MAGNA MEDIKA
Berkala Ilmiah Kedokteran dan Kesehatan
e-ISSN 2774-2318
p-ISSN 2407-0505

Research Article
MAGNA MEDIKA
Berkala Ilmiah Kedokteran dan Kesehatan
Journal Page: https://jurnal.unimus.ac.id/index.php/APKKM

In Vitro Antimicrobial Activity of *Eichornia crassipes* and *Pistia stratiotes* Extracts as Natural Disinfection Agents

Lia Yulia Budiarti[1], Nazla Puteri Azhari[2], Shofia Hilwa Ihsanti[3], Siti Kaidah[4]

[1,2,3,4] Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia

Article Info

Article history:
Received 03 January 2024
Revised 27 January 2024
Accepted 02 February 2024
Available online 15 August 2024

Keywords:
antimicrobial activity; *Eichornia crassipes*; *Pistia stratoites;* inhibition zone; phenol coefficient value

Correspondence:
siti.kaidah@ulm.ac.id

How to cite this article:
Lia Yulia Budiarti, Siti Kaidah, Shofia Hilwa Ihsanti. In Vitro Antimicrobial Activity of *Eichornia crassipes* and *Pistia stratiotes* Extracts as Natural Disinfection Agents. MAGNA MEDIKA Berk Ilm Kedokt dan Kesehat. 2024; 11(2): 154–168

Abstract

Background: Eichornia crassipes and Pistia stratoites are aquatic weeds that contain various antimicrobial compounds and have the potential to disinfectants.

Objective: This study aims to analyze the antimicrobial activity of extract of Eichornia crassipes and Pistia stratoites as In Vitro disinfection agents

Methods: This in vitro experimental study tested E.crassipes and P.statoites extracts in combined (EC+PS) forms against several species of bacteria and Candida albicans. The antimicrobial activity and effectiveness of the disinfectant were tested using the diffusion method and the phenol coefficient test.

Results: The results of the data analysis of the EC+PS 25%-100% (ratio 1:1) treatment using the ANOVA test and Duncan's posthoc (significance 95%) obtained different inhibition zones for gram-positive, gram-negative bacteria, and C.albicans; EC100%+PS100 extract produced the widest inhibition zone. The inhibitory power of EC+PS extracts ranged from moderate to strong (> 5 mm - > 20 mm). In the phenol coefficient test using a dilution of 1:20-1:250, a good coefficient value (> 1) was obtained from the EC+PS extract treatment and the disinfectant control for all test organisms.

Conclusion: E.crassipes and P.stratoites extracts can produce antimicrobial activity with a good phenol coefficient value, which makes their combination a natural disinfectant.

e-ISSN 2774-2318
p-ISSN 2407-0505

INTRODUCTION

The use of disinfectants is a vital thing to do in the community and hospitals as an effort to prevent transmission of pathogenic microbes either through direct or indirect contact.[1,2] What often happens is contact due to the use of water that has been contaminated with microbes to wash hands or wash equipment.[3,4] One of the widely used disinfectants is chlorine for the chlorination process.[5,6] Chlorine is effective in inhibiting and killing various types of opportunistic and pathogenic bacteria, and[2] it is also effective against bacteria that cause nosocomial infections.[1,7] Chlorine solution can reduce the growth of microorganisms by more than 98.2%.[8]

Chlorine is an efficient disinfectant at an affordable price; at very low concentrations (sub-toxic level), it is safe to use as a decontamination agent.[5,6] Chlorination of water sources generally uses a concentration of 2 ppm (0.0002%). The negative impact of chlorine can be felt when used in strong concentrations and for a long time.[9,10] This type of disinfectant can form trihalomethane compounds that are carcinogenic and mutagenic.[11] Not only chemicals can be used as disinfectants, but some natural plant ingredients are also effectively used as disinfectants. Plants containing antimicrobial secondary metabolites have been used extensively in traditional medicine for many years. Some of them are proven to act as skin disinfectants and antiseptics.[12]

Products of plant origin have been used as disease-control agents and are reported to have lower toxicity and fewer negative effects on the environment.[13] An alternative approach to identifying new and innovative medicinal products includes weed plants containing antimicrobial and antifungal compounds. Indonesia is a country with the second highest biodiversity after Brazil.[14] Various aquatic plants can potentially eliminate 90% of pathogenic microbes.[15] The abundant potential of aquatic weeds can be balanced by exploiting their properties as antimicrobials.[16]

Eichhornia crassipes (Mart) Solm (*E.crassipes*) and *Pistia stratiotes* L (*P.stratoites*) are two types of aquatic vascular plants that are very important for primary productivity and nutrient cycling of aquatic ecosystems but are also weeds. Water hyacinth (*E.crassipes*) belongs to the Pontederiaceae family, and Watercress (*P. stratiotes*) from the Araceae family.[14] Both of these weeds have activity degrading and absorbing organic and inorganic contaminants from wetlands.[17] Aquatic plants can improve water quality parameters, reduce detergent levels in waters18, and reduce most of the pathogens in aquatic waste.[15] phytochemical compounds in these two plants make them valuable as drugs and natural antimicrobial sources.[19,20,15] Most herbs have phenolic group compounds whose bioactivity plays a role in treating various infectious diseases.[21,22] The phytochemical content in *E.crassipes* are flavonoids, alkaloids, steroids, terpenoids, saponins, and anthraquinones;[16,23,24] whereas in *P.stratiotes* there are alkaloids, flavonoids, steroids, saponins, tannins, sterols, glycosides, and phenols.[16,25] The biological and pharmacological activities of *E. crassipes* and *P.stratoites* are anti-inflammatory, antioxidant, and antimicrobial.[19,20] Also as an analgesic, antipyretic, bronchodilator, and diuretic.[26]

e-ISSN 2774-2318
p-ISSN 2407-0505

Eichornia classiest activity as an antimicrobial has been reported previously; it affects opportunistic bacteria (*Staphylococcus aureus,*[19,27] *Staphylococcus epidermidis,*[28] *Escherichia coli,*[19,29] and *Pseudomonas aeruginosa*[30,31]; on pathogenic bacteria *Shigella flexneri* and *Salmonella var typhi,* and as an antifungal (*Candida albicans*).[27] The same thing has the antimicrobial effect of *P. stratoitestes*[32]; extract in *E.coli* [20, 34] and *S.aureus;* [20,33] *Pseudomonas aeruginosa, Streptococcus pyogens*, and *Salmonella.*[30]

