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Phytochemical Screening and Antibacterial Activities of Senggani (*Melastoma malabathricum* L.) Ethanolic Extract Leaves

(Skrining Fitokimia dan Aktivitas Antibakteri dari Ekstrak Daun Senggani (*Melastoma malabathricum* L.))

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ABSTRACT

The aim of the current research was to perform phytochemical screening and to know the pathogenic antibacterial activities of senggani leaves extract. Phytochemical screening was done by testing eight active compounds. Antibacterial activities testing was done by using well diffusion method at concentration level of 25%, 50%, 75%, 100%. Positive control was amoxicillin, while the negative control was aquadest. Meanwhile, pathogenic bacteria were *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Research design applied was complete random design, in which the data collected were analyzed using one-way ANOVA, continued by post-hoc test through Tukey method to know which concentration provide the most significant difference. Research results showed that ethanolic extract of senggani leaves has eight active compounds, those are phenolics, alkaloids, flavonoids, tannins, triterpenoids, glycosides, steroids, and saponins. This further proved that the ethanolic extract of senggani leaves have antibacterial activity and are able to inhibit the growth of all bacteria tested. The best ability shown to inhibit *E. coli* bacteria was at concentration of 100%, *Sh. dysenteriae* and *P. aeruginosa* started at the concentration of 75%, and *S. aureus* started at the concentration of 50%.

Keywords: active compounds, bacteria growth, inhibition, well diffusion

ABSTRAK

Penelitian ini bertujuan untuk skrining fitokimia dan mengetahui aktivitas antibakteri patogen dari ekstrak daun senggani. Skrining fitokimia dilakukan dengan menguji delapan senyawa aktif. Uji aktivitas antibakteri menggunakan metode difusi sumuran dengan taraf konsentrasi 25%, 50%, 75%, 100%. Kontrol positif yang digunakan adalah *amoxicillin,* dan akuades sebagai kontrol negatif. Bakteri patogen yang digunakan adalah *Escherichia coli, Salmonella dysenteriae, Staphylococcus aureus,* dan *Pseudomonas aeruginosa.* Rancangan yang digunakan dalam penelitian ini adalah rancangan acak lengkap. Analisis data menggunakan one-way ANOVA, dilanjutkan dengan uji post-hoc metode Tukey untuk mengetahui konsentrasi yang memberikan perbedaan paling bermakna. Hasil penelitian menunjukkan bahwa ekstrak etanol daun senggani memiliki delapan zat aktif yaitu senyawa fenolik, alkaloid, flavonoid, tanin, triterpenoid, glikosida, steroid, dan saponin. Penelitian ini menunjukan bahwa ekstrak etanol daun senggani mampu menghambat pertumbuhan semua bakteri uji. Kemampuan terbaik ekstrak etanol daun senggani menghambat bakteri E. *coli* pada konsentrasi 100%, *Sh. dysenteriae* dan *P. aeruginosa* mulai konsentrasi 75%, *S. aureus* mulai konsentrasi 50%.

Kata kunci: daya hambat, difusi sumuran, inhibisi, senyawa aktif

INTRODUCTION

Indonesian government recognized the utilization of various plants as herbs for public health. Therefore, the Ministry of Health of the Republic of Indonesia has issued several regulations concerning the use or utilization of plants as traditional medicines. This is integrated with the efforts made by the Indonesian government to utilize its biodiversity resources. However, many plants which were used as traditional medicine are still based on hereditary habits which were not provided by scientific data yet.

One of the plants widely used as a traditional medicine to treat various diseases is Senggani or Senduduk or Harendong or *Melastoma malabathricum* L. (Melastomataceae). Senggani as etnomedicinal that is popular in Bangladesh (Rahmatullah *et al.* 2009); India (Ringmichon *et al.* 2010) and Malaysia (Joffry *et al.* 2012; Samad *et al.* 2018); Thailand (Tangjitman *et al.* 2015) as well as Brunei Darussalam (Taha *et al*. 2021). In Indonesia, senggani is also used for etnomedicinal by Sundanese people (Roosita *et al*. 2008); Dayak people (Apridamayanti & Kurniawan 2018); Batak people (Silalahi *et al.* 2015); Talang

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Mamak community in Bukit Tigapuluh Jambi (Setyowati, 2006) and Batin ethnic society in Tabir District Merangin Regency Jambi (Jalius & Muswita 2013).

Several parts of a plant, such as the roots, stems, leaves, flowers, and the fruit of Senggani, are reported to have pharmacological activities, such as antivirus, antibacterial, anti-parasites, cytotoxicity, antioxidants, anticoagulants, Platelet Activating Factor (PAF) inhibition, antiulcer, anti-inflammatory, wound healing, antipyretic activities, antinociceptive, antivenom, and antidiarrheal, at different dosages/concentrations (Joffry *et al.* 2012; Silalahi 2020). Moreover, it also potentially has the source of antioxidant reported by (Susanti *et al.* 2007; Sari *et al*. 2018).

