 10 ml hot distilled water (dissolved) + 2N HCl, shake
5	Polyphenol	Extract 2 ml + 10 ml hot distilled water (dissolved and filtered). Filtrate + 5 drops of 5% $FeCl_3$.

research [21]. The 100 g of *meniran* and kenikir leaf powders were macerated with ethanol 96% added in a ratio of 1:10 (100 g powder + 1 L of ethanol 96%). The maceration was performed for 5 days with occasional stirring. The maceration results were filtered with a filter paper, until the filtrate was obtained. The filtrate was then concentrated by using a rotary evaporator at 400C to obtain a thick extract of *Meniran* and *Kenikir* leaves. The ethanolic extract of *Meniran* and *Kenikir* leaves used for antibacterial activity test was at 50% concentration [21, 22].

2.4 Extract Compound Group Identifications

The compound groups were identified to determine the compound classes: saponins, flavonoids, tannins, polyphenols, and alkaloids in each extract (*Meniran* and Kenikir leaves). The identification process was performed using several chemical reagents according to the previous method [23]. The determination of the group of compounds in Meniran and Kenikir can be seen in Table 2.

2.5 Measurement of Total Flavonoid, Total Tannins and Total Alkaloids in *Meniran* and *Kenikir* Leaves

Total flavonoid. A UV-Vis spectrophotometer was used to measure the total flavonoid content. With a few minor adjustments, measurements of total flavonoids are based on prior research (Chang et al., 2002). The standard used was quercetin. 10 mg of Quercetin was weighed and 10 ml of 70% ethanol were used to dissolve it, yielding a 1000 g/ml concentration. The quercetin stock solution was subsequently prepared serially at concentrations of 20 g/ml, 40 g/ml, 60 g/ml, 80 g/ml and 100 g/ml. One milliliter of each dosage of quercetin was ingested, along with one milliliter of 2% AlCl3 and 120 mM potassium acetate. After that, the solution was incubated for 60 min at room temperature. The absorbance of each solution was then assessed using a UV-Vis spectrophotometer with a wavelength of 435 nm. Measurements were carried out with 3 repetitions [25].

Ten milligrams of the ethanolic extracts of Meniran and Kenikir leaves were combined with ten milliliters of 70% ethanol. The concentration of each extract was 1000 g/ml. 1000 g/ml extract concentration was diluted in 1 mL with 1 mL of 2% AlCl3 and 1 mL of 120 mM potassium acetate. The solution was then incubated for 60 min at room temperature. The absorbance of each solution was then assessed using a UV-Vis spectrophotometer with a wavelength of 435 nm. Measurements were carried out with 3 repetitions [25].

Total Tannin Level. Using a UV-Vis spectrophotometer and tannic acid as the standard, the total alkaloid content was measured. 10 mg of tannic acid in total was weighed and dissolved in 10 mL of distilled water, to obtain tannic acid concentration of 1000 ppm. Then the tannic acid was produced in series at concentrations of 10, 15, 20, 25, 30 and 35 ppm. Each concentration of tannic acid was taken 1 ml and added 1 mL of Folin reagent and incubated for 5 min. The solution was then added to 1 mL of saturated Na2CO3 reagent and incubated for 40 min. Each solution was then measured its absorbance using a UV-Vis spectrophotometer with a wavelength of 649.9 nm. Measurements were carried out with 3 repetitions [25].

10 mg of the ethanolic extract of Meniran and Kenikir leaves were weighed and 10 ml (1000 ppm) of distilled water were added and replicated 3 times. Each replication was made at a concentration of 200 ppm. The extracts were then taken 1 mL each and added 1 mL of Folin Denis reagent, allowed to stand for 3 min, added 1.0 mL of saturated Na2CO3 solution and incubated for 40 min. The absorption was then measured at a wavelength of 649.9 nm [25].