Based on the antibacterial bioactive content of this type of weed, both have the potential to produce effects as natural disinfectants. The use of infusion combination preparations can increase antibacterial activity. The effectiveness of disinfection agents against standard laboratory microbes (for example, *S. aureus, S. epidermidis, E. coli, S. typhi, P. aeruginosa, B.subtilis,* and *C.albicans*) can be determined through the phenol coefficient test. A coefficient value >1 or the equivalent of a 5% phenol solution is categorized as an effective/good disinfection agent.[35,36] Examples of good phenol coefficient values were obtained from the test results of the combination of infusions of *A. blimbi* and *C. odorata*, [35] as well as the combination of *O. sanctum* and *P. betle.*[36]

Previous test results using a single preparation of *E. crassipes* and *P. stratoites* ethanol extract inhibited gram-positive and gram-negative bacteria, respectively.[31] The effect of water extract treatment (100% single preparation) of *E. crassipes* and *P. stratoides* is still below that of chlorine in reducing coliform bacterial colonization.[34] Its ability as an antimicrobial in combination form and its effectiveness based on the phenol coefficient test has not been widely reported. This study aimed to test the antimicrobial activity of *E. crassipes* and *P. stratoides* infusion as a disinfection agent in vitro.

METHODS

This experimental study used a posttest only with a control group design, and this was approved by the ethical commission of the Faculty of Medicine, University of Lambung Mangkurat (ULM); letter number: 243/KEPK-FKULM/EC/VIII/2022. The research was conducted at the Pharmacology and Microbiology Laboratory of the ULM Faculty of Medicine from October to December 2022.

Research procedure

Plant materials. The plants tested were collected from the Sungai Benua Anyar area, Sungai Jingah Village, North Banjarmasin. The collection time is September 2022 in the morning. Plant material stems and leaves are selected fresh from the banks of the river. The plant was identified at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, ULM Banjarbaru.

Sample preparation. Selected plants are separated and washed with tap water, followed by distilled water to remove all dirt and unwanted associated parts. The samples were then dried to a constant weight. The dried specimens were then blued to form a fine powder.

Extract preparation. Samples of the leaves and stems of *E.crassipes*, while the stems of *P.stratoites* were too small to be separated, so the extract was prepared from the whole plant. Dry extracts of the samples were prepared in 96% ethanol (20 g in 200 mL solvent). The suspension was shaken vigorously

e-ISSN 2774-2318
p-ISSN 2407-0505

in a shaker for three days. Then the suspension was filtered using gauze and filtered again with Whatman filter paper no.1. The filtrate was evaporated over a water bath. The concentrated extract was weighed, dissolved in 2 ml of DMSO (Dimethyl sulphoxide), and then stored in an airtight sample bottle in the refrigerator at 40 C for antimicrobial examination at various concentrations and phytochemical tests.[6,37] The extract combination was made by mixing 1 ml of each extract in a tube with a ratio of 1:1, and then several paper discs were added at each combination extract concentration.[38]

Preparation of stock extract solutions. Extracts of *E.crassipes* and *P.stratoites* were dissolved in 96% ethanol and 1% DMSO at a concentration of 100% w/v. The extract was prepared in a combined ratio of 1:1 and then analyzed for phytochemical tests. The extracts were made serial dilutions for the phenol coefficient test (1:20 - 1:250) and the concentrations of the combined extracts for the diffusion test (25%, 50%, 75%, and 100%).[35]

Phytochemical group test: According to Harborne (1987), a standard phytochemical test with modifications carried out to determine the composition of metabolic compounds in both extracts. Wagner and Dragendroff test for alkaloids, base test and lead acetate test for tannins, test for flavonoids, phenolics, steroids, terpenoids, anthraquinones, and foam test for saponins.[39,40]

Preparations of the test organism. The tested bacterial isolates were a collection from the Microbiology Laboratory of ULM Faculty of Medicine, namely *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 35983, *Escherichia coli* ATCC 25922, *Salmonella var typhi* ATCC 19430, *Pseudomonas aeruginosa* ATCC 19430, and *Candida albicans* ATCC 10231. Each bacteria and fungus was prepared in Brain Heart Infusion (BHI) media and homogenized equivalent to 0.5 Mc Farland standard solution.

Antimicrobial test of diffusion method. Petri dishes were prepared with 20 ml of Mueller Hinton agar (Oxoid) for bacteria and Sabaraud dextrose (Oxoid) agar for fungus. Test Organisms were wiped on compacted sterile media, and paper discs (5 mm in diameter) were added to the extract with varying concentrations. The discs were left for 1 hour and placed on a plate. Then, they were incubated at 37°C for 24 hours and 25 °C for 48 hours each for bacteria and fungi, with 0,0025% chlorine as a positive control. The diameter of the incubation inhibition zone (clearance) was recorded in millimeters.

Preparation of test disinfectants. A 5% (w/v) phenol solution was prepared by dissolving 5 g of phenol crystals in 95 ml of sterile distilled water. A 5% (v/v) disinfectant solution was prepared by adding 15 ml of dan 7.5 ml of test disinfectant into 300 ml and 150 ml of sterile distilled water. For dilution, doubling was then carried out on each 5% solution (1:20) of the extract and test disinfectant by adding 50 ml of the 5% solution into 50 ml of sterile distilled water to produce 1:40.[41] The same procedure was carried out up to 1:250 dilution.[35] Controls were 1% DMSO and sterile distilled water.

