Antibacterial activities of *Etlingera flexuosa* AD Poulsen (Zingiberaceae) from Central Sulawesi on *Staphylococcus aureus* and *Escherichia coli*

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Abstract. *E. flexuosa* which is endemic from Sulawesi is widely used by the local people for a lot of cultural activities. *E. flexuosa* fruit become the essential source to enhance the taste of fish dishes. The rhizome becomes a remedy to treat diarrhoea. This research aims to identify the antibacterial properties of *E. flexuosa* rhizome extract to two pathogen bacteria, *Staphylococcus aureus* and *Escherichia coli*. *E. flexuosa* sample were collected from Lore Lindu National Park, Indonesia. To detect the antibacterial activity, agar well diffusion was performed. The result showed that the extract of *E. flexuosa* rhizome has antibacterial properties to inhibit the grow of *Staphylococcus aureus* and *Escherichia coli* because of the secondary metabolites, such as flavonoid, tannin, saponin, terpenoid and alkaloid.

Keywords: Antibacterial property, *Etlingera flexuosa*, Central Sulawesi

1. Introduction

*Etlingera* Giseke is a genus in *Zingiberaceae* family comprises 150-200 species in the world [1], 74 species are recorded in Malesia region [2], 50 species are found in Sulawesi[3]. *Etlingera flexuosa*, one of endemic *Etlingera* of Sulawesi, is a terestrial herb which can reach 5 m in height. It is one of flowering plant species that was first time described from the province of Central Sulawesi, Indonesia [4]. It was very easy to recognize in the habitat due to leaves sheath colour from yellowish to purple. The flowering shoot is arising from rhizome with flowers pale pink in colour. The main characteristic of the species is the labellum bends outwards with age. It was known locally as *Karondo* (Topo Baria language) an indigenous ethnic in Central Sulawesi who live in Sedoa village, Lore Utara district, Poso Regency, Indonesia [5].

The species of *E. flexuosa* is used extensively by local community (Topo Baria ethnic) for a wide variety of cultures uses. The fruit is an important source for cooking fish dishes, It is used to enhance flavour of food. The young shoot are edible as vegetable while the leaves are used as roffing material.
E. flexuosa is playing important role in traditional Topo Baria medicine. The rhizome of this plants are used as medicine for diarrhoea [5,6].

So far, little is known about various important aspects of E. flexuosa, including the studies on exploration of phytochemical compounds (e.g. essential oils, antioxidants, appropriate extraction techniques), as a source of various new drugs against different kind of diseases (e.g. caused by pathogenic microorganisms, as nutritional, diabetes, hypertension, etc.), and also its cultivation and propagation effort (e.g. in-situ conservation, propagation by using tissue culture techniques.

Nowaday, the trend of using natural sources has raised and the bioactive chemical compounds are frequently studied for new drug invent as an antimicrobial agent. In our previous research, it was shown that E. flexuosa contained secondary metabolite compounds such as; flavonoid, tannin, alkaid, sapoain, terpenoid and steroid, and also shows antioxidant activity [6], even its hydroalcoholic rhizome extract had the ability to inhibit the growth of a pathogenic unicellular fungi [7]. There have been no widespread records of the antibacterial properties of the extract of E.flexuosa in pathogens.

This research aims to explore E. flexuosa’s antibacterial action using its rhizome extract against Staphylococcus aureus and Escherichia coli.

2. Materials and Methods

2.1. Plant Materials

Etlingera flexuosa sample was collected in January 2020 in montane forest of Lore Lindu National Park (LLNP), near Lake Kalimpa’a, Sedoa village, Lore Utara district, Poso regency Indonesia, about 80 km southeastern part of Palu, the Capital of Central Sulawesi, Indonesia (Figure 1). LLNP is one of protected area in central Sulawesi which has play important roles in various functions such as; habitat for Sulawesi’s flora and fauna [8,9], education and ecotourism destination [10] and as watershed protection [11].

2.2. Plant Extraction

Plant extraction was conducted at the Laboratory of Plant Biosystematic, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University. The extraction of the sample was carried out using known standar procedures [12,13]. E. flexuosa rhizome was washed in running tap water three time, cut to 3 cm length, washed more, and then kept in flowing tap water for five minutes then air dried. Cleaned dry plant sample was homogenized to a fine powder by a mixer grinder. These powder materials was then used for extraction of dyes. Dried powder of rhizome of the species was extracted with 95% ethanol with maceration and reflux technique. The extracts was filtered, evaporated in a vacuum evaporator to give yield of dry extract. Rhizome extracted by maceration technique was tested to S. aureus, while rhizome extracted by reflux technique was tested to E. coli.

2.3. Culture and growth media

Pure cultures of two experimental bacteria were obtained from the Laboratory of Microbiology, Department of Biology Faculty of Mathematics and Natural Sciences, Tadulako University, Palu. The pure culture of Staphylococcus aureus and Escherichia coli was maintained on Nutrient Agar medium (NA). Each bacterial species was further regularly maintained by sub-culturing on the same medium and stored at 4°C before use in experiments. S. aureus and E. coli were chosen based on their clinical and pharmacological importance [14].