Total Alkaloid Level. Measurement of total alkaloid content was carried out using a UV-Vis spectrophotometer and the standard used was chloroquine. Total alkaloid content can be determined by adding Bromocresol green (BCG) reagent. A 40 ppm chloroquine solution was created by weighing 10 mg of chloroquine in total and dissolving it in 25 mL of distilled water. Next, 0.5; 1.0; 1.5; 2.0; 2.5; 3.0 mL of the solution are taken and placed in a separating funnel with 5 mL of phosphate buffer and 5 mL of BCG. The solution was partitioned 2X with chloroform and the chloroform phase was taken. The chloroform phase was centrifuged for 10 min at 3000 rpm. Then 1 mL of the solution was taken and put in a 10 mL volumetric flask to obtain a standard solution of chloroquine with concentrations of 2, 4, 6, 8, 10 and 12 ppm. Each solution was

then measured its absorbance using a UV-V is spectrophotometer with a wavelength of 420 nm. Measurements were carried out with 3 repetitions [26].

The ethanolic extracts of Meniran leaves and Kenikir leaves were weighed at 10 mg each and diluted in 10 ml of distilled water. The solution was ultrasonicated for 10 min and centrifuged for 10 min at 3000 rpm. 1 mL of the solution was taken and added with 5 mL of phosphate buffer and 5 mL of BCG. The solution mixture was then partitioned by adding 5 mL of chloroform. The chloroform phase was taken 1 ml and put in a 10 ml volumetric flask. Each solution was then measured its absorbance using a UV-Vis spectrophotometer with a wavelength of 420 nm. Measurements were carried out with 3 repetitions [26].

2.6 Bacterial Isolate Characterization

Bacterial isolates were characterized by culture on Salmonella-Shigella-Agar (SSA) selective medium and stained using Gram stain. Colonies were identified using biochemical media including Kligler iron agar (KIA), Sulfide Indole Motility (SIM), Lysine Iron Agar (LIA) and Simmons Citrate Medium [27, 28].

2.7 Antibacterial Activity Combination Test of *Meniran* and *Kenikir* Leaf Extract with the Disc Diffusion Method

The antibacterial activity combination test of *Meniran* and *Kenikir* leaf ethanolic extracts were performed made with 50% concentration. Previous studies were used to establish the concentration, and it was found that pathogenic bacteria may be inhibited at a 50% concentration [21]. There were five combinations of Meniran and Kenikir leaf ethanolic extract, according to the Table 1, and the test was carried out with three replications, using ciprofloxacin as a positive control and DMSO 3% as a negative control. The *S. dysentriae* bacterial culture was grown on 5 ml of Brain Heart Infusion (BHI) media and incubated for 24 h at 370C in an incubator [28]. The BHI media with grown bacterial colonies were standardized using a *Mc. Farland* method at 1,5x108 cfu/ml [29]. The paper disk was prepared for antibacterial activity test of the *meniran* and *kenikir* leaf ethanolic extracts. Paper disk was soaked in five different ethanolic extracts based on the combination ratios in Table 1 at 50% concentration and stood for 24 h. The bacterial colonies grown on the BHI media were scratched evenly on the Muller-Hilton Agar (MHA) Media. The MHA medium was divided into seven sections for placing the paper disc samples (five sections for soaked paper disc samples), positive control (ciprofloxacin), and negative control (DMSO 3%). The incubation was performed for 24 h at 37 °C. The antibacterial activity test results were observed by measuring the inhibition zone formed around the paper disc samples [14].

2.8 Data Analysis

The data obtained from the antibacterial activity combination test of *Meniran* and *Kenikir* leaf extracts against *S. dysenteriae* bacteria were the inhibition zone diameter. Data were analyzed using statistical tests, following the Analysis of Variance (ANOVA) test.

Table 3. The moisture contents of *Meniran* and *Kenikir* leaf powders

Powder type	Material weight (grams)	Scale (ml)	Moisture content (%)
Meniran	20.0031	1.7	8.49
Kenikir	20.0032	1.8	8.99

Table 4. The yield of *Meniran* and *Kenikir* leaf ethanolic extract.