Standardization of test organism. Threefold serial dilutions of test organisms were carried out by standing six test tubes containing 2 ml of sterile normal saline. Serial dilutions were carried out by taking 1 ml of

e-ISSN 2774-2318
p-ISSN 2407-0505

24 h nutrient broth culture of the test organism using a micropipette and adding it into the first test tube. This mixture was mixed, and 1 ml was taken and inoculated into the second test tube. This activity was done until the sixth test tube.[41] Then, the dilution of the test organism that corresponds to the freshly prepared 0.5% McFarland standard (1.5×10^8 cfu/ml) was used.

Phenol Coefficient Test. Serial dilutions of extracts, disinfectants, and phenols were made in distilled water, starting with dilutions of 1:20 to 1:250. 0.5 ml of 24-hour standard culture was dispensed into each dilution of the extract, disinfectant, and phenol and incubated. Exactly 5 minutes after the culture was added to the first dilution, one loop from each tube was streaked onto the prepared nutrient agar plates. This activity was done for all dilutions and incubated at 37°C for 24 – 48 hours. The same thing was done at 10 minutes and 15 minutes of incubation. The result is expressed as growth or no growth.[35,36] The phenol coefficient was determined as the minimum dilution of phenol, extract, and test disinfectant that kills the test organism at 10 minutes but not at 5 minutes; calculate the phenol coefficient as follows:

$$\text{Phenol coefficient} = \frac{\text{The Concentration of test disinfectant killing at min but not at 5 min}}{\text{The Concentration of phenol killing at 10 min but not at 5 min}}$$

Disinfectant preparations with a phenol coefficient greater than one are more effective than phenol. The higher the phenol coefficient, the more effective the disinfectant is compared to phenol.

Data analysis.
Antimicrobial activity analysis was performed using the ANOVA test and Duncan's post hoc test, which had a significance level of 95%.[42] Analysis of the phenol coefficient values was carried out descriptively.

RESULTS

The results of the phytochemical screening test are shown in Figure 1 and Table 1. The bioactive compounds in both extracts were alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and steroids.

The results of the phytochemical screening showed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, and steroids in both the ethanol extract of *Eichornia crassipes* and the ethanol extract of *Pistia stratoites*. The EC+PS extract treatment generally produced an increased effect at increasing concentrations, with a broader inhibition zone against gram-positive bacteria than *C. albicans* yeast (Figure 2). The EC100%+PS100% extract treatment produced the most expansive inhibition zone. The analysis of inhibition zone data in Table 2 shows a different effect on each organism tested. Test organisms are the most common pathogens that cause infectious diseases in wetlands.[3]

e-ISSN 2774-2318
p-ISSN 2407-0505

a) *E.crassipes* infusion (b) *P.stratoites* infusion

Figure 1. The Phytochemical test results
(Budiarti et al., 2023)

Table 1. Results of phytochemical screening of the ethanol extracts of *Eichornia crassipes* and *Pistia stratiotes*

Phytochemical constituents test		Inference	
		Eicchornia crassipes	*Pistia stratoites*
Alkaloids	Dragendroff test	+	+
	Mayer test	+	+
Flavonoids	Alkaline Reagen	+	+
	Pb Acetate Test	+	+
Phenol	Iron (III) Chloride Tes	+	+
Tannins	Gelatin test	+	+
Saponins	froth test	+	+
Terpenoids	A grey solution	+	+
Steroids	Salkowski's test	+	+

(Budiarti et al., 2023)

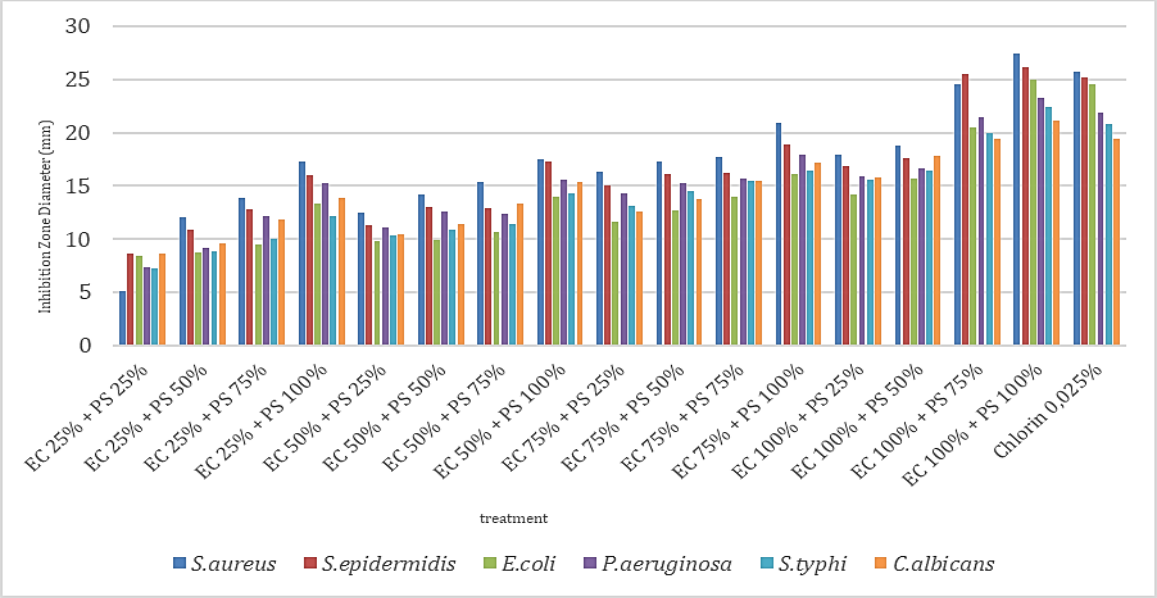

Figure 1. Inhibitory activity of *Echornia crassipes* and *Pistia stratoites* (EC+PS) extracts against five species of bacteria and *Candida albicans* (*Budiarti et al., 2023*)

doi: 10.26714/magnamed.11.2.2024.154-168
11 (2) August 2024

e-ISSN 2774-2318
p-ISSN 2407-0505

Table 2. The average inhibition zones of *Echornia crassipes* and *Pistia stratoites* (EC+PS) extracts against five species of bacteria and *Candida albicans*