2.4. Antimicrobial activities

This proposed work utilized gram positive and negative pathogens. S. aureus was used for gram positive bacteria, while for the gram-negative, E. Coli was picked. Antibacterial activities were examined for hydroalcohol extracts of rhizome of E. flexuosa by using agar well diffusion technique [7,15]. Both indicator bacteria were inoculated on NA medium by pour plate technique. The
experiment was designed by Completey Randomized Designed (CRD). The treatment was the four concentrations of rhizome extract (20, 40, 60 and 80%). Each treatment was replicated 3 times. The concentration of bacteria contained 1-2 x 10^8 CFU/ml each well. The diameter of well was 8 mm. Standard drugs Tetracycline 1% and Cloramphenicol 1%) diluted in sterile aquadest utilized as the positive controls for S. aureus and E.coli, respectively. While aquadest and Na-CMC 1% were picked to be the negative controls for S.aureus and E coli respectively. The inhibition zone in the well was measured after 18 to 24 hours of incubation at 37°C. The sensitivities of the microorganism species to the plant extracts were checked by looking at the diameter of inhibitory zones in the surface of the media. The diameter of inhibitory zone was calculated following the equation below:

\[(VD – WD) + (HD – WD) \]

\[ \frac{2}{2} \]

Explanation:
VD: Vertical Diameter
WD: Well Diameter
HD: Horizontal Diameter

Collected data were analyzed by One Way Anova using SPSS Software Statistics Version 26. DNMRT (Duncan New Multiple Range Test) was conducted if the treatment had significant effect.

3. Result and Discussion

*Etlingera flexuosa,* is one of 36 species of Etlingera was described as a new species from Sulawesi [6]. The natural distribution of the species is restricted in Central Sulawesi and South Sulawesi. It was firstsly collected from Gunung Nokilalaki, Lore Lindu National Park, Central Sulawesi. Additional collection was also made [5] in the humid tropical mountain forest of Lore Lindu National Park in Sedoa village, Lore Utara, Poso regency, Central Sulawesi Indonesia. It was known locally as *Karondo* and it has been used for many things by the indigenous ethnic in Central Sulawesi, Indonesia, Topo Baria.

It was proved that this extract can be used as antibiotic to *Stapylococcus aureus* (gram-positive bacteria) or *Escherichia coli* (gram-negative bacteria). With the same concentration of extract, the growth of *Escherichia coli* were more inhibited than the growth of *Staphylococcus aureus*. This result might be caused by different used extraction method and/or cell wall of bacteria. For *S. aureus*, rhizome was extracted by maceration while for *E. coli* rhizome was extracted by reflux technique. The cell walls of gram positive bacteria made of thick layers of peptidoglycan. While gram negative only made from a thin layer. The cell wall is also composed from lipopolysaccharide (LPS) molecules in the outer membrane area [16].

Plants of *Etlingera* have various traditional and commercial uses. It is important natural resources that provide many useful products for food, spices, medicines, dyes, perfume and aesthetics to human. In Sabah Malaysia, flower buds, fruit and young shoot of three species *Etlingera* are consumed by local people as condiment, eaten raw or cooked [2]. In North Sumatra Indonesia, the flowers and fruit of *E. elatior* are used as a mixture of traditional cuisine of Batak Karo sub-ethnic [17]. *E.elatior* is an aromatic plant that is widely cultivated as traditional spices for food flavouring and as ornamental plant. Farm in Australia and Costa Rica are cultivating the species as flower commodity [18]. In Southeast Sulawesi Indonesia, it was used as an herbal medicine to treat typhoid fever by Porehu community [19].
There are fifty (50) species of *Etlingera* in Sulawesi, one of them is *E. flexuosa* [5]. It has been used traditionally by local people in Lore Lindu National Park for various purposes such as for traditional medicines, food, material building and cooking spices [20].

3.1. Antibacterial activities

Antibacterial activity of rhizome extracts of *E. flexuosa* on both *Staphylococcus aureus* and *Escherichia coli* bacteria were assayed by using agar well diffusion technique with six (6) treatments namely extract concentrations of 20, 40, 65 and 80%, as well as positive control, and negative control. *E. flexuosa* showed antibacterial activity against experimented pathogens and different concentration of extract had different diameter of inhibition zone, which have been shown in Figure 1.
Figure 1. 1-A. Rhizome of *Etlingera flexuosa*, 1-B. Inflorescentia (flower) with labellum in white circle, 1-C. Infroductence. 2. Inhibition zone of rhizome extract of *E. flexuosa* against *S. aureus*. 2-A The growth media of bacteria was added by aquadest (negative control), 2-B. Extract concentration 20%, 2-C. Concentration 40%, 2-D. Concentration 60%, 2-E. Concentration 80%, 2 F. Positive control (Tetracycline). 3. Inhibition zone of rhizome extract of *E. flexuosa* against *E.coli*. 3-D Extract with concentration 60%, 3-E. Concentration 80%, 3F. Positive control (Chloramphenicol). White arrow is inhibition zone (clear zone).

