ANTIBACTERIAL ACTIVITY OF CYPERUS ROTUNDUS AND MIMOSA PUDICA BASED ON PARAMETERS OF NUMBER OF COLONIES OF TEST BACTERIA AND VALUE OF PHENOL COEFFICIENT

Lia Yulia Budiarti[1*], Husnul Khatimah[2], Dyna Aulia Millati[3], Tias Firdayanti[3], Shofia Hilwa Ihsanti[4]

[1]Department of Microbiology and Parasitology, Faculty of Medicine, Lambung Mangkurat University Banjarmasin (INDONESIA)

[2]Department of Biomedical, Faculty of Medicine, Lambung Mangkurat University Banjarmasin (INDONESIA)

[3]Undergraduate Student Program, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin (INDONESIA)

[4]Students of Medical Education Study Program, Faculty of Medicine Lambung Mangkurat University Banjarmasin (INDONESIA)

**Corresponding author: lybudiarti@ulm.ac.id*

Abstract

Weeds *Cyperus rotundus* and *Mimosa pudica* contain various antibacterial compounds that have the potential as antiseptics. The ability of an antiseptic is assessed by testing the coefficient of phenol and its antibacterial power. This study reported the infusion activity of *Cyperus rotundus* and *Mimosa pudica* on the number of bacterial colonies of *S.epidermidis, S.aureus, E.coli, P.aeruginosa,* and *S.typhi*, as well as the value of the phenol coefficient. This research design only with control group design uses the dilution method. The treatment effect of a single preparation and a combination of 50%, 75%, 100%, and 100% of *C.rotundus* rhizome infusion and 100% *M. pudica* leaves showed that the number of test bacteria was significantly different. The smallest number of test bacteria was obtained in the 100% infusion

combination treatment. Combination infusion of *C.rotundus* and *M. pudica* against *S.epidermidis, S.aureus, E.coli, and P.aeruginosa,* except for *S.typhi* produced an effect equivalent to 70% alcohol, and the phenol coefficient value was close to 5% phenol. In conclusion, the combined activity of *C.rotundus* and *M.pudica* infusion on the number of tested bacterial colonies and from the value of the phenol coefficient can be developed as an alternative antiseptic.
Keywords: antibacterial activity, *Cyperus rotundus*, *Mimosa pudica*, phenol coefficient value, number of bacterial colonies.

1 INTRODUCTION

Weed plant Java grass or *Cyperus rotundus (C.rotundus)* and the shy princess or *Mimosa pudica (M.pudica) are* widely found in the mainland region of Indonesia. *Cyperus rotundus* is a type of grass Family-Cyperaceae [1],[2],[3] and *Mimosa pudica* belongs to the family Mimosoideaee [4], [5] These two types of plants as been used empirically by several circles of society herbs to treat various diseases. Traditionally *C.rotundus* can be used to treat infections gastrointestinal tract, dyspepsia, diarrhea, dysentery, ascites, vomiting, cholera, fever [2] wounds, ulcers, and abrasions [1], [6] also for urinary stones, nail inflammation, nausea, and vomiting [7]. Pharmacological and biological activity *C.rotundus* is anti-inflammatory, antidiabetic, antidiarrheal, cytoprotective, antimutagenic, antimicrobial, antibacterial, antioxidant, cytotoxic, and apoptotic [6] as well as a sedative, antipyretic, analgesic, and wound healing [1],[2].

The *M.pudica* plant has been used as a medicine for urogenital disorders, piles, dysentery, and sinus, and can be applied to wounds [8] as an anti-infective tract, respiratory infections, herpes, diarrhea, asthma, insomnia, skin infections, and swelling due to wounds [4]. The pharmacological effects of *M.pudica* are antivenom, antifertility, anticonvulsant, antidepressant, aphrodisiac, antibacterial [4], antihepatotoxic, antioxidant, diuretic, anti-inflammatory, and antimicrobial (antibacterial, antiviral) [9]. The bioactive content in

these two plants is as efficacious as an antibacterial. The rhizome of *C.rotundus* contains flavonoids, alkaloids, cyperol, fatty oils, furokhromone, glycerol, linolenic acid, myristic acid, nootkatone, starch, saponins, sesquiterpenes, sitosterol, stearic acid, terpenoids, polyphenols, and valence [6] also contains compounds of quercetin, kaempferol, tannins, glycosides, gallic acid and p-coumarin acid [7] and cinema [10] Phytochemical compounds in *M.pudica* include glycosides, mimosine [4] tannins, flavonoids, steroids, terpenoids and sterols [9] alkaloids, quinine, phenols, saponins, and coumarins [5] triterpenes, quinines, c-glycosylflavones, phenols, and polyphenols [11].