Treatments	Average inhibition zones (mm)					
	S.aureus	*S.epidermidis*	*E.coli*	*P.aeruginosa*	*S.typhi*	*C.albicans*
25%EC+25%PS	5,16^{k}	8,69^{k}	8,42^{j}	7,37^{j}	7,28^{l}	8,63^{j}
25% EC+50%PS	12,07^{j}	10,85^{i}	8,75^{ij}	9,15^{i}	8,87^{k}	9,59^{i}
25% EC+75%PS	13,88^{i}	12,77^{i}	9,48^{hi}	12,19^{h}	9,98^{j}	11,89^{fg}
25% EC+100%PS	17,29^{f}	16,06^{f}	13,37^{de}	15,25^{e}	12,16^{g}	13,85^{e}
50 EC+25%PS	12,47^{j}	11,31^{i}	9,82^{gh}	11,12^{i}	10,30^{ij}	10,45^{h}
50 EC+50%PS	14,18^{i}	12,98^{i}	9,88^{gh}	12,58^{gh}	10,88^{hi}	11,40^{g}
50 EC+75%PS	15,32^{h}	12,90^{h}	10,64^{g}	12,34^{g}	11,37^{h}	13,36^{e}
50 EC+100%PS	17,47^{f}	17,33^{de}	13,95^{d}	15,59^{e}	14,35^{f}	15,37^{d}
75 EC+25%PS	16,29^{g}	15,07^{g}	11,65^{f}	14,25^{f}	13,11^{g}	12,58^{f}
75 EC+50%PS	17,27^{f}	16,13^{f}	12,65^{e}	15,25^{e}	14,47^{f}	13,76^{e}
75 EC+75%PS	17,69^{f}	16,23^{f}	14^{d}	15,64^{e}	15,51^{e}	15,50^{d}
75 EC+100%PS	20,94^{d}	18,85^{c}	16,15^{c}	17,91^{c}	16,45^{c}	17,20^{c}
100 EC+25%PS	17,96^{ef}	16,81^{ef}	14,21^{d}	15,90^{de}	15,56^{e}	15,75^{d}
100 EC+50%PS	18,76^{e}	17,66^{d}	15,60^{c}	16,68^{d}	16,41^{d}	17,77^{c}
100 EC+75%PS	24,57^{c}	25,56^{ab}	20,50^{b}	21,40^{b}	19,98^{c}	19,40^{b}
100%EC+100%PS	27,48^{a}	26,13^{a}	24,97^{a}	23,23^{a}	22,45^{a}	21,10^{a}
Chlorin 0,025%	25,71^{b}	25,23^{b}	24,59^{a}	21,84^{b}	20,85^{b}	19,44^{b}

*) the same alphabet in the column is not significantly different; P>0.05. (*Budiarti et al., 2023*)

Table 3. The average value of the phenolic coefficient of *E. crassipes* and *P. stratoites* (EC+PS) extracts and control of 5 species of bacteria and *Candida albicans*

Treatments	Phenol Coefficient Value					
	S.aureus	*S.epidermidis*	*E.coli*	*P.aeruginosa*	*S.typhi*	*C.albicans*
EC+PS extract	1,29	1,25	1,13	1,0	1,14	1,14
0,0025%chlorine	1,29	1,25	1,13	1,0	1,14	1,14
5% phenol	1,10	1,10	1,10	1,0	1,10	1,10

(Budiarti et al., 2023)

The inhibitory strength produced by all treatments on organisms, using the classification according to Davis and Stout.[43] In this study, the inhibition zone produced by EC+PS and chlorine was more effective against gram-positive bacteria than gram-negative bacteria and *C.albicans*. The inhibitory power of EC+PS extract on all organisms ranged from moderate to vigorous.

The smallest inhibition zone area was in the EC25%+PS25% treatment for *Salmonella var typhi,* which was 7,28 mm, and the largest in the EC100%+PS100% treatment for *Staphylococcus aureus* was 27,48 mm. The effectiveness of EC+PS extract as a disinfectant and control agent in this study used the phenol coefficient test with serial dilutions from 1:20 to 1:250. The results are shown in Table 3, and a

e-ISSN 2774-2318
p-ISSN 2407-0505

good effect is obtained with a phenol coefficient value >1.

The inhibitory strength produced by all treatments on organisms, using the classification according to Davis and Stout.[43] In this study, the inhibition zone produced by EC+PS and chlorine was more effective against gram-positive bacteria than gram-negative bacteria and *C. albicans*. The inhibitory power of EC+PS extract on all organisms ranged from moderate to vigorous. The smallest inhibition zone area was in the EC25%+PS25% treatment for *Salmonella var typhi,* which was 7,28 mm, and the largest in the EC100%+PS100% treatment for *Staphylococcus aureus* was 27,48 mm. The effectiveness of EC+PS extract as a disinfectant and control agent in this study used the phenol coefficient test with serial dilutions from 1:20 to 1:250. The results are shown in Table 3, and a good effect is obtained with a phenol coefficient value > 1.

The phenol coefficient values resulting from the EC+PS and chlorine extract treatment for *S. aureus, S. epidermidis, E. coli, P. aeruginosa*, and *S. typhi* bacteria, respectively were 1.29, 1.25, 1.13, 1.0, and 1.14 as well as for *C. albicans* of 1.14. These results indicate that the *E. crassipes* and *P. stratoites* extracts have the same effectiveness as chlorine disinfectants.

