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Coefficient Test of Antimicrobial Phenol Combination of *Eichhornia crassipes* and *Pistia stratiotes* Extract In Vitro as Alternative Disinfection Candidates

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Abstract:

Eichhornia crassipes and *Pistia stratiotes* are weeds that often pollute water areas. These two contain various active compounds that act as antimicrobials, which have the potential as disinfection agents to prevent the transmission of pathogenic bacteria and fungi. An antimicrobial substance is said to be effective as a good disinfectant if it has a phenol coefficient value equal to or more than the 5% phenol comparison substance. The aim of this research was to test the effectiveness of a combination of Eichhornia crassipes and Pistia stratiotes extracts as a disinfection candidate through the phenol coefficient test. This in vitro experimental study was carried out using a dilution technique. The combination treatment of *Eichhornia crassipes* and *Pistia stratiotes* extracts and 5% phenol was tested against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and also *Candida albicans* yeast. Observations on the test media suspension tubes that were clear or did not show the growth of the test microbes for a period of 5, 10, and 15 minutes. The phenol coefficient value of the combination treatment of Eichhornia crassipes and Pistia stratiotes extracts was ≥1 with 5% phenol as comparison. Higher coefficient values were obtained for Staphylococcus aureus, equivalent values of 5% phenol were found for *E.coli, P.aeruginosa*, and *C.albicans*; whereas in *S.typhi* the value is <1. The combination treatment of *E.crassipes* and *P.stratiotes* extracts resulted in a phenol coefficient value equivalent to that of 5% phenol comparator, for several tested bacteria and yeasts. So it has effectiveness as a preparation for disinfection. The combination of *Eichhornia crassipes* and *Pistia stratiotes* extracts has antimicrobial effectiveness and can be developed as an alternative disinfection candidate.

Keywords: phenol coefficient test; combined extract; *Eichhornia crassipes*; *Pistia stratiotes*; disinfection candidate

Introduction

Infectious diseases caused by bacteria and fungi are still frequently found in people living in wetlands with humid environmental conditions and unhygienic behavior. In this condition it is easier for infectious agents to be transmitted through the use of water, hand handling or equipment that has been contaminated by microbes. So maintaining environmental health is very important to note in minimizing the spread of infectious diseases.

One effort that can be made is the use of disinfectant substances. Disinfectants have different effectiveness in reducing the growth of bacteria and fungi.[1] The effectiveness of a disinfectant is determined based on the parameter of the phenol coefficient of the disinfectant, through the phenol coefficient test. In this test, a disinfectant substance is said to have good effectiveness if it has a value equal to 1 (one) or more than the value of 1 (one) pheno1 coefficient of 5%; effectiveness is better if the value is more than 1 (one).[2]

A common disinfectant used to clean the environment or water media is chlorine.[2] Chlorine is a relatively inexpensive, effective, easy to find and easy to use disinfectant.[3] In general, the process of inactivating bacteria and fungi by disinfectants is the same, that is disrupting cell metabolism.[1] The mechanism of action of chlorine is by damaging the cell structure of organisms, causing microbial cells to die.[4] Chlorine has activity in reducing the growth of bacteria and fungi.[1] In vitro, chlorine has an effect on the bacteria *Staphylococcus aureus* and *Salmonella typhi*.[5] Also on *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginos* from hospital liquid waste samples.[6]

Excessive use of chlorine substances has a negative impact, when chlorine enters the body when inhaled or swallowed with water consumed, it can also cause itching of the skin.[3] According to Sibarani et.al, several research reports mention the negative effects of chlorine on human health, including causing toxic effects, asthma, skin diseases, increasing serum cholesterol and low-density lipoprotein (LDL) levels, and triggering bladder cancer.[5]

An effort to reduce the use of chlorine is to use alternative disinfectants made from plants, which are known to have antimicrobial effectiveness.[7]