Based on the experiment, showed that the sample of *E. flexuosa* rhizome extract has a promising inhibitory power to inhibit *S. aureus* and *E. coli* bacteria, it can be seen in Figure 2 below. As a positive control, tetracycline formed 28.5 mm of inhibition zone to *Staphylococcus aureus* while Chloramphenicol formed 40.1 mm of inhibition zone to *Escherichia coli*. The highest inhibition zone of *S. aureus* and *E. coli* formed by rhizome extract was concentration of 80%. From this concentration, inhibition zone of *S. aureus* was 19.67 mm and of *E. coli* was 24 mm. These inhibition values from this concentration were equal with 69% of Tetracycline to inhibit growth of *S. aureus* and with 61.8% of Chloramphenicol to inhibit growth of *E. coli*. Tetracycline and Chloramphenicol are antibiotic to *S. aureus* and *E. coli* respectively. However, there was no significantly different among extract concentration of 40, 60 and 80% on inhibition of *S. aureus* growth.

Figure 2. Inhibition zone of rhizome extract of *E. flexuosa* against *S. aureus* and *E. coli* bacteria. The growth media of bacteria was added by either aquadest or Na-CMC 1% as a positive control (C+), Tetracyline or Chloramphenicol as a negative control (C-) for *S. aureus* and *E. coli* respectively and rhizome extract of *E. flexuosa* with many concentrations. The rhizome extract was obtained by maceration method for *S. aureus* and by reflux method for *E. coli*. Values are means and SD of three replicates of each treatment. Bars for each treatment with the same letter indicated not significant different (P<0.05).

In our previous study revealed that the *E. flexuosa* samples have the potential to use as an inhibitor agent for the growth of unicellular *Candida albicans* yeast. The highly potential anti-microbials activity possessed by *E. flexuosa* against fungal infections is due to its rhizome containing secondary metabolites compound such as flavonoid, tannin, saponin, terpenoid and alkaloid [6]. Secondary metabolite compounds are chemical compounds produced by plants which are not involved in the
normal growth but they are often play an important role in plant protection against herbivory and microorganism [19,20].

Based on the inhibitory test indicated that the *E. flexuosa* rhizoma extract was able to inhibit the growth of both *S. aureus* and *E. coli* bacteria. This can be seen from the presence of inhibition zones formed due to antibacterial activity. The *E. flexuosa* samples have the potential to inhibit the growth of bacteria. The highly potential antimicrobial activity possessed by *E. flexuosa* against bacterial infections is due to its rhizome containing flavonoid, tannin, saponin, terpenoid and alkaloid [6].

Secondary metabolite compound from various plants posses several bioactivity such as antibacterial or antioxidant [20]. *E. flexuosa* samples previously reported have the beneficial effect as an inhibitor agent for the growth of unicelluler yeast *Candida albicans* [5].

Flavonoids are a major natural compound which can be found in the nature. Flavonoid have significant effects to promote health, prevent disease and dietary supplements. This compound also exhibited several biological activities including antibacterial, antiviral and anti-inflammatory [21]. Saponins show the antibacterial activity by inhibiting the growth of gram-positive or gram-negative bacteria[22], whereas tannin can inhibit bacterial growth by destroying peptidoglycan from bacterial cell walls [23].

In the perspective of the microorganisms assay, *Staphylococcus aureus* and *Escherichia coli* were identified as gram positive and gram negative bacteria, respectively and commonly used for microbial assay. The scientific consideration of the selection of these two types of bacteria is due to clinical reasons. *Staphylococcus aureus* is found in individual cocci, which divide in more than one plane to form grape-like clusters. It is a major pathogen of increasing importance due to the rise in antibiotic resistance [24]. The colonies of this species commonly found in a golden colour in solid media [25]. *S. aureus* found naturally on the human skin and nasopharynx. It can cause wide range of infections diseases such as skin infection, nose, urethra, vagina and gastro intestinal tract, pneumonia and food poisoning [26, 27].

*E. coli* is a gram-negative bacteria belongs to family Enterobacteriaceae, which identified as facultative anaerobes and nonsporulating bacteria. These bacteria contain K1 capsular polysaccharide antigen that produced adverse effect including septicemia and meningitis. Diarrhoeogenic *E. coli* are spread throughout around the world with type of infection is fecal-oral by contaminated water and food [28].

4. Conclusions

*E. flexuosa* rhizoma extract was able to inhibit the growth of both *S. aureus* and *E. coli* bacteria because the ethanol extract of *E. flexuosa* rhizome contains some chemical components such as flavonoid, tannin, saponin, terpenoid and alkaloid.

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