DISCUSSION

The solvent in this study used 96% ethanol, which can dissolve polar and semipolar solvent compounds. As with previous research reports, 96% ethanol extracts of *E. crassipes* and *P. stratoites* found groups of phenolic compounds, flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids. [19,20,23,24] A group of phenolic compounds synthesized by plants in response to microbial infection, thus appearing in vitro as effective antimicrobial agents against various microorganisms.[12,27,28] These results were obtained in this study, and the effects of the two extracts were tested as antibacterial and antifungal.

The antimicrobial activity produced by *E. craasipes* and *P. stratoites* was relatively different from previous reports in which combined extracts were used in this study. Combining these extracts allows the activities of the bioactive compounds to interact with each other to produce a synergistic effect so that the inhibition is better.[44,45,46] 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 natural preparations for commercial scale hand washing.[47] Bioactive molecules in polyherbal have synergistic antimicrobial effects with lower side effects.[48] Polyherbal extracts have antimicrobial properties and stability, which are effective for skin disinfection.[49] Saradhajyothi and Subbarao (2011), active ingredients such as phenolic compounds, triterpenoids, steroids, flavonoids, ketones, and triterpenoids all contribute antimicrobial with good effect.[46] Herbs that contain these bioactive compounds can be used as chemotherapeutic agents and antiseptics.[21] As previously reported, the inhibitory activity as an antimicrobial extract was influenced by the type of solvent, the Concentration of the extract, pH, exposure time of the extract, and the type of microbe tested.[19,20,50] The phenolic compounds, saponins, tannins, and flavonoids dissolved in the ethanol extract, their effects can be bacte-

e-ISSN 2774-2318
p-ISSN 2407-0505

riostatic or bactericidal, depending on their Concentration.[32]

The antimicrobial activity produced by the extracts in this study Measurement of the antimicrobial activity produced by the extract in this study (Table 2) refers to Davis and Stout.[43] The resulting inhibitory effect is moderate (inhibition zone area >5mm) to firm (inhibition zone area >20mm); at extract levels <50%, it shows a bacteriostatic/fungistatic effect, and at extract levels of 75% it causes a bactericidal effect because the effect can be equivalent to the positive control. The active compounds produced by the two extracts produced an inhibitory effect on the five tested bacterial species and *C.albicans,* as well as the effect of chlorine. This result is consistent with previous research that chlorine disinfectants are effective against pathogens that cause nosocomial infections[51], water pollution microbes[10], including spore-forming microbes.[8]

In vitro, as mentioned in previous studies, this ethanol extract of E.crassipes and P.stratoites produced significant inhibitory activity against Gram-positive bacteria, Gram-negative bacteria, and yeast. [20] The extract was more effective on *S. aureus* and *S. epidermidis*, whereas *S. typhi* was more resistant to extract treatment, as found in previous research.[4,51,52] Moreover, extracts from aquatic plants or those submerged in estuaries can more easily enter the Gram-positive bacteria's cell wall structure, consisting of one layer. Tegos et al. (2002) hypothesized that plant antibiotics are effective if they can enter Gram-negative double membrane cells.[53] The cell wall of Gram-negative bacteria has a double-layer membrane. Surrounds the bacterial cell, preventing the cell membrane from being permeabilized to antimicrobial agents and delaying the bacterial cell's osmotic lysis. Membrane permeability causes resistance to the effects of ethanol.[53,54] Also, the periplasmic space contains enzymes that break down foreign molecules.[50,55]

Secondary compounds were found to have almost unlimited capabilities for all microorganisms.[56] Phenolic group phytochemical compounds, namely flavonoids and tannins.[53,57] Secondary compounds synergize with inhibitor components to increase their effectiveness.[46] Active flavonoids interfere with cell membrane formation and deactivate enzymes, inhibit replication in bacterial cells, disrupt the cytoplasm, and damage the cell membrane structure.[58,5960] In fungi, the flavonoid group works by inhibiting nucleic acid synthesis, inhibiting cell division, and cell proliferation.[61,62] Alkaloids work by interfering with the function of the outer membrane, depolarizing the microbial cell membrane.[63,64] In fungi, alkaloids can inhibit fungal cell respiration and inhibit nucleic acid synthesis.[65] Tannins work by inhibiting enzymes (catalase) produced by bacteria and affecting nucleic acid synthesis and the integrity of cell wall composition.[66,67] The mechanism of tannins in fungal cells is to inhibit the integrity of the cell membrane by inhibiting the bonds of ergosterol and polyphenols in the cell membrane.[57] Saponins increase the permeability of bacterial cell membranes, dissolve proteins, and destroy bacterial cell walls.[55,63] In fungi, saponins interact through sterols in the cell membrane and induce damage to the fungal cell membrane.[62,63,68] Terpenoid compounds destroy porins69, reducing the permeability of bacterial cell walls and inhibiting bacterial growth.[56,57,58] Terpenoids in fungi work by dis-

e-ISSN 2774-2318
p-ISSN 2407-0505

solving cell walls and weakening tissue membranes.[56.65] Steroids work by inhibiting cell membrane synthesis, cell leakage, and the release of intracellular bacterial material. In fungi, steroids are lipophilic and inhibit spore germination.[70,71]

On the phenol coefficient parameter, EC+PS and chlorine extracts were effective against all test organisms with a coefficient value of >1. These results indicate that the incorporation of extracts produces antimicrobial properties and enhances its activity as a disinfecting agent. The biocidal effect of chlorine (hydrogen peroxide) is due to the -OH radical, which is formed by the decomposition of peroxides in the presence of a catalyst (Fe, Cu ions) in microorganisms, resulting in an oxidative mechanism against membranes, DNA, and other cellular constituents of microorganisms.[2,47] The efficacy of disinfectants such as chlorine can decrease after three weeks of storage.[46] For this extract, it is not yet known how effectively it is stored. In addition, the effectiveness of disinfectants also needs to be evaluated for biofilm-forming bacteria and fungi, which are among the causes of increased resistance rates in nosocomial infections.[1]