Ratio of *Meniran* leaf powder: *Kenikir* leaf powder	*Meniran* leaf powder (*P. niruri* L.) (g)	*Kenikir* leaf powder (*C. caudatus* Kunth.) (g)	Extract (g)	Yield (%)
1:0	100	0	11.996	11.9
2:1	67	33	12.502	12.5
1:1	50	50	13.028	13
1:2	33	67	12.564	12.5
0:1	0	100	12.735	12.7

3 Results

3.1 Moisture Content of *Meniran* and *Kenikir* Leaf Powders

The moisture contents of *Meniran* and *Kenikir* leaf powders can be shown in Table 3. The moisture content of *Meniran* leaf powder was 8.49%, while *Kenikir* leaf powder was 8.99%. The moisture contents of *Meniran* and *Kenikir* leaf powders were less than 10%.

3.2 The Combination of *Meniran* and *Kenikir* Leaf Ethanolic Extract

The combination of *Meniran* and *Kenikir* leaf ethanolic extract can be shown in Table 4. The extraction results showed the highest yield at 13% (ratio of *Meniran* and *Kenikir* = 1:1), while the smallest was at 11.9% (ratio of *Meniran* and *Kenikir* = 1:0).

3.3 Group Compound Identifications

The *Meniran* and *kenikir* leaf ethanolic extract compound groups were identified by various chemical reactions. The identification results can be seen in Table 5, which indicate that the ethanolic extracts contain saponins, flavonoids, tannins, polyphenols, and alkaloids.

Table 5. Compound group identification of *meniran* and *kenikir* leaf ethanolic extracts

Group Compounds	Results	
	Meniran	*Kenikir*
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Polyphenols	+	+
Alkaloids	+	+

Table 6. Total flavonoid, total tannin, total alkaloid of Meniran leaves and kenikir leaves

No	Group of compound	Leaf extract	
		Meniran	Kenikir
1	Flavonoids	37.6 mgQE/g	33.8 mgQE/g
2	Taninns	141 mgTAE/g	76 mgTAE/g
3	Alkaloids	4.28 mgK/g	4.603 mgK/g

3.4 Total Flavonoid, Total Tannins and Total Alkaloids of *Meniran* and *Kenikir* Leaves

The results of measurements of total flavonoids, total tannins and total alkaloids can be seen in Table 6. Compared to flavonoids and alkaloids, tannins are present in greater amounts in Meniran and Kenikir leaves.

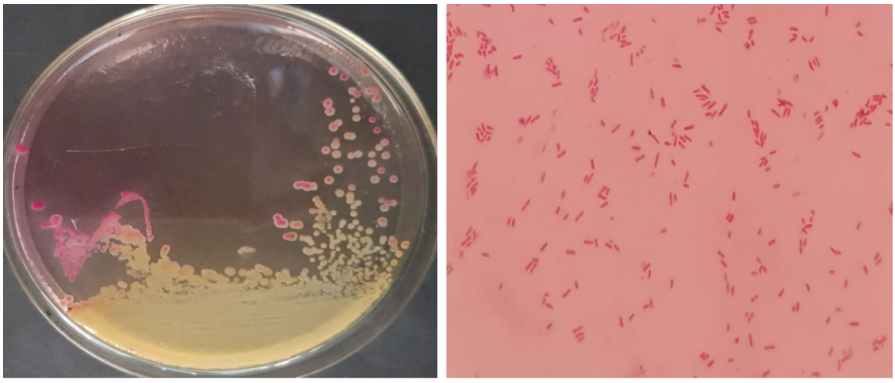
3.5 Bacterial Isolate Characterization

The results of bacterial isolate culture on Salmonella-Shigella-Agar (SSA) medium can be seen in Fig. 1.A. Gram staining revealed the bacterial isolates to be gram negative bacteria (Fig. 1.B).

The findings of the biochemical assays used to characterize bacterial isolates are shown in Table 7.

3.6 Antibacterial Activity of *Meniran* and *Kenikir* Leaf Ethanolic Extracts Against *S. Dysentriae*

The antibacterial combination activity results of *Meniran* and *Kenikir* leaf ethanolic extracts against *S. dysenteriae* can be seen in Table 8. Table 8 shows the calculated value of inhibition zone diameter (*Meniran* and *Kenikir* leaf ethanolic extracts) against *S. dysentriae.*



A B

Fig. 1. A: image of bacterial isolates cultured on Salmonella-Shigella-Agar (SSA) media, B: staining of bacterial isolates.