Among the plants that have been studied as having pharmacological activity as antimicrobials are plants that live in aquatic areas, namely *Eichhornia crassipes* and *Pistia stratiotes*. The pharmacological and biological effects of E*. crassipes* are to treat boils, hot throats, fainting due to hot air, swelling due to inflammation of the kidneys, inability to urinate, and hives.[8] Antimicrobial power is generated by the active compounds contained in these plants. The results of phytochemical screening tests on *E. crassipes* and *P. stratiotes* plants are known to contain various phytochemical compounds such as alkaloids, glycosides, steroids, terpenoids, saponins and tannins.[9]

Several previous research results have proven the existence of antimicrobial activity from extracts of *Eichhornia crassipes* (water hyacinth) and *Pistia stratiotes* (apu-apu); has an inhibitory effect on the fungus *Candida albicans* and on several types of bacteria.[3,9,10,11,12]

According to Dhir (2020), around 90% of waterborne pathogenic microbes such as *Enterococcus, E. coli, Klebsiella pneumoniae, P. aeruginosa, Clostridium perfringens, Staphylococcus aureus*, and *Salmonella sp*. Its

growth can be inhibited by various aquatic plants including *E. crassipes* and *P. stratiotes*.[13] Some of the results of previous studies stated that there was an antimicrobial effect in vito in the treatment of *E. crassipes*14 and *P. stratiotes*.14,15 However, the resulting effect is still under the positive control tested.

The potential of the aquatic plants *E. crassipes* and *P. stratiotes* are known to contain various antimicrobial compounds, making it possible that they can be developed as alternative disinfectant preparations. This study aims to test the effectiveness of a combination of *Eichhornia crassipes* and *Pistia stratiotes* extracts as a disinfection candidate through the phenol coefficient test.

Combination preparations of a drug that have the same ingredients and mechanism of action can form synergistic or antagonistic effects; good effect is cybergistic so that it can increase its antimicrobial power.[16] A herbal combination treatment is known to produce a better antimicrobial effect than a single preparation; for example, the effect of a combination of *Stenochlaena palustris* (kelakai) and *Sauropus androgynus* (katuk) leaves on the bacteria *S.aurues, E. coli*, and on the fungus *C.albicans*.[17]

Research Method

Instrument

The plant materials used in this research were roots, stems and leaves. The two types of test plants were obtained from the Banua Anyar River area. The test plants have been identified as Eichhornia crassipes and Pistia stratoites at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat, Banjarbaru.

The test bacterial isolates studied were *Staphylococcus aureus* ATCC 29523, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 19430, and *Candida albicans* ATCC 10231. The microbial isolates tested were collections of the Microbiology Laboratory, Faculty of Medicine, Universitas Lambung Mangkurat Banjarbaru. The microbial suspension tested was made homogeneous by growing it on BHI media and made equivalent to 0.5 Mac Farland solution (1.5x106 CFU/ml).

The test media used are Nutrient Agar (NA), Nutrient Broth (NB), and Brain Heart Infusion (BHI). The solvent for making infusions and dilution series is sterile distilled water. The controls used in the phenol coefficient test are 0.002% chlorine and 5% phenol.

Preparation of *Eichhornia crassipes* and *Pistia stratiotes* Ethanol Extracts

The extraction method used for this research is maceration. As much as 1000 grams each of *Eichhornia crassipes* and *Pistia stratiotes* powder samples were put into the maceration tool, then 96% ethanol solution was poured slowly into the maceration tool.[6] The maceration process is carried out within 3 x 24 hours by stirring until evenly distributed, every 1 x 24 hours the filtrate is filtered, and the solvent is replaced with a new one. After that, the extract was put into a rotary evaporator at a temperature of 60◦C until a thick ethanol extract was obtained, then evaporated in a water bath to obtain a constant weight. The extraction results can be stored in the refrigerator at a temperature of 4◦C.[2]