Various studies have proven the activity of *C.rotundus* and *M.pudica* extracts on various types of bacteria*. C.rotundus* extract has antibacterial effect against *Staphylococcus aureus*, *Bacillus subtilis* [6], [12] *Staphylococcus epidermidis* [13] and *Propionibacterium Acnes* [7] *Enterococcus aureus, Escherichia coli, Pseudomonas aeruginosa* [2],[15] *Shigella dysenteriae, Salmonella typhimurium,* and *Pseudomonas aeroginosa Shigella dysenteriae* [6]. While *M.pudica* extract affects *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae* [8]**,** [11], *Bacillus subtilis* [5],[16],[17] also affects *Escherichia coli, Klebsiella pneumoniaea, Pseudomonas spp., Klebsiella pneumonia, Proteus vulgaris* [8], *Pseudomonas aeroginosa, Salmonella sp*., *Shigella flexineri* [18] and *Salmonella typhi* [5],[16],[17]. The ability of herbs as antibacterial becomes the basis for using them in various dosage forms, to prevent the transmission of bacteria to humans. Pathogenic bacteria such as *Staphylococcus aureus (S.aureus), Staphylococcus epidermidis (S.epidermidis), Escherichia coli (E.coli), Pseudomonas aeruginosa (P.aeruginosa),* and *Salmonella typhi (S.typhi),* can be transmitted through the mediation of hand skin, feces, contaminated equipment [19], [20], [21]. Efforts to prevent bacterial transmission are by applying hand washing using soap [8] or antiseptic [21]. Antiseptics are used to inhibit bacterial colonization and prevent bacterial transmission through the skin of the hands [21].

Preparations of antibacterial drugs that also function as antiseptics can be obtained from a combination of several plant extracts. The dosage form of herbal combinations (polyherbal) is more widely used than single preparations, because of the synergistic effect of the combination preparations which is more beneficial in increasing drug activity [3]. An antiseptic can be said to have good effectiveness, it has a phenol equivalent coefficient value of 5% based on the phenol coefficient test. The phenol coefficient test is a test to determine the effectiveness of an antiseptic preparation compared to 5% enol liquid which has been proven to be effective as an antiseptic [23]. The availability of *C.rotundus* and *M.pudica* plants which are quite abundant in the community can be developed as alternative antiseptic candidate preparations. This study aimed to analyze the antibacterial activity of the infusion of *Cyperus rotundus* rhizome and *Mimosa pudica* leaves in inhibiting the growth of several pathogenic bacteria in humans. Based on the presence of various antibacterial compounds in the rhizome of *C.rotundus* and *M.pudica* leaves, their activity in inhibiting bacterial growth can be determined based on the parameters of the number of tested bacterial colonies in vitro, while the ability of the combined preparation of the two test plants to act as an antiseptic is known through the coefficient test. phenol. The method of infusion preparation was chosen in this study because the infusion form has advantages in the availability of materials and ease of manufacture.

2 METHODOLOGY

This true experimental research uses a posttest control group design. Infusion treatment test rhizome combination of *C.rotundus* and *M.pudica* leaves and control treatment using the dilution method. The antibacterial activity of the infusion treatment and 70% alcohol control was observed based on the number of bacterial colonies growing on the test medium, while the activity of the treatment as an antiseptic was calculated and analyzed based on the magnitude of the phenol coefficient value. This research is a laboratory experiment conducted

at the Laboratory of Microbiology, Faculty of Medicine, the University of Lambung Mangkurat in September 2021. The phenol coefficient test method was carried out conventionally, using bacterial isolates of *Staphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922*, Salmonella typhi* ATCC 19430*,* and *Pseudomonas aeruginosa* ATCC 27853. The main ingredients used in this study were the test plant *Cyperus rotundus* rhizome and *Mimosa pudica* leaves as well as samples of pure isolates of bacterial colonies tested from the Microbiology Laboratory of FK ULM. The chemicals used were Nutrient Broth (NB) media, Brain Heart Infusion (BHI) media, sterile distilled water, 5% phenol solution, and 0.5 Mc Farland standard solution (equivalent to 1.5 X 10^8 CFU/mL).

2.1 Research procedure

2.1.1. Preparations of test Bacteria

Pure isolates of the test bacteria were prepared, then 1 ose was taken and put into the BHI media to make a suspension of the test bacteria cultures. Bacteria were incubated at 37°C for 24 hours. Then, sterile distilled water was added to the bacterial suspension in BHI media so that the turbidity was the same as the standard 0.5 Mc Farland solution.

2.1.2. Making Simplicia and a Combination Infusion of Test Plants

The simplicia material of *C.rotundus* rhizome and *M.pudica* leaves were washed thoroughly with running water until separated from dirt, then drained and avoided contamination. Furthermore, the simplicia material is sliced into small pieces to expand the surface so that the drying process is faster. The simplicia material is dried by aerating and avoiding direct sunlight until the moisture content is <10% (easily broken); dried in a blender to a powder. Simplicia powder is stored in clean plastic and then placed in a room with low humidity, room temperature (15°-30°C), smooth air circulation, and not exposed to direct sunlight [24].