Table 7. Biochemical assays of *S.dysentriae* bacterial isolates

Assay	**Tested *S. dysentriae***	**Reference**
Motility	-	-
Glucose	+	+
Lactose	-	-
Sucrose	-	-
Indole	-	-
H_2S	-	-
Methyl Red	+	+
Sulfide	-	-
Lysine	-	-
Simmons Citrate	-	-

4 Discussions

The main materials of this study were *Meniran* and *Kenikir* leaf powders. The moisture contents of both materials were tested. The moisture content results showed that *Meniran* and *Kenikir* leaf powders had a moisture content below 10% (Table 3). The moisture content is important to prevent the fungal and bacterial growth in the material. High moisture content can also cause enzymatic reactions that affect the metabolite contents. This condition was similar to Tran and Nguyen [30], who stated that dry ingredients can avoid bacterial and fungal contaminations, related to the good quality and healthy product substance contents. In addition, medicinal plant drying is also beneficial in maintaining the quality of the harvested plants, besides reducing the plant weight and mass for cheaper

Table 8. The antibacterial combination activity of *meniran* and *kenikir* leaf ethanolic extracts against *S. dysenteriae*

Ratio of *Meniran* and *Kenikir* leaf powders	Replications (mm)			Average inhibition zone diameter (mm)
	One	Two	Three	
1:0	15	14	14	14.3^{c}
2:1	14	14	13	13.6^{c}
1:1	13	12	13	12.6^{bc}
1:2	11	12	12	11.6^{b}
0:1	12	11	10	11^{b}
Negative control (DMSO 3%)	0	0	0	0^{a}
Positive control (Ciprofloxacin 5μg/ml)	21	22	24	22.3^{d}

Note: The $^{a-d}$ symbols indicate significant differences between treatments ($p < 0.05$).

transportation and storage. Previous studies showed that [31] the medicinal plant drying at less than 700C can increase the availability of phenolic compounds in plants.

Extraction is an active compound transfer process from the test plant cells into the solvent. The active compounds that can be extracted are determined by the solvent type used in the extraction [32]. This study used ethanol 96% solvent, based on the previous study [20], which can dissolve polar to non-polar active compounds. Previous studies [33] have shown that ethanol 96% solvent can dissolve several compounds: flavonoids, tannins, saponins, alkaloids, phenolics, and steroids. The extraction results (Table 4) showed that there were variations among the *Meniran* and *Kenikir* leaf ethanolic extracts. These variations showed the plant diversity. Plant diversity is not only shown by morphological variations and genetic variations, but also variations in extraction product results, known as plant secondary metabolites. These variations can be caused by different plant species [34].

The Meniran leaf ethanolic extract contained: saponins, tannins, flavonoids, polyphenols, and alkaloids (Table 5). This was in accordance with several previous studies that *meniran* leaves contained saponins, tannins, flavonoids [12], alkaloids [10, 16], polyphenols [14]. A previous study has shown that *Kenikir* leaves contained saponins, tannins, alkaloids, flavonoids, and polyphenols [20, 35–37], following the present study (Table 5). These plant secondary metabolites can inhibit and kill the bacteria. Also, these compounds can damage the building blocks of bacterial cells and cause structure and working alterations in bacteria [10].

The active compounds in the *Meniran* and *Kenikir* leaf ethanolic extracts can inhibit the bacterial growth. Tannins can inhibit the bacterial growth by precipitating protein from enzymes produced by bacteria, causing an inactive protein and inhibited bacterial growth [9]. Tannins are also toxic compounds that can damage the bacterial cell membranes by inhibiting certain enzyme productions. Other antibacterial compounds are saponins. Saponins can increase the membrane permeability, causing a membrane proteins denaturation and cell lysis. Alkaloids can damage the peptidoglycan component

in bacterial cells, so that the cell wall layer is incompletely formed, resulting in a cell death [10]. Flavonoids are antibacterial and antioxidant properties, which can enhance the immune system performance, because leukocytes as antigen-eaters are produced faster and lymphoid systems are activated faster [9].

