Contents lists available atIOCS

Science Midwifery

journal homepage:www.midwifery.iocspublisher.org

Inhibitory Test of Dayak Onion Extract (Eleutherine palmifolia) (L) Merragainst Gram Negative and Gram Positive Bacteria

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ARTICLEINFO

Article history:

Received Aug 30, 2022
Revised Sep 18, 2022
Accepted Sep 28, 2022

Keywords:

Dayak Onion,
Inhibitory,
Gram Positive bacteria,
Gram Negative Bacteria

ABSTRACT

Dayak onion tubers contain chemical compounds such as alkaloids, glycosides, flavonoids, phenolics, steroids and tannins which are potential sources to be developed as medicinal plants. Dayak onions grown in different soil topography produce different secondary metabolite content even though they are from the same species. The purpose of this study was to determine the potential of Dayak onion (Eleutherine palmifolia) (L) Merr extract to have inhibitory potential and the amount of inhibition on the growth of Gram-positive and Gram-negative bacteria. This study is a laboratory experiment using a post test with a group design with 10 treatment groups, namely 1% Dayak onion extract, up to 10% with 5% DMSO solvent, control using 0.85% NaCl tested on Gram positive and Gram negative bacteria. Dayak onion extract (Eleutherine palmifolia) (L) Merr which was tested on the Gram negative bacteria group Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa gave weak inhibitory results, while the Dayak onion extract (Eleutherine palmifolia) (L) Merr which was tested on the bacterial group Gram positive Stapylococcus aureus, Staphylococcus epidemicdis, Staphylococcus saprophyticus give strong inhibitory results.

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INTRODUCTION

Infectious diseases in humans can be treated with the use of antibiotics. The need for the development of herbal-based antibiotics to overcome them. Great opportunity to obtain antibacterial compounds by utilizing the diversity of plants in Indonesia (Abdelmegeed et al, 2015).This development is supported by the tendency of humans to treat nature or return to nature (back to nature). Traditional medicine is considered more practical because it has been hereditary.One of the plants that can be used as traditional medicine is Dayak onion (Eleutherine palmifolia) (L) Merr. Dayak onion bulbs contain chemical compounds such as alkaloids, glycosides, flavonoids, phenolics, steroids and tannins which are potential sources to be developed as medicinal plants.Masfria and Tampubolon (2019).Alkaloids can function as antimicrobials, glycosides and flavonoids have hypoglycemic functions, while tannins are commonly used as stomach ache drugs.Wijayanti, 2018).

Dayak onion also grows in Lembata Regency, East Nusa Tenggara, known as "forest onion", used for the treatment of stomach pain, festering wounds, ulcers. Dayak onions grown in different soil topography produce different secondary metabolite content even though they come from the same species(Endang and Iswarin, 2016). Based on the description above, the author intends to conduct further studies on the potential of extractsDayak onion bulbs (Eleutherine palmifolia) (L) Merrhas the potential for inhibition and the amount of inhibition on growthGram positive and Gram negative bacteria

RESEARCH METHOD

Dayak onion samples were taken in Lusilame Village, Atadei District, Lembata Regency, East Nusa Tenggara. Microbiological testing was carried out at the Kupang City Laboratory Technical Implementation Unit. The time of the study was carried out in June-July 2022. 300 grams of Dayak onion (Eleutherine palmifolia) (L) Merr powder was extracted using the maceration method with 3 liters of 95% ethanol and evaporated, obtained a thick extract (paste) of Dayak onion powder as much as … ..grams. The propagation of the test bacteria was carried out on selective media and observed colony growth and Gram staining. This research is a laboratory experimental study using a post test with a group design with 10 treatment groups, namely 1% Dayak onion extract, up to 10% with 5% DMSO solvent, control using NaCl 0, 85% were tested on Gram positive and Gram negative bacteria. Furthermore, the extract was made in several dilutions using 5% DMSO solution, and absorbed into blank discs to be tested with test bacteria, namely Stapylococcus aureus ATCC 25923, Staphylococcus epidemics ATCC 14990, Staphylococcus saprophyticus ATCC 15305 and Gram negative Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC 27853, the media used is Muller Hiton Agar by doing 3 (three) repetitions.The inhibition formed was measured and averaged in mm.

RESULTS AND DISCUSSION

Dayak onion extract (Eleutherine palmifolia) (L) Merr was tested on bacteria in Muller Hilton media to see the inhibition formed around the discs and the zone of inhibition was calculated in mm units. Interpretation of inhibition according to the Clinical Laboratory Standards Institute (CLSI) (Novaryatiin, et al.,2019) ie if <15 mm it means resistant (weak), 16-18 mm means intermediate (strong) and 19 means susceptible (very strong).