Simplicia made infusion preparations, namely by weighing the test plant simplicia as much as 100 grams each and placing them in a

beaker glass container and adding 100 ml of distilled water, to make a liquid preparation with a concentration of 100% w/v. Next, the glass containing the herbal preparation was placed in an infusion pot bath at 90℃ and heated for 15 minutes, stirring occasionally [24]. The results of the aqueous extract were filtered through filter paper while hot and made several test concentrations. Then a single preparation and a combination of infusion extracts were made according to the concentration to be tested. The concentrations of the prepared *C.rotundus* infusions were 50%, 75%, and 100% and 100% of *M.pudica* infusions. The combination treatment was made with an infusion mixture of each concentration of *C.rotundus* and *M.pudica* infusion, with a ratio of 1:1.

2.1.3. Antibacterial Activity Test

Methods of testing antibacterial activity against bacterial cultures using the dilution method (dilution) and standard culture. For each test treatment of single and combined infusion, 1 ml of 70% alcohol control was prepared and 0.1 ml of the test bacterial isolate suspension (equivalent to 0.5 Mac Farland) was prepared, to be inserted into the test medium aseptically. Infusion treatment and test control and suspension of test bacteria were poured into a petri dish and a warm NA liquid medium was added to cover the test solution. The crucible was gently shaken to homogenize, then wrapped in aluminum foil and incubated at 37°C for 24 hours. The results of the incubation will show some colonies of test bacteria that grow on NA media, then the number of bacterial colonies is calculated using a colony counter.

2.1.4. Preparation of phenol stock solution:

Phenol stock solution was prepared by dissolving 5 grams of phenol crystals with 100 ml of sterile distilled water, so a phenol solution with a concentration of 5% was obtained.

2.1.5. Dilution Phenol 5%

The 5% phenol solution was put into a sterile test tube that had been labeled 1-13 as much as 2 ml, then distilled water was added to it. After being added and then homogenized, then the phenol solution mixture was transferred to the 13-36 tubes as much as 2 ml, then the

dilution of the phenol solution was 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1: 80, 1:90, 1:100, 1:110, 1:150, 1:200, 1:250. Dilution infusion of *cyperus rotundus* and *mimosa pudica.* a total of 1 ml of the combined infusion of *cyperus rotundus* and *mimosa pudica was* put into a sterile test tube and then diluted with sterile distilled water without concentrations 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:100, 1:110, 1:150, 1:200, 1:250. The solution was homogenized and transferred to another sterile tube so that a final volume of 2ml was obtained [21],[22],[23].

2.1.6. Phenol Coefficient Test

Prepared suspensions of test bacteria (*S.aureus, S.epidermidis, E.coli, P.aeruginosa*, and *S.typhi*), tube racks, and sterile test tubes containing NB media that have been labeled according to the dilution and length of contact time (5, 10, and 15 minutes). 0.5 ml of the test bacterial suspension was put into a tube containing the test treatment (infusion of *C.rotundus-M.pudica* and control and 5% phenol) in various dilution series starting from the tube with a dilution of 1:20 to 1:250, then homogenized. After 5 minutes, 1 ose is taken from each test dilution series tube and put into each test tube containing the test dilution series with a contact time of 5 minutes, then the used ose is sterilized with Bunsen fire. After the second 5 minutes, it was carried out in the same way for the test treatment with a contact time of 5 minutes (total contact time 10 minutes), and after the third 5 minutes for the test treatment with a contact time of 5 minutes (total contact time 15 minutes). The same phenol coefficient test steps were carried out on each of the bacteria tested. All observation tubes were incubated at 37°C for 24 hours, then observed for turbidity. The presence of bacterial growth (+) is indicated by the medium becoming cloudy, and the absence of bacterial growth (-) is indicated by the medium remaining clear. Furthermore, the value of the phenol coefficient is calculated with the following formula [21],[22],[23].

$$Phenol\ Coefficien = \frac{\left\{\frac{\text{Lowest dilution of phenol that kills bacteria}}{\text{Lowest dilution of antiseptic that kills bacteria}}\right\}}{\left\{\frac{\text{Highest dilution of phenol that kills bacteria}}{\text{Highest dilution of antiseptic that kills bacteria}}\right\}}$$

2.1.7. Data analysis

Research data to analyze antibacterial activity was tested using the ANOVA test (one-way analysis of variance) and Duncan's post hoc test, at a significance level of 95% (α = 0.05). [25] Data analysis of the results of the phenol coefficient test to assess the effectiveness as an antiseptic was carried out descriptively, namely by comparing the coefficient value of the test treatment with the phenol coefficient value of 5% phenol solution.

3 RESULTS

Infusion treatment of a single dosage form and a combination of *C.rotundus* and *M.pudica* generally produced a better inhibitory effect against gram-positive bacteria than gram-negative (***Figure. 1***). The least number of test bacterial colonies was obtained from the 100% (1:1) infusion combination treatment. The results of the ANOVA test revealed that there were differences in the effect of all the treatments tested. Duncan's test results (***Table. 1.)***, showed that the average number of colonies from the single infusion and combination treatments of *C.rotundus* and *M.pudica* was significantly different. A combination infusion of 100% (1:1) provides an effect equivalent to 70% alcohol treatment, which was tested on *S. aureus* and *S. epidermidis* bacteria, except *S.typhi*.