Preparation of Treatment Dilution Series for Phenol Coefficient Test

The 26 sterile tubes were prepared each for treatment with a combination of 100% *E.crassipes* and *P.stratoites* extracts (1:1) and 5% phenol control and labeled 1-26. In each

tube 1-13 filled with test infusion treatment and 5% phenol control as much as 2 ml and added sterile distilled water from each tube 1-13 in a row, namely 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 13, 18, and 23 ml; Then each tube is homogenized. Next, 2 ml of each tube containing the infusion and test control was pipetted aseptically and put into tube 14-26. The results obtained were serial dilution of each treatment and 5% phenol control test 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; and 1:250. Preparation of treatment dilution series was made in the same steps for each treatment of the bacteria tested.[2]

Phenol Coefficient Test

Prepared suspensions of test bacteria (*S. aureus, E.coli, P.aeruginosa*, and *S. typhi*), as well as *Candida albicans* fungi on tube racks and sterile test tubes containing NB media which have been labeled according to the dilution and the length of contact time (5, 10, and 15 minutes), as well as equipment such as alcohol burner, micropipettes, and tubes. Put 0.5 ml of the test bacterial suspension into a tube containing the test treatment (*Eichhornia crassipes* and *Pistia stratoites* extract and 5% phenol) in various dilution series starting from a 1:20 to 1:250 dilution tube, then homogenize. After 5 minutes, 1 loop was taken from each tube of the test dilution series and put into each test tube containing the test dilution series with a contact time of 5 minutes, then the loop was sterilized with an alcohol burner. After the second 5 minutes, 1 loop was taken from each tube of the test treatment dilution series into a test tube of the test treatment dilution series with a contact time of 5 minutes (total contact time was 10 minutes), then the loop was sterilized with an alcohol burner. After the third 5 minutes, 1 ose was taken from each dilution tube of the test treatment into the series test tube dilution of the test treatment with a contact time of 5 minutes (total contact time of 15 minutes), then the ose was sterilized with an alcohol burner. The same steps were carried out for the treatment of each test bacteria. All tubes were incubated at 37°C for 24 hours, then the turbidity was observed. The presence of (+) bacterial growth was indicated by the medium becoming cloudy, and the absence of test microbial growth (-) was indicated by the medium remaining clear.

Data Analysis

The data of phenol coefficient values were analyzed descriptively.

Results

The effectiveness of an antimicrobial agent as a good disinfection agent is equal to or greater than the phenol liquid coefficient value of 5%, which is equal to 1 (one). Observations of the phenol coefficient test for each treatment tested can be seen in Tables 1 and 2. For treatments that show no microbial growth, their effectiveness is calculated using the phenol coefficient value formula. The average phenol coefficient value for each treatment is shown in Table 2. Based on the phenol coefficient test, it was found that the average phenol coefficient value of the combination extract treatment of *E. crassipes* and *P. stratoites* on *S. aureus* and *E. coli*, as well as on *C. albicans* was > 1.00. On *P.aeruginosa* and *S.typhi* = 1.00.

Table 1. Antimicrobial Strength of 5% Phenol and 0.0002% Chlorine Solutions in Several Concentrations Against Several Test Microbes Per Contact Length

Treatment Concentration	Average Treatment Strength on Phenol 5% and Chlorine 0,0002%														
	Time of Contact per Minute for 3 Repeatation														
	S.aurues			*E.coli*			*P.aeruginosa*			*S.typhi*			*C.albicans*		
	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	-	-	+1	-	-	-	-	-	-	-	-	-	-	-
1:90	+2	+1	+1	+3	+1	+1	+2	-	-	+1	-	-	+1	-	-
1:100	+3	+3	+3	+3	+3	+3	+2	+2	+2	+2	+1	+1	+2	+1	+1
1:110	+3	+3	+3	+3	+3	+3	+3	+2	+2	+3	+2	+2	+2	+2	+2
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 was no growth of the test microbes, which was observed on 3 different observations
+: there was growth of the test microbes, which was observed on 3 different observations