Table 8 shows the antibacterial combination activity of *Meniran* and *Kenikir* ethanolic extracts. The ratio combination of *Meniran*: *Kenikir* at 1:0 obtained the strongest antibacterial activity compared to other combinations. In this study, the combination of *Meniran* and *Kenikir* leaf ethanolic extracts had no synergistic properties to inhibit the *S. dysentriae* growth. This was supported by a previous study that the combination of medicinal plants had no synergistic properties in bacterial growth inhibition [16]. Kristen and Kasmiyati, 2021 [22] stated, that the combination of *Phyllanthus niruri*, *Euphorbia hirta*, and *Loranthus* sp. leaf extracts at a ratio = 0:0:1 obtained the strongest antioxidant activity. This means that the combination of herbal plant extracts can have antagonistic properties. The findings of this study show that meniran can inhibit *S. dysentriae* bacteria without combination with Kenikir. Not all herbal plants can be combined to provide more potent antibacterial activity. The combination of Meniran and Kenikir used in this study in ratios of 1:0, 2:1, 1:1, 1:2, 0:1, and this combination was only tested on *S. dysentriae* bacteria (this was a limitation of the problem in this study).

5 Conclusion

The combination of ethanolic extract of *Meniran* leaves and *Kenikir* leaves can inhibit *Shigella dysentriae*. The strongest inhibition against *Shigella dysentriae* was the ethanolic extract of meniran leaves without the combination of kenikir.

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References

1. Ranjbar R, Farahani A. Shigella: Antibiotic-resistance mechanisms and new horizons for treatment. Infect Drug Resist. 2019; 12: 3137–67.
2. Sheikh AF, Moosavian M, Abdi M, Heidary M, Shahi F, Jomehzadeh N, et al. Prevalence and antimicrobial resistance of shigella species isolated from diarrheal patients in Ahvaz, Southwest Iran. Infect Drug Resist. 2019; 12: 249–53.
3. Herwana E, Surjawidjaja JE, Salim OC, Indriani N, Bukitwetan P, Lesmana M. Shigella-associated diarrhea in children in South Jakarta, Indonesia. Southeast Asian J Trop Med Public Health. 2010; 41(2): 418–25.
4. Orrett FA. Prevalence of Shigella serogroups and their antimicrobial resistance patterns in Southern Trinidad. J Heal Popul Nutr. 2008; 26(4): 456–62.
5. Hussen S, Mulatu G, Yohannes Kassa Z. Prevalence of Shigella species and its drug resistance pattern in Ethiopia: A systematic review and meta-analysis. Ann Clin Microbiol Antimicrob [Internet]. 2019; 18(1): 1–11. Available from: https://doi.org/10.1186/s12941-019-0321-1