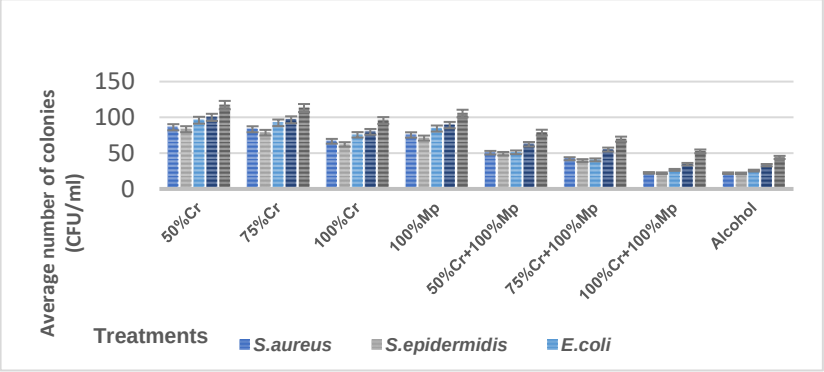

***Figure 1**. Infusion activity of Cyperus rotundus (Cr) rhizome and Mimosa pudica (Mp) leaves against their age number of colonies of Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi.*

The results of the combined infusion of C.rotundus and M.pudica proved to show better antibacterial activity than a single preparation. Furthermore, the effectiveness of combination infusions was tested by comparison with antiseptic fluids, the effect was obtained as well as antiseptics. This is because it produces a phenol coefficient value equal to or more than the 5% phenol coefficient value. The results of the phenol coefficient test are shown in ***Table. 2-4*** and the coefficient values are in ***Table. 5.***

The phenol coefficient value of the combined infusion of *C.rotundus* rhizomes and M.*pudica* leaves and 70% alcohol against *S.epidermidis*, *S.aureus*, and *E.coli* was 1.10; for *P.aeruginosa* close to the coefficient value of 5% phenol solution, which is 0.99, while for *S.typhi*, it is 0.89. These results indicate the effectiveness of combination infusions is relatively the same as the effectiveness of antiseptics. The combination treatment of *C.rotundus* and *M.pudica* infusion was proven to have better antibacterial activity than the single preparations. The inhibition of bacterial colonization was affected by an increase in the concentration of the infusion, so the number of viable bacterial colonies decreased. This is the same as the

combination treatment of *Piper battle* L. and *Ocimum sanctum* L. infusion on the number of test bacterial colonies.[22] The results of this study are not much different from previous studies; gram-positive bacteria were more sensitive to *C.rotundus* extract than gram-negative bacteria.[13],[26] The effect of infusion on the number of colonies of *E.coli* and *P.aeruginosa* is relatively the same, and the effect is less on *S.typhi*.[22],[23]. It is like the previous research reports; there was the antibacterial activity of *M.pudica* extract against *E.coli*, *P.aeruginosa*, and *S.aureus*, but had no effect on *S.typhi*.[5] Based on AgNPS biosynthesis, *M.pudica* leaf extract did not have different antibacterial activity against *E.coli* and *P.aeruginosa*.[16] Research using the diffusion method stated that the inhibition zone of *M.pudica* showed the highest activity against *E.coli* and *S.aureus*, while against *P.aeruginosa* the activity was moderate.[11]

Table 1. *Differences in the mean number of test bacteria colonies in the treatment of Cyperus rotundus (Cr) and Mimosa pudica (Mp) infusion*

Infusion treatments	**Number of bacterial colonies (CFU/ml)**				
	S.aureus	***S.epidermidis***	***E. coli***	***P.aeruginosa***	***S.typhi***
50%Cr*	86,20 + 6,56^{f}	83,50 ±1.48^{g}	96,00 ±5,0^{f}	100,00 + 7,00^{f}	117,20 ±5.21^{g}
75%Cr*	83,40 + 5,02^{f}	78,60 ±1.14^{f}	92,40 ±3.78^{f}	96,60 + 4,50^{f}	113,00 ±4.74^{g}
100%Cr*	66,60 + 3,64^{e}	62,20 ±1.92^{e}	75,60 ±3.36^{e}	79,80 + 3,03^{e}	95,60 ±4.61^{f}
100%Mp*	75,40 + 7,23^{d}	71,00 ±1.58^{d}	84,40 ±3.50^{d}	89,20 + 7,53^{d}	105,40 ±5.02^{e}
50%Cr+100%Mp*	50,60 + 1,52^{c}	49,20 ±1.48^{c}	51,20 ±1.30^{c}	62,40 + 2,30^{c}	78,80 ±5.16^{d}
75%Cr+100%Mp*	42,20 + 6,65^{b}	39,60 ±1.14^{b}	40,60 ±1.14^{b}	55,00 + 2,82^{b}	69,60 ±2.07 ^{c}
100%Cr+100%Mp*	22,40 + 1,52^{a}	22,00 ±1.58^{a}	26,60 ±1.14^{a}	34,60 + 3,50^{a}	52,50 ±1.92 ^{b}
70%Alcohol	22,00 + 2,00^{a}	21,80 ±1.48^{a}	25,60 ±1.14^{a}	33,20 + 1,30^{a}	44,00 ±1.58 ^{a}

*) the same alphabet in the same column is not significantly different (P>0.05).