Tabel 2. Antimicrobial Strength on Combination Extract of *Echornia crassipes* and *Pistia stratoites* in Several Concentrations Against Several Microbes Tested Per Time of Contact

Treatment Concentration	Average of Treatment Power on Combination Extract														
	Time of Contact per Minute for 3 Repeatation														
	S.aurues			*E.coli*			*P.aeruginosa*			*S.typhi*			*C.albicans*		
	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+			+1	-	-	-	-	-	-	-	-	-	-	-
1:90	2+	1+	-	+3	+1	-	+2	-	-	+1	-	-	+1	-	-
1:100	3+	3+	1+	+2	+1	+1	+2	+2	+2	+2	+1	+1	+1	-	-
1:110	3+	3+	3+	+3	+3	+2	+3	+2	+2	+3	+2	+2	+2	+2	+1
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 was no growth of the test microbes, which was observed on 3 different observations
+: there was growth of the test microbes, which was observed on 3 different observations

Table 3. Average Value of Phenol Coefficient for Disinfection Preparations on Microbes in Vitro

Treatment	Average Value of Phenol Coefficient				
	S.aureus	*E.coli*	*P.aeruginosa*	*S.typhi*	*C.albicans*
Combination Extract of *E.crassipes* & *P.stratiotes*	1,13	1,13	1,00	1,00	1,10
Chlorine	1,00	1,00	1,00	1,00	1,00
Phenol 5%	1,00	1,00	1,00	1,00	1,00

Discussion

The combination of *Eichhornia crassipes* and *Pistia stratiotes* extracts has a phenol coefficient value of ≥1 with a phenol ratio of 5%; demonstrated to be effective as a disinfection preparation. Phenol substances are the standard for disinfecting power. The mechanism of action of phenol as a bacteristatic antiseptic works by interaction between phenolic compounds and bacterial cells through an absorption process involving hydrogen bonds. At low concentrations, phenol will form protein complexes with weak bonds and will soon undergo decomposition, followed by penetration of phenol into microbial cells and cause deposition and protein denaturation. At high concentrations, phenol will cause the coagulase protein of the bacterial cell and the cytoplasmic membrane to undergo lysis.[18]

The antimicrobial activity produced by an extract treatment is due to the role of the active compounds it contains. *Eichhornia crassipes* extract contains secondary compounds flavonoids, alkaloids, steroids, saponins, terpenoids and anthraquinones.[19] While the secondary compounds contained in *Pistia stratiotes* are alkaloids, flavonoids, steroids, phenols, saponins and tannins.[3]

Antimicrobial effectiveness is influenced by the nature of the active compounds in the extraction solvent, extract concentrations and microbial cell components. Ethanol solvent, capable of dissolving polar and semi-polar secondary compounds so that the secondary compounds in the combined extract will dissolve easily and can work optimally as antimicrobials.[20]

The mechanism of action of flavonoids as antimicrobials is by inhibiting cell membrane function, nucleic acid synthesis, and bacterial energy metabolism. When inhibiting cell membrane function, flavonoids form complex compounds with extracellular proteins that can damage bacterial cell membranes, followed by the release of intracellular compounds in the bacteria. Flavonoids inhibit the synthesis of nucleic acids which play a role in the process of interclassification or hydrogen bonding by accumulating bases in nucleic acids which inhibit the formation of DNA and RNA. This causes damage to the permeability of bacterial cell walls and lysosomes. Flavonoids can inhibit energy metabolism by inhibiting the use of oxygen by bacteria. Energy is needed by bacteria for the biosynthesis of macromolecules, so that if their metabolism is hampered, the bacterial molecules cannot develop into complex molecules.[21,22]

The mechanism of action of alkaloids as antimicrobials is by inhibiting and disrupting the constituent components of peptidoglycan in bacterial cells because the nitrogen in the alkaloid base group can bind amino acids, so that the cell wall layer is not formed intact and causes cell death.

