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Antibacterial Activity of *Leucaena leucocephala* Extracts on Growth of *Escherichia coli*

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Enhancement of pathogenic bacteria such as gram-negative bacteria (*Escherichia coli*) and gram-positive bacteria (*Staphylococcus aureus*) can cause diseases such as diarrhea, nausea, abdominal pain and fever. Utilization of medicinal plants as an herbal remedy for preventing and treating the disease continues to evolve in development of the medical field to obtain material resources as new antibiotics. The use of *Leucaena leucocephala* extracts with various solvent to determine the antibacterial activity was a novelty research of *leucaena*. Analysis of antibacterial activity was done by two methods, inhibition zone and Total Plate Count. In this study used two factors. The first factor was the type of leucaena seeds (mature and immature seeds) and the second factor was type of solvent (water, ethanol and hexane). The results of leucaena extract identificated compound of saponin, tannin, alkaloid and terpenoid, total phenol of 523.87 mg/L. The highest antibacterial activity of *Escherichia coli* was founded in mature seed extract with water solvent at a concentration of 100% had inhibition zone of 19 mm (positive control of 29 mm) and Total Plate Count of bacterial growth of 2.11 × 107 (CFU/ml) in line a negative control 7.78 × 105 (CFU/ml) and positive control 0.94 × 107 (CFU/ml).

Keywords: *L. leucocephala*, Active Compounds, Antibacterial, *Escherichia coli*, Inhibition Zone.

1. INTRODUCTION

Leucaena leucocephala is a plant of the leguminous family, and it is widely used in Indonesia as a shade plant. A lot of research on the plant revealed a high protein content and a relatively high polar amino acids than red beans.[1,2] *Leucaena* has high (40–70%) antioxidant activity, and this has been shown in various products; as such sauces, tempeh and tauco.[3] As functional foods are important in prevention or spreading of diseases, and it is beneficial to search for plants with medicinal properties, as plants generally contain active compounds in the form of secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids, and tannins.[6] Active compound affected to the ability as antioxidants and antibacterials. Leucaena leucocephala seeds extract had high antioxidant activity.[3,9]

Resistance to antimicrobial agents has become an increasingly important and pressing global problem. The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind.[11]

Increased bacterial pathogens such as Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*) can cause diseases such as diarrhea, nausea, abdominal pain, fever due to food contamination by these bacteria. The use of medicinal plants as an herbal remedy for preventing and treating diseases continues to evolve in line with the development, in accordance with the development of technology and science, especially in the medical field to obtain material resources as new antibiotics.[6]

The screening 14 flavonoids of different structures for inhibitory activity against *Escherichia coli* DNA gyrase, and for antibacterial activity against *Staphylococcus epidermidis*, *S. aureus*, *E. coli*, *S. typhimurium* and *Stenotrophomonas maltophilia*. It was found that *E. coli* DNA gyrase was inhibited to different extents by seven of the compounds, including quercetin, apigenin and 3,6,7,3,4-pentahydroxyflavone. Interestingly, with the exception of 7,8-dihydroxyflavone, enzyme inhibition was limited to those compounds with B-ring hydroxylation.[4,5] However, since the level of antibacterial activity and enzyme inhibition did not always correlate, they also suggested that other mechanisms were involved.[4]

Leucaena leucocephala contain tannin, alkaloid, terpenoid, phenol and high antioxidan.[9] The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. Hence the present study was carried out to find out the antibacterial activity of *leucaena* against the selected pathogenic bacteria. The aims of the present investigation were to assess the antimicrobial activities of the phytochemistry compound and compare these to the effect of the antibiotics

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upon bacterial growth; to assess the components determined to be present in leucaena. The use of *Leucaena leucocephala* apart type extracts with various solvent to determine the antibacterial activity was a novelty research.

2. MEASUREMENT OF ANTIBACTERI ACTIVITY

2.1. Preparation of *Leucaena leucocephala* Extracts

Leucaena Leucocephala Powdered of seeds (mature, immature) and leaves weighed as much as 50 g of maceration using solvent water, ethanol and hexane (1:10 (w/v)) for 24 jam. The filtrate was evaporated using a Rotary Evaporator for 90 minutes at a temperature corresponding to the boiling point of solvent. Extracts obtained was placed on the bottle vial for testing identification bioactive compounds and the measurement of total phenols as well as antibacterial activity test.

2.2. Test Organisms/Bacterial Organisms

The test organisms used in this study was obtained from the Laboratory of Food Safety, University of Brawijaya, Malang, Indonesia. The organisms used in this study was: *Escherichia coli.*

2.3. Propagation and Maintenance of Test Organisms

The test organisms were streaked on the Nutrient Agarslants and was incubated overnight at 37 °C. The cultures were kept under refrigerated conditions and were subcultured after every fifteen (15) days.

2.4. Preparation of Concentrations of Lemongrass Oil

The different concentrations (v/v) of *leucaena* seeds extract 80%, 90% were prepared aseptically insterile tween-80.