Table 2. *The average results of the phenol coefficient test 5% phenol liquid on some test bacteria.*

Dilutions of each treatment	treatment of phenol 5% Average contact length per unit time (minutes)														
	S.aurues			***S.epidermidis***			***E. coli***			***P.aeruginosa***			***S.typhi***		
	5'	**10'**	**15'**	**5'**	**10'**	**15'**	**5'**	**10'**	**15'**	**5'**	**10'**	**15'**	**5'**	**10'**	**15'**
1:20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:80	-	-	-	-	-	-	-	-	-	1+	-	-	3+	2+	-
1:90	-	-	-	-	-	-	2+	-	-	2+	1+	1+	3+	3+	3+
1:100	2+	2+	2+	2+	-	2+	3+	2+	2+	3+	3+	3+	3+	3+	3+
1:110	2+	3+	3+	3+	1+	2+	3+	2+	2+	3+	3+	3+	3+	3+	3+
1:150	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
1:200	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
1:250	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

\+ = There is the growth of bacteria (cloudy medium)

\- = No growth of bacteria (clear medium)

Table 3. *The average results of the phenol coefficient test were 70% Alcohol on some test bacteria.*

Dilutions of each treatment	70% alcohol treatment														
	Average contact length per unit time (minutes)														
	S.aurues			*S.epidermidis*			*E. coli*			*P.aeruginosa*			*S.typhi*		
	5'	10'	15'	5'	10'	15'	5'	10'	15'	5'	10'	15'	5'	10'	15'
1:20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:80	-	-	-	-	-	-	-	-	-	-	-	+1	2+	2+	-
1:90	-	-	-	-	-	-	-	-	-	2+	1+	1+	3+	3+	3+
1:100	2+	2+	2+	2+	-	3+	3+	2+	2+	3+	3+	3+	3+	3+	3+
1:110	2+	3+	3+	2+	2+	2+	3+	2+	2+	3+	3+	3+	3+	3+	3+
1:150	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
1:200	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
1:250	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

\+ = There is the growth of bacteria (cloudy medium)

\- = No growth of bacteria (clear medium)

Table 4. *The average results of the phenol coefficient test Infusion of the combination of Cyperus rotundus rhizome and Mimosa pudica le on several test bacteria.*

Dilutions of each treatment	Combination treatment														
	Average contact length per unit time (minutes)														
	S.aurues			*S.epidermidis*			*E. coli*			*P.aeruginosa*			*S.typhi*		
	5'	10'	15'	5'	10'	15'	5'	10'	15'	5'	10'	15'	5'	10'	15'
1:20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:70	-	-	-	-	-	-	-	-	-	-	-	-	+1	-	-
1:80	-	-	-	-	-	-	-	-	-	-	-	+1	2+	2+	-
1:90	-	-	-	-	-	-	1+	-	-	2+	1+	1+	3+	3+	3+
1:100	-	-	1+	2+	-	2+	1+	1+	2+	3+	3+	3+	3+	3+	3+
1:110	2+	2+	2+	3+	2+	2+	3+	2+	2+	3+	3+	3+	3+	3+	3+
1:150	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
1:200	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
1:250	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+

\+ = There is the growth of bacteria (cloudy medium)

\- = No growth of bacteria (clear medium)

Table 5. *Average Phenol Coefficient Value of Treatment on each Test Bacteria.*

Treatments	Phenol Coefficient Value				
	S.aureus	**S.epidermidis**	**E. coli**	**P.aeruginosa**	**S.typhi**
Combination infusion	1,10±0,01	1,10±0,00	1.00±0,01	0.99±0,04	0.89±0,07
Alkohol 70%	1,10±0,00	1,10±0,00	1,00±0,00	0,99±0,00	1,00±0,00
5% phenol	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00

The combination of *C.rotundus* 100% and 100% *M.pudica* infusion had an effect that was not significantly different from 70% alcohol on all bacteria, except for *S.typhi* which had a different effect at concentrations <100%. This proves the achievement of a synergistic effect, which strengthens each other at the combined concentrations. The results of this study are in accordance with the results of the combined infusion test of *Stanochlaena palustris* and *Sauropus androgynous* leaves against *E.coli, S.aureus*, and *C.albicans* bacteria; the inhibitory effect was greater than that of the single preparation and the effect was equivalent to that of the positive control.[27] The synergistic effect of several herbs can provide more beneficial effects.[3] Significant inhibitory effect of polyherbal plant extracts on *S.aureus, P.aeruginosa, B.subtillis,* and *E.coli*; so that polyherbal formulations can be used in commercial scale hand washing herbal preparations.[8]