6. Williams PCM, Berkley JA. Guidelines for the treatment of dysentery (shigellosis): a systematic review of the evidence. Paediatr Int Child Health [Internet]. 2018; 38: S50–65. Available from: https://doi.org/10.1080/20469047.2017.1409454.
7. Chang Z, Zhang J, Ran L, Sun J, Liu F, Luo L, et al. The changing epidemiology of bacillary dysentery and characteristics of antimicrobial resistance of Shigella isolated in China from 2004–2014. BMC Infect Dis [Internet]. 2016; 1–10. Available from: https://doi.org/10.1186/s12879-016-1977-1.
8. Wambe H, Noubissi PA, Fokam Tagne MA, Foyet Fondjo A, Fankem GO, Kamtchouing I, et al. Anti-Shigellosis Activity of Cola anomala Water/Ethanol Pods Extract on Shigella flexneri- Induced Diarrhea in Rats. Biomed Res Int. 2019; 9(1): https://doi.org/10.1155/2019/6706230.
9. Sabdoningrum EK, Hidanah S, Wahjuni RS, Chusniati S, Arimbi A. An In Vitro Antibacterial Activity Test of Meniran Herbs'(Phyllanthus Niruri L.) Ethanol Extract Against Mycoplasma gallisepticum causes Chronic Respiratory Disease (CRD) in Broiler Chickens. KnE Life Sci. 2017; 3(6): 48.
10. Hidanah S, Sabdoningrum EK, Wahyuni RS, Dewi AR, Safitri E-. Effectiveness of Meniran (Phyllanthus Niruri Linn) As Antibacterial for Resistance Antibiotics Prevention of Enterotoxin Escherichia Coli. Indones J Trop Infect Dis [Internet]. 2018; 7(2): 35. Available from: https://doi.org/10.20473/ijtid.v7i2.7328.
11. Rameli NM, Kader MA, Aznan AS, Musa N. Effect of cosmos caudatus extract on antibacterial activity and lethality activity of brine shrimp. AACL Bioflux [Internet]. 2018; 11(3): 606–12. Available from: http://www.bioflux.com.ro/aacl.
12. Dwinanti SH, Savacka MA, Sasanti AD. In Vitro Analysis: Inhibitory Effect of Phyllanthus niruri Extract Against Aeromonas salmonicida. Bioscience. 2021; 5(2): 94.
13. Ibrahim D, Hong LS, Kuppan N. Antimicrobial activity of crude methanolic extract from phyllanthus niruri. Nat Prod Commun. 2013; 8(4): 493–6.
14. Shilpa V, Muddukrishnaiah K, Thavamani Bs, Dhanapal V, Arathi K, Vinod K, et al. In vitro immunomodulatory, antifungal, and antibacterial screening of Phyllanthus niruri against to human pathogenic microorganisms. Environ Dis. 2018; 3(3): 63.
15. KH TD, Sudirman A, Juniarti DE. Daya Antibakteri EkstrakMeniran (Phyllanthus niruri linn) Terhadap Bakteri Enterococcus faecalis (Antibacterial Activity Of Phyllanthus niruri linn Extract Against Enterococcus faecalis Bacteria). Conserv Dent J [Internet]. 2016; 6(2): 99. Available from: https://doi.org/10.20473/cdj.v6i2.2016.99-104.
16. Agustin BA, Puspawaty N, Rukmana RM. Aktivitas Antibakteri Kombinasi Ekstrak Etanolik Daun Beluntas (Pluchaea indica Less.) dan Meniran (Phyllanthus niruri L.) terhadap Bakteri Staphylococcus aureus. Biomedika. 2018; 11(2): 79–87.
17. Yusoff NAH, Rukayadi Y, Abas F, Khatib A, Hassan M. Antimicrobial stability of Cosmos caudatus extract at varies pH and temperature, and compounds identification for application as food sanitiser. Food Res. 2021; 5(3): 83–91.
18. Nor Hafipah Md Rasdi1, Othman Abd. Samah1* AS and QUA. Antimicrobial studies of Cosmos caudatus Kunth. (Compositae). J Med Plants Res. 2010; 4 (8)(April): 669–73.
19. Bunawan H, Baharum SN, Noor SNB, Amin NM, Mohd N. Cosmos Caudatus Kunth: A Traditional Medicinal Herb. Glob J Pharmacol. 2014; 8(3): 420–6.
20. Nurhayati B, Rahayu IG, Rinaldi SF, Zaini WS, Afifah E, Arumwardana S, et al. The antioxidant and cytotoxic effects of Cosmos caudatus Ethanolic Extract on Cervical Cancer. Indones Biomed J. 2018; 10(3): 243–9.
21. Agustin BA, Puspawaty N, Rukmana RM. Aktivitas Antibakteri Kombinasi Ekstrak Etanolik Daun Beluntas (Pluchaea indica Less.) dan Meniran (Phyllanthus niruri L.) terhadap Bakteri Staphylococcus aureus. Biomedika [Internet]. 2018; 11(2): 79–87. Available from: https://doi.org/10.31001/biomedika.v11i2.425.

