Test of Antibacterial Activity 70% Ethanol Extract of Seeds Black Pepper (*Piper Nigrum* L) Against Bacteria *Escherichia Coli* Atcc 25922

Arina Zulfah Primananda*, Laksmi Anggun, and Rika Murhayati

Study of Program of Bachelor of Pharmacy Muhammadiyah University of Kudus

*arinzulfah@umkudus.ac.id*

**Abstract.** Seeds of black pepper (*Piper nigrum* L) had antimicrobial compounds was efficacious for treating diarrhea. This purpose of research to determine the activity of the of black pepper seed extract in varying concentrations. Seeds of black pepper extracted by maceration method. Black pepper seeds of 70% ethanol extract made with the series a concentration of 100 mg / ml; 50 mg / ml; 25 mg / ml; 12.5mg / ml; 6.25 mg / ml; 3.125 mg / ml; 1.565 mg / ml; 0.781 mg / ml; 0.3905 mg / ml; negative control, and positive control and cotrimoxazole as controls for comparison. Tests conducted on the bacteria *Escherichia coli* ATCC 25922 in dilution. Antibacterial test results showed black pepper seeds extract had an antibacterial activity against *Escherichia coli* ATCC 25922 to minimum bactericidal concentration (MBC) was 50 mg / ml. Antibacterial activity found on 50 mg/ml of minimum concentration  because black pepper seeds extract contained flavonoids, alkaloids, tannins, saponins, essential oils, and piperine. Piperine was a chemical content that highest.

1. **Introduction**

The use of traditional medicine has attracted attention and its popularity in our society is increasing. Plants are one of the traditional medicines that have been used for a long time [1]. One of them is maintaining the health of the body from infection. Diseases caused by bacterial infections that are very easily transmitted from one patient to another [2].

Infection problems can be overcome with the use of antibacterial drugs with the aim of inhibiting the growth of bacteria and kill bacteria [3]. Potential medicinal plants to be developed as antibacterial include black pepper seeds. Pepper plants have various antimicrobial compounds such as flavonoids, alkaloids, saponins, tannins, piperins, and essential oils [8]. Pepper plants have properties that are to overcome diarrhea [10]. Research previously demonstrated Minimum inhibitory concentration (MIC) of seed ethanolic extract of black pepper (*Piper nigru*) into bacteria *Escherichia coli* is 1 mg / ml [2].

The content of compounds and properties of pepper seeds can be used to inhibit and kill bacteria. The content of black pepper seeds contains saponins whose mechanism of action decreases surface tension and the content of flavonoids and alkaloids whose mechanism of action is by denaturing proteins and damaging bacterial cell membranes by dissolving lipid contained in cell walls, causing cell wall damage to occur in
the end. Causing cell death [9]. The advantage of black pepper seeds is the presence of alkaloid compounds, namely piperin, which is capable of lysis of bacterial cells while [7]. Research ethanol extract of the seeds of black pepper needs to be done, to determine its potential as an antibacterial.

2. Research Methods

2.1 Tool

The tools used in this research include erlenmeyer, thermometer, stove, test tube, petri dish, sterile ose, micropipette, autoclave, incubator, bunsen lamp, slide glass, object prop, boor prop, deck glass, microscope, coarse and fine gram balance, sticky cotton swab teryl, blender, oven, beaker glass, and flannel.

2.2 Material

The main ingredient used in this study was black pepper (Piper nigrum L.) seeds taken from Lampung. The comparison material used is cotrimoxazole antibiotics obtained from drugstore in the Kudus area, Central Java. Materials used to test the antibacterial activity is Escherichia coli ATCC 25922. Media Endo order (EA) and Brain Heart Infusion (BHI) and sterile distilled water. The solvent used for the manufacture of extracts is 70% ethanol.

3. Results And Discussion

The results of the test of the antibacterial activity of 70% ethanol extract of black pepper seeds against Escherichia coli ATCC 25922 can be seen in table 1.

| No. | Concentration (mg / ml) | Black Pepper Seed Extract |
|-----|-------------------------|----------------------------|
|     |                         | I  | II | III |
| 1   | Control (-)             | -  | -  | -   |
| 2   | 100                     | -  | -  | -   |
| 3   | 50                      | -  | -  | -   |
| 4   | 25                      | +  | +  | +   |
| 5   | 12.5                    | +  | +  | +   |
| 6   | 6.25                    | +  | +  | +   |
| 7   | 3.125                   | +  | +  | +   |
| 8   | 1.56                    | +  | +  | +   |
| 9   | 0.781                   | +  | +  | +   |
| 10  | 0.3905                  | +  | +  | +   |
| 11  | Control (+)             | +  | +  | +   |