The same results were obtained for the phenol coefficient parameter. Comparison of the results of the phenol coefficient test between the combination infusion preparations with antiseptic control and 5% phenol proved the effectiveness of this combination infusion as a good antiseptic, i.e. the equivalent value of the phenol coefficient is obtained ≥1.[22],[23] Its effectiveness as an antiseptic preparation against gram-positive bacteria is stronger than gram-negative bacteria.The infusion preparations from the two test plants contained various bioactive compounds to inhibit bacterial growth/colonization. The antibacterial effect is influenced by the level, type, and polarity of the secondary compound content. In the infusion method, most of the polar compounds and some semi-polar compounds are dissolved in aqueous solvents. Compounds in *C.rotundus* such as alkaloids, cineol, pinene, cyproterone, retinol, eplerenone, tannins, cyperols, and flavonoids;[7] as well as glycosides, anthraquinones, saponins, steroids, and triterpenoids, have antibacterial properties.13,26 Secondary compounds in *M.pudica* that are antibacterial include tannins, saponins, steroids, flavonoids; glycosides, non-protein amino acid leucine (mimosine), alkaloids, steroids, tannins, triterpenes, flavonoids, glycosides, quinine, phenols, saponins, coumarins, c-glycosyl-flavones; [28],[29] also tyrosine, and mimosinamine.[5]

The antibacterial activity of active compounds is also influenced by bacterial cell surface components (cell membrane, cell permeability), cytoplasmic components, and bacterial virulence factors.[30],[31] Polar secondary compounds are effective in inhibiting the growth of gram-positive bacteria, penetrating the peptidoglycan layer which is also polar, and more easily penetrated by polar antibacterial compounds.[32],[33] The peptidoglycan structure plays a role in maintaining osmotic stability and antiphagocytosis with fewer lipids and contains polysaccharides (teichoic acid). Teichoic acid is a water-soluble polymer that acts as a carrier for positive ions in or out.[31],[34] Polar compounds that have entered the bacterial cell immediately work to destroy the bacteria by denaturing proteins (enzyme catalysts) and causing the bacterial cell's metabolic activity to stop and resulting in the death of the bacterial cell.[33],[34] The outer cell membrane of Gram-negative bacteria is a double layer consisting of phospholipids (inner layer) and lipopolysaccharide (outer layer), with a high lipid composition (non-polar) and little peptidoglycan.[32],[33] This makes it difficult for antibacterial compounds to penetrate gram-negative bacterial cells. There are relatively more secondary compounds in *M.pudica*, thus showing a better antibacterial effect in combination preparations. It is suspected that the phytochemical groups of flavonoids, alkaloids, and tannins are more responsible for the antibacterial activity, but it depends on the dose and type of bacteria.[17],[28]

The mechanism of action of flavonoids as antibacterials is to form complex compounds with extracellular and soluble proteins. Flavonoids can damage cell membrane function, as well as inhibit DNA-RNA synthesis and energy metabolism.[26],[35] Alkaloid compounds have an inhibitory mechanism by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer does not form completely and causes cell death.[34],[36] Saponin compounds are antibacterial, work by diffusion through the outer membrane and vulnerable cell walls, then bind to the cytoplasmic membrane thereby disrupting and reducing membrane stability, and ultimately causing cell death due to leakage of cytoplasm from the cell.[37] Terpenoid compounds can react with porins

(transmembrane proteins) on the outer membrane of the bacterial cell wall and form strong polymeric bonds that damage the porins.[38] Damage to the porin, which is the entry and exit point for compounds needed by the bacterial cell, will reduce the permeability of the bacterial cell wall so that the bacterial cell lacks nutrients so that the growth of the bacteria is inhibited or dies.[35],[39] Tannins are polyphenolic compounds that dissolve in water. Tannins are derivatives of 3,4,5-trihydroxy benzoic acid. Tannins can bind to proteins, forming complex compounds with proteins through hydrogen bonds. Hydrogen bonding between tannins and proteins is affected by temperature, pH, solution composition, and the tannin: protein ratio.[40]

The results of this study were relatively the same as previous studies, namely the combined infusion test results of *Averrhoa bilimbi* and *Cananga odorata* [22] and the combined infusion of *Piper battle* L. and *Ocimum sanctum* L. [23]; both tests use the same type of test bacteria. This study can prove the effectiveness of the infusion combination as an antiseptic based on the parameters of the number of bacterial colonies and the value of the phenol coefficient. So that the combination infusion of *C.rotundus* and *M.pudica* has the potential to be developed as a natural antiseptic candidate. The next stage can be tested organoleptic and its effectiveness in vivo.

4 CONCLUSIONS

The antibacterial activity of *C.rotundus* rhizome infusion and M.pudica *leaves* as natural antiseptic candidates was proven in this study, with parameters of the number of bacterial colonies and their phenol coefficient values against several laboratory standard bacteria. In conclusion, the combination of *C.rotundus* and *M.pudica* infusion had good antibacterial activity in inhibiting test bacterial colonization and had a phenol coefficient value equivalent to that of the antiseptic control.

ACKNOWLEDGEMENTS

Appreciation and gratitude for the support of the ULM FK Microbiology Laboratory team in this research activity.