The average inhibition of the extracts tested against groups of Gram-negative bacteria Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC 27853 in the formed media can be seen in the table below.

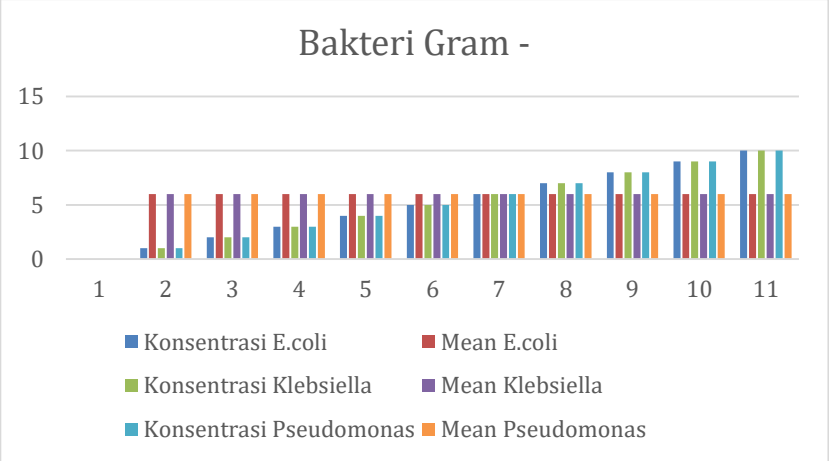
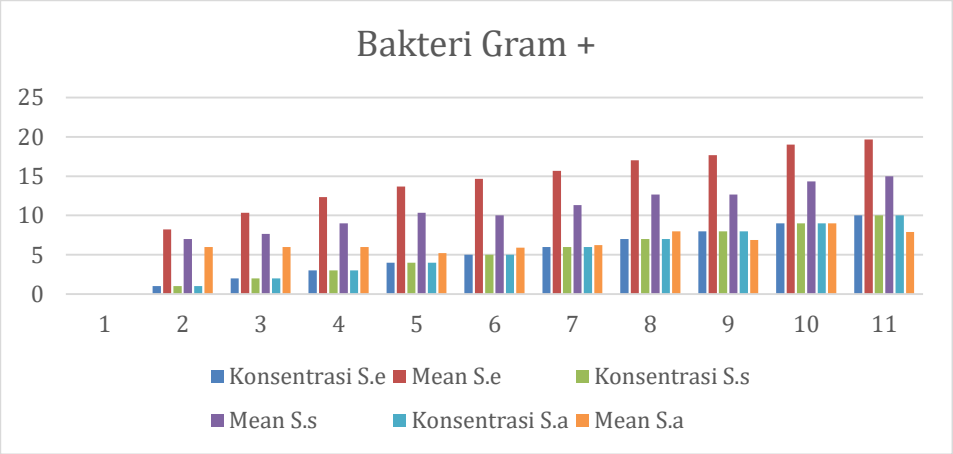


Table 1. Inhibitory activity of the tested extracts against groups of Gram-negative bacteria Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC 27853

Based on the test results of the diameter of the inhibition zone of the ethanolic powder extract of Dayak onion (Eleutherine palmifolia) (L) Merr), against Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa bacteria which were carried out three times with a concentration of 1-10%. Seen to have a weak inhibition zone at concentrations of 1%, 2% ,3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, all of which are 6 mm, so they are included in the weak category because the zone visible resistance below 15 mm.

These results when compared with the results of research fromJulianti (2020), the inhibition zone produced in this study was smaller than the inhibition zone produced by the ethanol extract of the Dayak onion on bacterial growth. Based on CLSI , the inhibition zone formed after being tested on all concentrations of Dayak onion extract (Eleutherine palmifolia) (L) Merr on Gram negative bacteria was categorized as resistant (weak). The control test was carried out on one of the test bacteria of the Gram negative group of bacteria, namely Klebsiella pneumoniae bacteria where the inhibitory power formed was 17 mm while the negative control with DMSO 5% was 6 mm. This indicates that the solvent used does not affect the inhibition formed.