Based on this study, it is possible to investigate further the stability of polyherbal formulations made from *E.crassipes* and *P.stratoit* as a substitute for the effect of antimicrobial and alternative disinfectants. This in-vitro-research was limited to testing the antimicrobial effectiveness of *E. crassipes* and *P. stratoites* as disinfectant agents based on the phenol coefficient test and diffusion test. In vitro studies, however, do not fully replicate the complex interactions and environments found in vivo. Knowing more about the influence of contact time and its stability as a disinfection agent is essential. The efficacy of disinfection also needs to be tested on biofilm-forming pathogenic microbes and evaluated using appropriate methods.

CONCLUSION

Several bioactive compounds were found in *E. crassipes* and *P. stratoites,* which are soluble in 96% ethanol solvent. The activity of both extracts is antibacterial and antifungal, which in vitro produces the same effect as chlorine activity, with moderate to extreme inhibitory power. The combination at a concentration of 75% is effective against *S.aureus*, *S.epidermidis, E.coli,* and *P.aeruginosa,* while on *S.typhi* and *C.albicans* at a concentration of 100%. Combining these two extracts produces effectiveness as a disinfectant with a phenol coefficient value of >1. In conclusion, *E. crassipes* and *P. stratoites* extracts can produce antimicrobial activity with good phenol coefficient values, so combining the two can be an alternative natural disinfectant.

Authors contributions

The authors declare that they have no conflict of interest. Budiarti LY conceived and designed research. Budiarti LY and Kaidah S conducted experiments. Budiarti LY, Azhari NP, and Ihsanti SH contributed reagents and analytical tools. Budiarti LY and Azhari NP analyzed the data. Budiarti LY, Kaidah S, and Ihsanti SH wrote the manuscript. All authors read and approved the manuscript.

e-ISSN 2774-2318
p-ISSN 2407-0505

REFERENCES

1. Gargi R, et al. Efficacy Study of some Antiseptics and Disinfectants; International *J. of Life Sciences.* 2017;5;(4):593-598.
2. Montagna MT, *et al.* Study on the In Vitro Activity of Five Disinfectants against Nosocomial Bacteria. Int. *J. Environ. Res. Public Health*. 2019;16(11):1-9.
3. 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*:2477-3522:336-347.
4. Heriyani F, et al. 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.
5. Obi CN, Onyekaozuru AO. Antimicrobial Activities of Some Household Disinfectants on Selected Human Pathogens in Umuahia, Abia State, Nigeria. *World J. Pharmaceutical Research*. 2015;4(12):171-183.
6. Aminu AI, Abdullahi MS. Evaluation of the Antibacterial Effectiveness of Some Antiseptics and Disinfectants. *UJMR,* 2021;6(1):175 – 181
7. Al-Sa'ady AT, Nahar HS, Saffah FF. Antibacterial activities of chlorine gas and chlorine dioxide gas against some pathogenic bacteria. *J.BioSciences Eurasia.* 2020;14:3875-82.
8. Jui-Wen M, et al. Efficacy and Safety Evaluation of a Chlorine Dioxide Solution. Int. *J. Environ. Res. Public Health,* 2017; 14(329):1-12
9. Herawati D, Yuntarso A. Penentuan dosis kaporit sebagai desinfektan dalam menyisihkan konsentrasi ammonium pada air kolam renang. *J SainHealth.* 2017;1(2):66-74.
10. Supriyadi, Sumantri I, Hartati I. Pengaruh dosis klorin pada pertumbuhan bakteri coliform total dan *Escherichia coli* pada sungai Kreo, sungai Garang dan sungai Tugu Suharto. *Momentum,* 2016;12(1):30-5.
11. Indriana et al., Potential of Plantation Commodities Products as Raw Materials of Natural Disinfectant Products (Review). *Jurnal Industri Hasil Perkebunan.* 2021; 16 (1), 18-31
12. Khanam S, Afsar Z. Herbal Disinfectants: A Review. World *J Pharmaceutical research.* 2013;3 (1):258-273.
13. Rufchaei R, et al., Evaluation of the Chemical Compounds and Antioxidant and Antimicrobial Activities of the Leaves of *Eichhornia Crassipes* (Water Hyacinth). *Jundishapur J. Nat Pharm*: e101436.2021:1-11.
14. A'tourrohman M. Inventarisasi dan Kajian Etnobotani Tanaman Akuatik di Taman Akuatik Kebun Raya Eka Karya Bali. *Biologi Sel,* 2020;9(1):2541-1225.
15. Dhir B. Effective control of waterborne pathogens by aquatic plants. *Waterborne*

e-ISSN 2774-2318
p-ISSN 2407-0505

Pathogens, 8.00017-7 (Chp.7).2020:339–61.

16. Tyagi T, Agrawal M. Pharmaceutical potential of aquatic plant *Pistia stratiotes (L.)* and *Eichhornia crassipes*. *Journal of plant sciences. Medicinal Plants*, 2015;3(1):10-18.

17. Arias JAV. Pharmaceutical and Personal Hygiene Products (PPcPs): A Threat Little Studied in Colombian Waters. *Agri Res& Tech: Open Access J.* 2019; 22(3): 1-8.

18. Al-Idrus SW, et al. Phytoremediation of Detergent Levels in Waters Using Water Plants: *Eichornia crassipes, Ipomoea aquatica, Pistia stratoites* and Their Combinations. European *J. Advanced Chemistry Research,* 2020;1(5):1-5.