REFERENCES

[1] Imam, M.Z. and C.D. Sumi. Evaluation of the antinociceptive activity of hydroethanolic extract of Cyperus rotundus in mice. BMC Complementary and Alternative Medicine. 2014;14(83).1-5

[2] Kabbashi, A.S., S.E.A. Mohammed, A.Z. Almaggboul, I.F. Ahmed. Antimicrobial activity and Cytotoxicity of Ethanolic Extract of Cyperus rotundus L. American Journal of Pharmacy and Pharmaceutical Sciences. 2015;2(1): 1 - 13

[3] Sutalangka, C and J. Wattanathorn. Neuroprotective and cognitive-enhancing effects of the combined extract of Cyperus rotundus and Zingiber officinale. BMC Complementary and Alternative Medicine. 2017:17.

[4] Durgadevi, G and N. Karthika. Screening Of Phytochemicals and Pharmacological Studies On Mimosa pudica L. Asian Journal of Innovative Research. 2018; 3(2), 19-28.

[5] Sharma S. and Kaur J. Phyto. 2019. Pharmacological and Patented Review On mimosa Pudica. Think India Journal. 2019;22(30): 788-796.

[6] Kumar, M., M. Rani, B. Meher Review on Pharmacology and Phytochemistry of Cyperus rotundus L. Current Research in Pharmaceutical Sciences. 2017; 07 (01): 11-15

[7] Nurjanah S , Rokiban A, Irawan E. 2018. Ekstrak Umbi Rumput Teki (Cyperus Rotundus) Sebagai Antibakteri Terhadap Staphylococcus Epidermidis Dan Propionibacterium Acnes. BIOSFER: Jurnal Tadris Pendidikan Biologi. 2018;9(2): 165-175.

[8] Sandeep DS, Narayana CR, Prashant N, Maharjan A, Ghalan I, Formulations of Antimicrobial Polyherbal Hand wash. Research J. Pharm. and Tech. 2016; 9(7):864-866.

[9] Mambou, AHMY et al. Antifatigue properties of an aqueous extract of Mimosa pudica Linn. (Fabaceae) in mice subjected to weight loaded force swimming test. GSC Biological and Pharmaceutical Sciences. 2022; 18(02), 224–233

[10] Sukandar, D., Hermanto, S., Amelia, E. R., & Zaenudin, M. (2016). Aktivitas Antibakteri Ekstrak Biji Kapulaga (Amomum compactum Sol. Ex Maton). Jurnal Kimia Terapan Indonesia. 2016: 17(2):119–129.

[11] Ahmed, SR. et al. Screening, Antioxidant and Antibacterial Activity of Some Medicinal Plants Grown In Sylhet Region. Journal Of Pharmacy And Biological Sciences. 2019:14(1): 26-37.

[12] Lamaga, F. E., Nazila, H., Fitri, R., & Putri, F. M. S. Formulasi Krim Ekstrak Rimpang Rumput Teki (Cyperus rotundus) dan Uji Aktivitas Antibakterinya Terhadap Staphylococcus aureus ATCC 25923.2019;10(1):20-26

[13] Masfria and Permata YM. Total Phenolic Content and Antibacterial Activity of Nut Grass (Cyperus rotundus L.) Extract. Talenta. Indonesian Journal of Pharmaceutical and Clinical Research (IDJPCR). 2018;1(1): 28 – 36.

[14] Ermawati E , Kusumaningrum NSD , Sari AAY , Anggraini MH, Pringgani VD. Akar Mimosa Pudica L yang Diozonasi Terhadap Penyembuhan Luka Ulkus Diabetikum. Jurnal Kesehatan Perintis. 2021;8 (1): 17-25.

[15] Lina, R. N., & Rahmawaty, A. Uji Efek Antidiare Kombinasi Ekstrak Etanol Biji Pepaya (Carica papaya L.) dan Rumput Teki (Cyperus rotundus L.) pada Mencit Jantan. Cendekia Journal of Pharmacy. 2021: 5(1); 8-15.

[16] Mahato T.K., Sharma K. Study of Medicinal Herbs and Its Antibacterial Activity: A Review. Journal of Drug Delivery & Therapeutics. 2018; 8(5-s):47-54.

[17] Fatimah I, Mutiara NAL. Biosintesis Nanopartikel Perak Menggunakan Ekstrak Daun Putri Malu (Mimosa pudica) dan Iradiasi Gelombang Mikro. Molekul. 2016;11(2): 288 – 298.

[18] Sharmin Sultana, Md. Lokman Hossain. Sultana S and Hossain ML. Evaluation of Anxiolytic and Antibacterial Activity of Methanol Extract of Leaves of Mimosa pudica. Universal Journal of Plant Science. 2019; 7(3): 44-49.

[19] Lubis, IA, Prenggono MD, Budiarti LY. Identifikasi Jenis Bakteri Kontaminan PadaTangan Perawat Di Bangsal Penyakit Dalam RSUD Ulin Banjarmasin Periode Juni-Agustus 2014. Berkala Kedokteran. 2015. 11(1): 11-5.

[20] Budiarti LY, Khariyati L, Fakhriyadi R. The relationship between the existence of bacterial type from hand and feces with water piping on elementary school students on the riverbanks of Kuin in Banjarmasin. Proceeding international Seminar: development of tropical disease research based on wetland and Indonesian local 2017. ISSN:2477-3522:p.336-347.