The average formation of the area (zone) of inhibition of the growth of bacterial colonies of Stapylococcus aureus, Staphylococcus epidemics, Staphylococcus saprophyticus in the formed media can be seen in the table below:



Based on the test results of the diameter of the inhibitory zone of the ethanol extract of the Dayak onion (Eleutherine palmifolia) (L) Merr) powder against Stapylococcus aureus, Staphylococcus epidemics, Staphylococcus saprophyticus, three repetitions were carried out at a concentration of 1-10%. There was a difference in the inhibition formed by each bacterium but showed the same thing, which was seen to have an inhibition zone, the greater the concentration of the tested Dayak onion extract, the greater the inhibition formed.

Phytochemical compounds in herbal ingredients have more potential to inhibit Gram-positive bacteria than Gram-negative bacteria because Gram-positive bacteria have a thinner and more polar phospholipid layer so that the extract penetrates the cell wall more easily.Susilawati, 2016). This is what causes the Dayak onion to have a very weak activity in inhibiting the growth of the Gram negative group of bacteria even though it uses an ethanol type of solvent, where ethanol is a polar solvent so that the filtered bioactive compounds are also polar.

In this study, phytochemical screening was not carried out to identify the content of secondary metabolites in the tested Dayak onion powder extract, so it is not known what active compounds are contained in it. By Harlita et al (2018) on Dayak onion bulbs extracted with 96% ethanol solvent, positive results were found on flavonoid, triterpenoid and tannin tests while negative on saponin and steroidal alkaloids. In contrast to Mierza et al (2021) where flavonoids, triterpenoids, tannins, alkaloids, saponins and steroids the results are positive. The difference in the phytochemical test results caused by the Dayak onion extract can be caused by several factors including the topography of the soil in the plant, the unknown age of harvest (tuber age), freshness, and the sterility test of the Dayak onion bulbs which will be used as extract material is not carried out.

According to research conducted byArmanda et al (2017) qualitatively, every 1 mg or 1 mg/mL contains 100.71 ug of total flavonoids. Flavonoids function as antifungal and antibacterial. The mechanism of occurrence is denaturation of bacterial cell proteins, so that their characteristic properties are lost. Flavonoids also cause

changes in organic components and nutrient transport that result in toxic effects on bacteria. Flavonoids have antibacterial effects due to their ability to interact with bacterial DNA. Each flavonoid component has the ability to break the hydrogen bridge bonds of the double-stranded DNA sequence. Flavonoid compounds will contact the DNA in the cell nucleus and through the difference in polarity between the lipids that make up DNA and the alcohol groups on the flavonoid compounds a reaction will occur, thereby damaging the lipid structure of DNA and the bacterial cell nucleus will also lyse and cause death.

Flavonoids denature bacterial cell proteins and damage bacterial cell membranes (Afiff and Aminah 2017). Alkaloids interfere with the peptidoglycan constituent components of bacterial cells, so that the cell wall layer is not completely formed and causes cell death.Hasibuan,2016). Tannins lead to protein denaturation and lower surface tension, so that bacterial permeability increases (Azizah, 2016). Sponins can inhibit protein synthesis, causing damage to the components that make up bacterial cells.Hasibuan, 2016). Terpenoids penetrate the bacterial cell wall and then damage the porin so that the bacteria experience a lack of nutrients so that it inhibits their growth.Hasibuan, 2016).

The results of the inhibition zone formed in this study were different from previous studies. This can be caused by differences in solvents used because different solvents can affect the compounds that will be dissolved in the extraction process for their polarity (Susana, et al.,2018). Research conducted byJulianti (2020) using ethanol solvent which is polar so that it is able to attract almost all good substances or secondary metabolite compounds that are polar, semipolar and nonpolar (Aminah, et al., 2016). The use of solvents affects the susceptibility test of various pathogenic bacteria for extracts drawn by a certain organic solvent because this is related to one or more active substances that have biological activation as antimicrobials. While in the negative control test in this study the solvent used was DMSO 5% which is organic which is not bactericidal and has no effect on the function of secondary metabolites in the Dayak onion extract (Eleutherine palmifolia L Merr) so it does not affect the inhibition zone in the antibacterial activity test of the extract. Dayak onion ethanol (Warsiti,2018).

CONCLUSION

Dayak onion extract (Eleutherine palmifolia) (L) Merrwhich was tested on the group of Gram negative bacteriaEscherichia coli,Klebsiella pneumoniaeandPseudomonas aeruginosa gave weak inhibitory results, while the Dayak onion extract(Eleutherine palmifolia) (L) Merrwhich was tested on the group of Gram positive bacteriaStapylococcus aureus, Staphylococcus epidemicdis, Staphylococcus saprophyticusprovide strong inhibitory results.

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