19. Tyagi T, et al. Phytochemical Screening of Active Metabolites present in *Eichhornia crassipes* (Mart .) Solms and *Pistia stratiotes* (L .): Role in Ethanomedicine. *Asian J. Pharm Educ Res,* 2017;6(4):40–56.

20. Tyagi T, Parashar, P. Antimicrobial And Antioxidant Activity Of *Pistia stratiotes (L.) Int J. Pharma Bio Sci*; 2017;8(3):391-399.

21. Raharjo SJ, Ningsih RW. Cytotoxic Activities of Ethyl Acetate Fractions from Petroleum Ether Extract and Methanol Extract Of *Pistiae* Leaves. *Trad. Med. J.,* 2015;20(3):134-139.

22. Hossain J, Khan A, Uddin MA. Antimicrobial Efficacy and Phytochemical Analysis of Three Aquatic Plant Species in Bangladesh. *Bangladesh J. Microbiol*, 2018;35 (1):07-11.

23. Kumar S, et al. In Vitro Antioxidant, Antibacterial, and Cytotoxic Activity and In Vivo Effect of *Syngonium podophyllum* and *Eichhornia crassipes* Leaf Extracts on Isoniazid Induced Oxidative Stress and Hepatic Markrs. *BioMed Research International*. 2014;1:1-11.

24. Saraf KR, Barate DL. Antimicrobial Activity of *Eichhornia crassipes* Against MDR Clinical Pathogens. *International J. Recent Scientific Research*. 2018. 9(6)27260-27264.

25. Yuzammi. Kiambang Kecil (*Pistia stratiotes* L.): Gulma Unik Yang Kaya Manfaat. *Warta Kebun Raya*. 2016;16(1):47-54.

26. Dianasari D, Firdiyansari I. Potency of Ethanolic Extract of *Pistia stratiotes* Herbs and Its Fractions as Antioxidants using DPPH Method. *Pharmacon J.* 2019;16(2):83-88.

27. Rehman R, et al. Biological Screening of *Eichornia crassipes* against Different Pathogenic Microbes: An In Vitro Study. *Recent Advances in Biology and Medicine,* 2016;(2):79-82.

28. Wali S. et al. Efficiency Of Common Water Hyacinth (*Eichhornia crassipes*) In Controlling Growth Of Fungal And Bacterial Clinical Strains. *Pure and Applied Biology*. 2019;8(4):2178-2186.

29. Haggag MW, Alla AE, Abouziena HF. Phytochemical Analysis, Antifungal, Antimicrobial Activities And Application of *Eichhornia crassipes* Against Some Plant Pathogens. *Planta Daninha*. 2017;(35):1-11.

30. Khanal S, Neupane K. Photochemical

e-ISSN 2774-2318
p-ISSN 2407-0505

Screening And Antimicrobial Screening Of Potential Invasive Species Found At Around Paklihawa, Nepal. *Himalayan Biodiversity*. 2018;6: 8-45.

31. Budiarti LY, et al. Antibacterial Effectiveness of *Eichornia Crassipes* Solms. Extract Against Selected Bacteria Isolates In Vitro. *Proceeding Seminar of Science and Technology for Society 5.0 Development. ISST* 2021. e- ISBN: 978-623-312-830-8:210-26.

32. Tyagi T, Agarwal M. Phytochemical and GC-MS analysis of bioactive constituents in the ethanolic of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. *J. Pharmacognosy and Phytochemistry*. 2017;6(1*)*:195-206.

33. Dianasari D, Iftitah MB. Aktivitas Antibakteri Ekstrak Etanol Herba Apu-Apu (*Pistia stratiotes*) terhadap *Staphylococcus aureus*. J-PhAM. 2019; 2 (1):1-7.

34. Budiarti LY, et.al. Antibacterial Test of Water Extract *Eichhornia crassipes* (Mart.) Solms. and *Pistia stratiotes* (L.) on Coliform MPN Value of Water Sample. Seminar of Science conference- *ISST*. 2022.

35. Budiarti LY, Wydiamala E, Ulfa, N. Phenol Coefficient Test Combination Infusion of *Cananga odorata - Averrhoa bilimbi* L. against *staphylococcus aureus* and *Salmonella typhi* in vitro. *BBRJ*. 2021;4 (1):19 – 26.

36. Budiartii LY, et al. The Antibacterial Activity of Betel (*Piper battle* L.) and Basil (*Ocimum sanctum* L.) Leaves Infusion as Antiseptic Preparations Against Some Bacteria *In Vitro*. *BBRJ*. 2021;4(2):48 - 56.

37. Bhoora HSG, et al. Comparative study of antibacterial assay of *Mentha piperita* (in vivo and in vitro cultured) leaves extract on enveloped human pathogenic bacteria and its phytochemical screening. *J. Pharmacognosy and Phytochemistry,* 2020;9(4):15-19.

38. Budiarti LY, Wydiamala E, Madani RA. Antibacterial Activity of Extract Combination of Leaves and Peels Kaffir Lime (*Citrus Hystrix* Dc.) Against Some Test Bacteria. *BBRJ*. 2021;4(2):39 - 47.

39. Pandey A, Tripathi S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem.* 2014;2(5):115-119.

40. Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. *International J. Chemical Studies*. 2020;8(2):603-608.

41. Ononugbo CM, et al.The Effect of pH and Temperature on Phenol Coefficients of Two Common Disinfectants Using Clinical Isolates of *Escherichia coli* and *Staphylococcus aureus*. *JAMB*. 2018;10(2):1-7.