22. Kristiani EBE, Kasmiyati S. The Combination of Phyllanthus niruri, Euphorbia hirta, and Loranthus sp. as a Source of Antioxidant Agents. Biosaintifika J Biol Biol Educ. 2021; 13(2): 201–11.
23. Dian Riana Ningsih1*, Zusfahair1 DK, 1Jurusan. Identification of Secondary Metabolites Compounds and Antibacterial Activities on the Extract of Soursop Leaf Dian. Molekul,. 2016; 11(M1): 101–11.
24. Sari GNF, Rejeki ES. Uji Sitotoksik Ekstrak Etanol Daun Stevia (Stevia Rebaudiana Bertoni) pada Kultur Sel Hela. J Farm Indones. 2021; 18(2): 189–99.
25. Rebaya A, Belghith SI, Baghdikian B, Leddet VM, Mabrouki F, Olivier E, et al. Total Phenolic, Total Flavonoid, Tannin Content, and Antioxidant Capacity of Halimium halimifolium (Cistaceae). J Appl Pharm Sci. 2015; 5(1): 052–7.
26. Chelvin Ari Kusnanto1, Andayana Puspitasari Gani2, Subagus Wahyuono2 NF. Optimasi Penggunaan High Shear Mixer pada Pembuatan Fraksi Alkaloid dari Daun Awar-awar (Ficus septica) dengan Desain Faktorial Optimization. J Kefarmasian Indones [Internet]. 2021; 11(2): 98–108. Available from: 10.2%0A2435/jki.v11i2.4874.
27. Kusuma SAF, Wahyuni UT, Zuhrotun A. Evaluation of antibacterial activity of indonesian varieties sweet potato leaves extract from cilembu against Shigella dysenteriae ATCC 13313. Asian J Pharm Clin Res. 2017; 10(2): 377–80.
28. Gaurav A, Singh SP, Gill JPS, Kumar R, Kumar D. Isolation and identification of shigella spp. from human fecal samples collected from pantnagar, India. Vet World. 2013; 6(7): 376–9.
29. Turahman T. Aktivitas Antibakteri Ekstrak Dan Fraksi Herba Kemangi (Ocimum Sanctum L) Terhadap Staphylococcus Aureus Dan Pseudomonas Aeruginosa. J Farm Indones [Internet]. 2019; 16(2): 90–7. Available from: https://doi.org/10.31001/jfi.v16i2.596.
30. Tran TTA, Nguyen HVH. Effects of spray-drying temperatures and carriers on physical and antioxidant properties of lemongrass leaf extract powder. Beverages. 2018; 4(4).
31. Sousa AD, Ribeiro PRV, Canuto KM, Zocolo GJ, Pereira R de CA, Fernandes FAN, et al. Drying kinetics and effect of air-drying temperature on chemical composition of Phyllanthus amarus and Phyllanthus niruri. Dry Technol. 2018; 36(5): 609–16.
32. Prastiyanto ME, Rukmana RM, Saraswati DK, Darmawati S, Maharani ETW, Tursinawati Y. Anticancer potential of methanolic extracts from pleurotus species on raji cells and antibacterial activity against methicillin-resistant staphylococcus aureus. Biodiversitas. 2020; 21(12): 5644–9.
33. Hikmawanti NPE, Fatmawati S, Asri AW. The effect of ethanol concentrations as the extraction solvent on antioxidant activity of Katuk (Sauropus androgynus (L.) Merr.) leaves extracts. IOP Conf Ser Earth Environ Sci. 2021; 755(1): 1–7.
34. Rukmana RM, Soesilo NP, Pratiwi R. The Effect of Ethanolic Extract of Black and White Rice Bran (Oryza sativa L .) on Cancer Cells. Indones J Biotechnol [Internet]. 2016; 21(1): 63–9. Available from: https://doi.org/10.22146/ijbiotech.26814.
35. Moshawih S, Cheeme MS, Ahmad Z, Zakaria ZA, Hakim MN. A comprehensive review on cosmos caudatus (ulam raja): pharmacology, ethnopharmacology, and phytochemistry. Int Res J Educ Sci eISSN 2550-2158 Vol. 2017; 1(1): 14–31.
36. Firdaus MD, Artanti N, Hanafi M, Rosmalena. Phytochemical constituents, and in vitro antidiabetic and antioxidant properties of various extracts of kenikir (cosmos caudatus) leaves. Pharmacogn J. 2021; 13(4): 890–5.
37. Seyedreihani SF, Tan T, Alkarkhi AFM, Easa AM. Total phenolic content and antioxidant activity of Ulam raja (Cosmos caudatus) and quantification of its selected marker compounds : Effect of extraction. Int J Food Prop [Internet]. 2017; 20(2): 260–70. Available from: https://doi.org/10.1080/10942912.2016.1155055.