The mechanism of saponin's action as an antimicrobial is by causing the leakage of proteins and enzymes from bacterial cells. Saponin is an active substance that can increase membrane permeability resulting in cell hemolysis. When saponins interact with bacterial cells, the bacteria will decompose or lyse which interferes with the survival of these bacteria.[22]

Differences in the antimicrobial effectiveness of extracts are influenced by the composition of the membrane and microbial cell components. Gram-positive microbes tend to be more sensitive to antimicrobial compounds such as flavonoids, alkaloids and saponins. This is because the structure of the cell wall of gram-positive microbes is relatively

simpler compared to the structure of the cell wall of gram-negative microbes. The composition of gram-positive microbial cell walls consists of more than 50% peptidoglycan and teichoic acid content which are polar and low lipid content (1-4%) which are non-polar. Meanwhile, flavonoids, alkaloids, and saponins are polar compounds.[23] Therefore, this antimicrobial compound more easily penetrates the cell membrane of gram-positive microbes. Whereas gram-negative microbes have a microbial cell wall that is more difficult for compounds to penetrate by flavonoids, alkaloids and saponins because it consists of a high lipid content (11-22%) which is non-polar and peptidoglycan content is only about 5-10% which is polar and the membrane outer cells that function as a selective defense of toxic compounds that enter and leave the cell. In addition, gram-negative microbes have an outer membrane consisting of phospholipids (inner layer) and non-polar lipopolysaccharide (outer layer). This is why it is more difficult for polar secondary compounds (flavonoids, alkaloids and saponins) to enter into gram-negative microbial cells so that their antimicrobial activity is less strong than gram-positive microbes.[24]

The outer membrane of gram-negative microbes consists of three layers, namely lipopolysaccharide (LPS), lipoprotein, and phospholipids. In phospholipids there are porins which are formed from proteins. Porins are channels through which some molecules can pass. This outer membrane serves as a barrier against antibiotics, digestive enzymes, and dry conditions, but cannot be a barrier to all substances. The main factors for cell wall damage are lipopolysaccharide (LPS) and porins. Antimicrobial compounds that work by penetrating LPS (lipopolysaccharide), hydrophilic molecules will more easily pass through LPS than hydrophobic molecules.[25]

Gram-negative microbes have hydrophilic properties, namely carboxyl, amino acids and hydroxyl. The antimicrobial mechanism of each secondary metabolite compound is different. Secondary metabolite compounds inhibit microbial growth starting with damaging the cell wall. Polar compounds can penetrate polar peptidoglycan, as well as some polar compounds such as phenolic compounds which can break peptidoglycan bonds in microbial walls. Antimicrobial compounds that are capable of reacting with porins (trans membrane proteins) on the microbial outer membrane and microbial cell walls will form strong polymeric bonds, resulting in damage to the porin. Damage to the porin which functions as a place for entry and exit of nutrients will cause the permeability of the microbial cell wall to decrease so that microbial growth is inhibited, or the microbial cell will die.[24]

The treatment of the extract as an antimicrobial in a combination preparation was able to produce an inhibitory effect on the microbes tested, which is known as a synergistic effect.[16] A synergistic effect that produces an effect as a disinfectant was reported in previous studies, namely the combination treatment of the combination of *Averhoae blimbi* and *Cananga odorata* infusions and the combination of *Cyperus rotundus* and *Mimosa pudica* infusions.[23,26]

In this study it was possible to prove the effectiveness as a candidate for a disinfectant preparation from a combination of *Eichhornia crassipes* and *Pistia stratiotes* extracts, based on the magnitude of the phenol coefficient value produced. Further research that can be carried out is the effectiveness of contact

duration through in vivo tests and organoleptic tests.

Conclusion

Based on the phenol coefficient value, the combination of *Eichhornia crassipes* and *Pistia stratiotes* extracts has antimicrobial activity. A combination of *Eichhornia crassipes* and *Pistia stratiotes* extracts can be developed as an alternative disinfection candidate.

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