42. Dahlan SM. Statistik untuk kedokteran dan kesehatan: deskriptif, bivariat, dan multivariate, dilengkapi aplikasi dengan menggunakan SPSS. 2019; Ed.5. Jakarta. Salemba Medika

43. Buton J, Mangesa R, Sehol M. Uji efektifitas hand sanitizer alami dalam menghambat pertumbuhan bakteri *Staphylococcus aureus*. *J. Innovation*. 2022; 2(2):317-22.

e-ISSN 2774-2318
p-ISSN 2407-0505

44. Sutalangka C, Wattanathorn J. Neuroprotective and cognitive-enhancing effects of the combined extract of *Cyperus rotundus* and *Zingiber officinale*. *BMC Complementary and Alternative Medicine*. 2017;17 (35):1-11.

45. Song S, et al. Antifungal activity of hypocrellin compounds and their synergistic effects with antimicrobial agents against *Candida albicans*. *Microbial Biotechnology*. 2021;14(2):430–443.

46. Azelee NIW, et al. Antimicrobial disinfectants and sanitizers: An effective tool for breaking the circle of pandemic disease. *Asian J. Agric. Biol.* 2018;8(3):348-367.

47. Sandeep DS, et al. Formulations of antimicrobial polyherbal hand wash. *Res. J. Pharm. Technol.* 2016;9(7):864-866.

48. Shuja S, et al.. The Use, Phytochemical and Antimicrobial Activity Evaluation of *Foeniculum vulgare*, *Cuminum cyminum*, and *Trachyspermum ammi* Were Reviewed for the Treatment of Gastric Diseases As Future Compounds. *J. Pharmacy and Pharmacology*. 2022;10:273-282.

49. Mulani SA, et al., Formulation and evaluation of polyherbal human health amiable hand sanitiser: A need to fight COVID 19. *Indian J. Anatomy and Surgery of Head, Neck and Brain.* 2021;7*(2)*:47–50.

50. Tensay GK, et al. Evaluation of in vitro antibacterial potential of *Eichhornia crassipes* leaf extracts. *Drug Invention Today*. 2018;10(5):3824-31.

51. Al-Sa'ady AT, Nahar S, Saffah FF. Antibacterial activities of chlorine gas and chlorine dioxide gas against some pathogenic bacteria. Eurasia *J Biosci.* 2020;14:3875-3882.

52. Septiani et.al, Antibacterial Activities of Lintut Leaves (*Hemigraphis* sp) Extract Against *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis,* and *Salmonella typhi* Bacteria's. *Conf. 13th Proc. Mul. Pharm.* 2021; 62-6. e-ISSN:2614-4778.

53. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern. *Plants. Front. Microbiol.* 2019;10.(911):1-28.

54. Fajrih N, et al. Bioactive compounds and its inhibitory effect of banana corm. *Biodiversitas 23*. 2022;(1):195-204.

55. Khan MI, et al. Green Tea Seed Isolated Saponins Exerts Antibacterial Effects Against Various Strains of Gram Positive and Gram Negative Bacteria A Comprehensive Study In Vitro and In Vivo. *Evidence-Based Complementary and Alternative Medicine.* 2018;1–12. ID 3486106.

56. Suurbaar J, Mosobil R, Donkor AM. Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of *Ricinus communis* against selected pathogens. *BMC research notes*. 2017;10(1):1-6.

57. Carvalho RS, et al. 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 J. of Botany*. 2018;114:181-187.

e-ISSN 2774-2318
p-ISSN 2407-0505

58. 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.

59. Al-Aboody MS, Mickymaray S. Antifungal Efficacy And Mechanisms Of Flavonoids. *Antibiotics (Basel).* 2020;9(450):1-42

60. Sarbu, et al.. Synthetic flavonoids with antimicrobial activity: a review. *J. Applied Microbiology*. 2019;127:1282—1290.

61. Musiam, et al., Aktivitas Antifungi Flavonoid Dari Ekstrak Daun *Citrus aurantifolia* Kalimantan Selatan Terhadap Pertumbuhan *Candida albicans*. *J. Farmasi Indonesia AFAMEDIS*. 2020;1(1):55-63.

62. Kusuma SAF, Herawati IE, Darniasih C. Appraisal of *Jatropha curcas* Leaf Characteristic As A Natural Anti-Vaginal Candidiasis. *Drug Invention Today*. 2020;13(6):1001-5.

63. Zaynab M, et al., Review Saponin toxicity as key player in plant defense against pathogens. *Toxicon.* 2021;193:21–27.

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

65. Mejdad NMA. Antifungal activity of some plant extracts against two yeasts isolates in vitro. Research *J. Pharmaceutical, Biological and Chemical Sciences*. 2014;5(2):1992-8.

66. Septiadi. Uji Fitokimia dan Aktivitas Antifungi Ekstrak Teripang Keling (*Holoturia atra*) dari Pantai Bandengan Jepara Terhadap Fungi *Candida albicans*. *J. Marine Research*. 2013;2(2):76-84..

67. Miftahulaila, Pengaruh waktu perendaman plat resin akrilik polimerisasi panas dalam ekstrak kulit durian (*Durio zibethinus* L.) terhadap jumlah koloni *Candida albicans*. *Prima J. Oral and Dental Sciences*. 2021;4(2):33-38.

68. Yang L, et al. Antifungal Effects of Saponin Extract from Rhizomes of *Dioscorea panthaica* Prain et Burk against *Candida albicans*, *Evidence-Based Complementary and Alternative Medicine*. 2018, Article ID 6095307:1-13.

69. Hamza M, et al. In vitro effectiveness of triterpenoids and their synergistic effect with antibiotics against *Staphylococcus aureus* strains. *Indian J Pharmacol.* 2016;48(06):710–714 .

70. Carvalhal F, et al. Sources and biological activities of marine sulfated steroids. *Journal of Molecular. Endocrinology.* 2018;61(2):T211–T231.

71. Syawal H, Hakim L, Effendi I. Phytochemical analysis of *Rhizophora apiculata* leaf extract and its inhibitory action against *Staphylococcus aureus, Aeromonas hydrophila* and *Pseudomonas aeruginosa*. *AACL Bioflux*. 2020;13(4):2242-2249.