Information:

(-) : no bacterial growth
(+): there is bacterial growth

A single extract of black pepper seeds was tested for antibacterial activity against Escherichia coli ATCC 25922. Each of them was made with a concentration series of 100 mg / ml; 50 mg / ml; 25 mg / ml; 12.5 mg / ml; 6.25 mg / ml; 3,125 mg / ml; 1,565 mg / ml; 0.781 mg / ml; 0.3905 mg / ml; negative control, and positive control. Tests carried out obtained results for a single extract of black pepper seeds at a concentration of 50 mg / ml can inhibit bacterial growth.

A single extract of black pepper seeds is effective because black pepper seeds contain essential oils and alkaloid compounds, namely piperine, which can cause cells to become lysis and morphological changes in
bacteria. Piperin is the most active compound of black pepper seeds so that 70% ethanol extract of black pepper seeds is effective.

The antibacterial activity of cotrimoxazole is based on its work which lasts two sequential stages in an enzymatic reaction to form tetrahydrofolic acid. Sulfamethoxazole inhibits the entry of PABA molecules into folic acid molecules and trimethoprim inhibits the occurrence of a reduction reaction and hydrofolate to tetrahydrofolate [3]. Trimethoprim inhibits the enzyme dehydrofolate reductase in microbes very selectively. The interaction between sulfamethoxazole and trimethoprim is estimated from their respective mechanisms [6]. The most effective ratio of these two drugs for most microorganisms is 20 parts sulfamethoxazole: 1 part trimethoprim. The combination is formulated to achieve sulfamethoxazole in vivo which is 20 times greater than trimethoprim [5].

Sulfamethoxazole can inhibit sequential stages in folate synthesis and can produce significant increases or synergistic activity of both drugs. This combination has a bactericidal effect is more effective than just be right sulfonamides alone [6]. Further research needs to be done to isolate pure chemical compounds from each of the simplicia which is expected to increase the antibacterial activity because the smaller the Minimum Kill Rate (CBC) of the simplicia, the better it is to be used as an antibacterial. Side effects and the risk of antibiotic resistance obtained will also be smaller.

4. Conclusion

Based on the results of the study it can be concluded that:

Extract of ethanol 70% grains of black pepper with concentrations ranging from 50 mg / ml have activity as an antibacterial against bacteria Escherichia coli ATCC 25922

References

[1] Evans, W.C. 2002. Trease and Evans Pharmacognosy. Ed 15. University of Nottingham. Nited Kingdom. 334.
[2] Ganesh P, Kumar Suresh R, Saranraj P. 2014. Phytochemical Analysis and Antibacterial Activity of Pepper (Piper Nigrum L.) Against Some Human Pathogens .Department of microbiology Annamalai University. India.
[3] Ganiswara, SE, 1995. Pharmacology and Therapy. IV Edition. Pharmacology Department. University of Indonesia. Jakarta. Pp. 571-573.
[4] Gillespie SH, Bamford KB. 2008. At a Glance Mikrobiologi Medis dan Infeksi Edisi ketiga. Astikawati R, Safitri A. editor. Jakarta: Erlangga
[5] Goodman & Gilman. 2010. Pharmacology and Therapeutic Manual. Jakarta: EGC Medical Book Publisher.
[6] Katzung BG 2011. Basic Pharmacology and Clinics. 10th edition. Translator; Pharmacology Section, Faculty of Medicine, Airlangga. University. Jakarta: Salemba Medika. Page 790
[7] Ministry of Health Republic of Indonesia 2000. Materia Medika Indonesia. Ji. Jakarta: Ministry of Health of the Republic of Indonesia. Pp. 34-39.
[8] RI Ministry of Health]. 1989. How to make Simplisia. Volume V. Ministry of Health of the Republic of Indonesia
[9] Parhusip, AJN 2006. Study of Ant- bacterial Mechanisms of Andaliman Extract (Zanthoxylum acanthopodium DC) on Food Pathogenic Bacteria. Bogor Agricultural Institute
[10] Risfahei, Nurdjannah N. 2012. Pepper Processing-The Indonesian Scenario In Black Pepper. In: Ravindran P.N, editor. Medicinal and Aromatic Plants-Industrial. Harwood Academic Publishers, 2000. p.355-366