2.5. Test of Antimicrobial Activity

A total of 20 mL of sterile Nutrient media was poured into petridish on 50 °C temperature and allowed to solidify. Based on the Kirby-Bauer method,[17] each inoculated with 1 ml diswab manner *E. coli* of bacterial culture media Nutrient Broth aged 24 h. Paper disc diameter of 8 mm had sterilized to drip with 1 ml seed extract of *leucaena* with a concentration of 100%, 90%, and 80% and the antibiotic *amoxicillin* 500 mg as a positive control.

Each Paper disc that will spilled seed extract elderly, extracts were placed above media Nutrient Agar. Petridish keep to one hour at room temperature then they Incubated for 24 hours at a temperature of 37 °C. Antimicrobial inhibitory activity shown by the clear zone around the seed extract Paper disc against extracts of *leucaena* were measured using calipers.

3. RESULTS AND DISCUSSION

3.1. Active Compounds of *Leucaena leucocepahala*

Based on early identification results, they founded that extract contained saponins on the mature seeds, immature seeds, leaves of *leucaena* with water and ethanol solvent was identified very strong. On the solvent hexane wasn't identified saponins.[9] Saponins are polar compounds tend to extraction with polar solvents like water. This is presumably because the magnitude of the dielectric constant of water amounted to 80.40 so that the aqueous solvent can extract saponins optimally.

The identification of tannin compounds, solvent extraction using water and ethanol on the seeds of mature, immature and leaves of *leucaena* can be identified tannin compounds, whereas with hexane unidentified tannin.[9] Tannin is a polyphenolic compounds that are in plants that are soluble in water.[7] The tannins soluble in alcohol and water, but not soluble in non-polar organic solvents.[8]

The test results of extract with water, ethanol and hexane solvent identified terpenoids compounds and alkaloids. The three types of leucaena parts: the mature, immature seeds and leaves of leucaena with all three types of solvent identified terpenoids and alkaloids compounds.[9] This showed that the terpenoids can be extracted by polar or non-polar solvents. In this study, hexane can identify compounds terpenoids and alkaloids stronger. Terpenoid compounds can be found in the water extract, ethanol, methanol, chloroform and ether.[8]

Total phenol of mature seeds and immature seeds of *leucaena* obtained by 100.55–523.87 (mg/L). Bioactive compounds of *leucaena* (mature seeds, immature seeds) had inhibitory activity against free radicals. The antioxidant activity of *leucaena* ranged between 36.49%–98.87%. In water solution with a long maceration of 24 hours produced antioxidant activity of mature leucaena seeds the highest (98.87%). Increasing the amount of antioxidant activity of leucaena extract, along with the longer maceration on three types of good solvent of water, ethanol and hexane.[9]

Phenolic components are generally soluble in organic solvents that are polar.[10] The results of this study on the mature seeds and immature seeds using a solvent of water and ethanol can extract better phenolic compounds.

The extraction process by maceration or immersion allegedly also affect the total phenols be produced. In extraction process, events solvent diffusion into the fuel cell. Solvent into the fuel cells will dissolve the compound when the solubility of the compound that is extracted together with solvent.[11] The process of extracting chemical components in plant cells with a predetermined solvent will penetrate the cell wall and into the cavity of the cell that contains the active substance (e.g., phenol). The active substance will dissolve in the solvent because of the similarity of polarity and this will cause the solution becomes concentrated.

3.2. Inhibition Zone and Total Plate Count of *Leucaena Extracts* Against Bacteria *Escherichia coli*

The extract *leucaena* seeds of ethanol, water and hexane solvent stable in inhibition. On smaller concentration indicated to give little inhibitory effect (Table I). Water solvent chosen to extract polar compounds, ethanol and hexane for semipolar compounds for nonpolar compounds contained in *leucaena*. The three types of solvents had the ability to identify compounds polar, semipolar and non-polar in the seeds and leaves of *leucaena* so that the compound had the function work separately in inhibiting bacterial growth.

Antibacterial activity that was possessed the seeds and leaves of leucaena were phenolic compounds include alkaloids, tannins, terpenoids and saponins. Antibacterial is defined as material that can interfere with the growth and metabolism of bacteria. It works antibacterial among others by damaging the cell walls, changing the permeability of cells, inhibits the action of enzymes, changing the protein molecules and nucleic acids, as well as inhibit the synthesis of nucleic acids and proteins. Working power

Table I. Inhibitation zone and total plate count of *Escherichia coli* growth of *leucaena* extract.