[21] Heriyani F, Budiarti LY, Nursantari W, Apriliani A. Hand Soap Activity Against The Number of Bacterial Colonies From The Housewife's Hand Swab Samples In A Temporary Landfill In Kelurahan Gadang Banjarmasin. Berkala Kedokteran. 2021;17(2): 87-94.

[22] Budiarti LY, Nurichwan PW, Muthmainah N. Metode Pencegahan Penyakit infeksi. Banjarmasin: Sari Mulia Indah. 2021.

[23] Budiarti, L. Y., Wydiamala, E., Ulfa, N. Phenol Coefficient Test Combination Infusion of Cananga odorata – Averrhoa bilimbi L. Against Staphylococcus aureus and Salmonella typhi in Vitro.

Bioinformatics and Biomedical Research Journal. 2021:4(1):19-26. doi: 10.11594/bbrj.04.01.03

[24] Ditjen POM.Farmakope Indonesia. Edisi Ketiga. Jakarta: Departemen Kesehatan RI.1979 Hal. 32-33.

[25] Dahlan MS. Statistik untuk Kedokteran dan Kesehatan : Deskriptif, Bivariat Multivariat, Dilengkapi Aplikasi dengan Menggunakan SPSS. Edisi ke-5. Jakarta: Salemba Medika. 2011.

[26] Taheri A. et all.. Cyperus spp.: A Review on Phytochemical Composition, Biological Activity, and Health-Promoting Effects. Hindawi Oxidative Medicine and Cellular Longevity. 2021; 1-17.

[27] Budiarti LY, et al.. Antimicrobal Activity of Stanochlaena palustris and Sauropus androgynus in Staphyloccus aureus, Esherichia coli and Candida albicans. Bioinformatics and Biomedical Research Journal. 2021; 4 (1), 32 – 38.

[28] Ermawati E, et al.. Akar Mimosa pudica L yang Diozonasi Terhadap Penyembuhan Luka Ulkus Diabetikum. Jurnal Kesehatan Perintis. 2021; 8 (1), 17-25

[29] Tomar RS, Shrivastava, Kaushik S. In vitro efficacy of methanolic extract of Mimosa pudica against selected microorganisms for its broad spectrum antimicrobial activity. International Journal of Current Microbiology and Applied Sciences. 2014; 3(4), 780-84.

[30] Zhang Z, et all.. Antimicrobial susceptibility among gram-positive and gram-negative blood-borne pathogens collected between 2012-2016 as part of the Tigecycline Evaluation and Surveillance Trial. Antimicrobial Resistance and Infection Control. 2018, 7(1), 1–13.

[31] Septiani S, Dewi EN, Wijayanti I. Aktivitas antibakteri ekstrak lamun (Cymodocea rotundata) terhadap bakteri

Staphylococcus aureus dan Escherichia coli. Saintek Perikanan. 2017;13(1), 1-6.

[32] Sudewi S, Lolo WA. Campuran ekstrak buah mengkudu (Morinda citrifolia L.) dan daun sirsak (Annona muricata L.) dalam menghambat bakteri Escherichia coli dan Staphylococcus aureus. Kartika-Jurnal Ilmiah Farmasi, 2016; 4(2), 36-42.

[33] Suerni EA, Muhammad, Guli MM. Uji Daya Hambat Ekstrak Buah Nanas (Ananas comosus L. Merr.), Salak (Salacca edulis Reinw.) dan Mangga Kweni (Mangifera odorata Griff.) terhadap Daya Hambat Staphylococcus aureus. Jurnal Biocelebes, 2013; 7(1), 35-47.

[34] Ngajow M, Abidjulu J, Kamu VS. Pengaruh antibakteri ekstrak kulit batang matoa (Pometia pinnata) terhadap bakteri Staphylococcus aureus secara in vitro. Jurnal Mipa, 2013; 2(2), 128-32.

[35] Khalid M, Bilal M, Dan-feng H. Role of flavonoids in plant interactions with the environment and against human pathogens - A review. J. Integr. Agric. 2019;18(1), 211–30.

[36] Yan Y, et al.. Research Progress on Antibacterial Activities and Mechanisms of Natural Alkaloids: A Review. Journal Antibiotics. 2021;10(318), 1-30.

[37] Yu-Pu J and Pi-Hu L. (2020). Biological and Pharmacological Effects of Synthetic Saponins. Molecules, 2020; 25, 49714, 1-22.

[38] Broniatowski M & Mastalerz P. (2015). Studies of the interactions of ursane-type bioactive terpenes with the model of Escherichia coli inner membrane Langmuir monolayer approach. Biochimica et Biophysica Acta. 1848, 469–76.

[39] Carvalho R.S., et al.. (2018). Antibacterial and antifungal activities of phenolic compound-enriched ethyl acetate fraction from Cochlospermum regium (mart. Et. Schr.) Pilger roots: Mechanisms of action and synergism with tannin and gallic acid. South African Journal of Botany 114, 181–87.

[40] Sieniawska E & Baj T. (2017). Tannins. Pharmacognosy, 199–232.