Sample	Treatment: Solvent	Treatment: Concentration (%)	Inhibition zone (mm) *Escherichia coli*	Total plate count (CFU/mL) *Escherichia coli*
Mature seeds	Water	100	19	2.11×10^7
		90	18	2.13×10^7
		80	17	2.18×10^7
	Ethanol	100	15	2.22×10^7
		90	14	2.25×10^7
		80	13	2.29×10^7
	Hexane	100	16	2.12×10^7
		90	15	2.14×10^7
		80	14	2.19×10^7
Immature seeds	Water	100	13	2.70×10^7
		90	11	2.76×10^7
		80	10	2.81×10^7
	Ethanol	100	12	2.77×10^7
		90	11	2.87×10^7
		80	10	2.97×10^7
	Hexane	100	11	2.70×10^7
		90	10	2.85×10^7
		80	9	2.90×10^7
Leaves	Water	100	17	2.15×10^7
		90	16	2.22×10^7
		80	14	2.25×10^7
	Ethanol	100	12	2.27×10^7
		90	11	2.30×10^7
		80	10	2.31×10^7
	Hexane	100	11	2.11×10^7
		90	10	2.15×10^7
		80	9	2.20×10^7
Positive control	*Amoxicillin*	100	29	0.94×10^7
		90	27	0.96×10^7
		80	25	0.97×10^7

antibacterial mechanism against microorganisms vary for example by making dehydration bacteria, oxidize the bacterial cells, bacteria or coagulation fluid surrounding the poisoning bacteria. The bioactive compounds are phenols, alkaloids, saponins, tannins, terpenoids.[8]

The presence of the active compounds in *leucaena* such as terpenoids, tannins, saponins and alkaloids can be antibacterial compounds. Terpenoids as antibacterial mechanism is reacted with Porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming a strong bond polymers that cause damage Porin. Porin destruction that are the entry and exit of the compounds will reduce the permeability of bacterial cell walls that would result in a bacterial cell would be a lack of nutrients, so that bacterial growth is inhibited or die.[12] In this study, the higher the concentration used, the greater the inhibition zone was produced. The higher the concentration of an antibacterial agent, the higher the antibacterial power.[13]

The testing of antimicrobial compound of *leucaena* seeds and leaves extract against bacterial growth inhibition was based on bacterial ability to grow at some concentration of *leucaena* extract. It can be seen Table I that the higher concentration of *leucaena* extract, the less bacteria can grow and the larger the inhibition zone produced the smaller the growing bacteria. This can be caused by bioactive compounds contained in *leucaena* extracts that can inhibit bacterial growth. The bioactive compounds are phenol, alkaloids, saponins, tannins, terpenoids.

The results of inhibition zone of *leucaena* extract against *Escherichia coli* resulted smaller compared with the positive control was *Amoxicillin*. *Amoxicillin* is a penicillin class antibiotic which mechanism of action with the damage so that the bacterial cell wall synthesis inhibition zones produced greater than *leucaena* extract (Table I). Condensed tannins has antibacterial activity because it can bind to the bacterial cell wall, inhibit the growth and activity of proteases.[12] Examples of condensed tannins, namely: B-3 (katekin-(4α->8)-katekin) and B-4 (katekin-(4α->8)-katekin).[14]

The natural products, it is generally accepted that phytochemicals are less potent anti-infectives than agents of microbial origin, i.e., antibiotics.[15] However, new classes of antimicrobial drug are urgently required and the flavonoids represent a novel set of leads. Future optimisation of these compounds through structural alteration may allow the development of a pharmacologically acceptable antimicrobial agent or group of agents. Existing structure–activity data suggest that it might be possible, for example, to prepare a potent antibacterial flavanone by synthesising a compound with halogenation of the B ring as well as lavandulyl or ger-anyl substitution of the A ring.[18]

Terpenoids mechanism as antibacterial is reacted with Porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming a strong bond polymers that cause damage Porin. Porin destruction that are the entry and exit of the compounds will reduce the permeability of bacterial cell walls that would result in a bacterial cell would be a lack of nutrients, so that bacterial growth is inhibited or die. Terpenoids compounds having polar and non-polar parts, but parts of the non-polar in terpenoids far more than in the polar so terpenoids tend to be more soluble in nonpolar solvents.[7] The mechanism of triterpenoids as antibacterial is reacted with Porin (transmembrane protein) on the outer membrane of the bacterial cell wall, forming a strong bond polymers that cause damage Porin.[12]

Apart from the phytochemicals found in *A. Digitata* extract, previous studies showed the presence of an alkaloid namely adansonin. The antibacterial activity of the aqueous and ethanol extract of this plant has already been reported against *E. coli*.[7] Therefore, the inhibitory activity found herein against reference and multi-resistant strains of *E. coli* as well as other Gram-negative species is complementary.[16]

4. CONCLUSION

Leucaena extract of mature seeds, immature seeds and leaves contained active compounds as antibacterial. *Leucaena* extract had antimicrobial activity as bacterial inhibition zone *Escherichia coli*. The mature seeds of water solvent on concentration of 100% resulted in highest inhibition zone against bacteria *Escherichia coli* was 19 (mm), while the the immature seed with hexane concentration of 80% had a lowest zone of inhibition against the bacteria *Escherichia coli* was 9 (mm).

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