These functions of sengani leaves are basically for the prevention and treatment of diseases caused by infections of bacterials. To complete the data about *M. malabathricum* as a medicinal plant from Indonesia that potentially becomes medicine to cure a disease caused by the infection of pathogenic bacteria, this study was conducted to screen phytochemical active compounds and antibacterial activities of the ethanolic extract of senggani leaves against four pathogenic bacteria, those are *Escherichia coli, Shigella dysenteriae, Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

MATERIAL AND METHODS

Sample, Extraction and Phytochemical Screening

The senggani leaves used for the study were collected from Candika Village, Rimbo Tengah District, Muara Bungo Regency, Jambi Province. The senggani leaves extract ethanol 70% was made by using maceration method (Sembiring *et al.* 2018). Rotary evaporator was using for concentrated the filtrate. Qualitative screening was done on the phytochemical content of senggani leaves ethanolic extract consisted of eight phytochemical tests, those are phenolics, flavonoids, alkaloids, tannins, triterpenoids, glycosides, saponins and steroids. Phytochemical screening was done based on Harbone (1987). The process of making ethanolic extract and screening for phytochemical content of senggani leaves was carried out at the Research Center for Spices and Medicinal Plants, Bogor, West Java.

Antibacterial Activities

The bacterial in the current research were *E. coli* (ATCC 8739), *Sh. dysenteriae* (UnasCC 310), *S. aureus* (ATCC 6539), and *P. aeruginosa* (ATCC 9027) which were cultured on 24-hour Muller Hinton agar at room temperature. The bacterial suspension was prepared using a physiological solution of NaCl which was adjusted to the McFarland Standard 0,5. The method used was diffusion well with a well width of 6 mm on Muller Hinton agar in petri dish.

Amoxicillin 10 µg was used for the positive control, while sterile distilled water was used for the negative control. Senggani leaves ethanolic extract used were at the concentration levels of 25%, 50%, 75%, 100%. Approximately 50 µL of senggani leaves ethanolic extract was put into a well in petri dish containing Muller Hinton agar medium on which the bacterial had been spreaded using the spread plate method (Dhuha 2016). Furthermore, the placement of bacterial suspension and control was done using random technique. The plates with the bacteria were incubated for 24 hours at 37°C. The antibacterial activities was measured by the zone of inhibition in the form of a clear area formed around the well. The clear area formed around the well was observed and measured using a caliper. The antibacterial activites of senggani leaves ethanolic extract against the bacterial were carried out at the Microbiology and Genetics Laboratory of Universitas Nasional.

Research Design

The current research applied a completely randomized design (CRD-F) using two factors of (T1) four types of the bacterial and (T2) the senggani leaves ethanolic extract concentration which consisted of four concentration levels, those are 25%, 50%, 75%, 100%, one positive antibiotic control (amoxicillin) and one negative control (aquadest). Each treatment was repeated three times.

Data Analysis

The data obtained were analyzed using IBM SPSS Statistics 23.0. Concentration variation was considered as the independent variable, while the zone of inhibition of each bacteria was considered as the dependent variable. The resulting data were analyzed using one-way ANOVA and then continued by using a post-hoc test of Tukey method aiming to determine senggani leaves ethanolic extract concentration which gave the most significant difference.

RESULTS AND DISCUSSIONS

Phytochemical

The qualitative phytochemical screening results of senggani leaves ethanolic extract are shown in the following Table 1. In this research, senggani leaves

Table 1 Phytochemical screening results of senggani leaves ethanolic extract

No	Active compound	Results
1	Phenolics	+
2	Alkaloids	+
3	Flavonoids	+
4	Tannins	+
5	Triterpenoids	+
6	Glycosides	+
7	Steroids	+
8	Saponins	+

ethanolic extract contained eight active substances in the form of phenolics, flavonoids, alkaloids, tannins, triterpenoids, glycosides, saponins and steroids. The phytochemical screening result in this study is more complete than those by Noviyanty & Linda (2020) in the 96% ethanol extract of senggani fruits. From the six active compounds that were tested, senggani fruits have flavonoids and tannins. The phytochemical screening result of the water extract of *M. malabathricum* leaves was known to have flavonoids, triterpenes, saponins, steroids and tannins (Zakaria *et al.* 2006). The result of a study by Danlandi *et al.* (2015) for the methanolic extract of leaves, flowers, fruits, and stems showed that all parts contained tannins, steroids, phenols, and flavonoids. The result of a study by Sembiring *et al*. (2018) showed that 70% ethanol extract from the stems, leaves, flowers, and fruits had flavonoids, terpenoids, alkaloids, tannins and only leaves that contained saponins.

The active or secondary metabolite compounds in the plant were alkaloids, sterols, triterpenes, glycolsides, saponins, steroids, phenolics, tannins, and flavonoids had an antibacterial activity that can inhibit or kill pathogenic bacteria using the chemical, physical, and biological activities (Ncube *et al.* 2008). The antibacterial mechanism of alkaloids compounds are inhibiting nucleic acid synthesis (Rao & Venkatachalam 2000), disturbing membrane integrity and activity on the cytoplasmic membrane (Alhanout *et al*. 2010; Salmi *et al*. 2008). Saponins inhibiting bacterial growth by lowering the surface tension, resulting in increased cell permeability and the release of intracellular compounds (Ngajow *et al*. 2013). Tannins inhibiting the growth of bacteria due work like a siderophore to chelate iron from the substrate and make iron unavailable to bacteria (Akiyama *et al.* 2001) Tannins also inhibiting DNA topoisomerase works (Nuria *et al.* 2009), and inhibiting perform of formation on cell wall polypeptides (Fahriya & Shofi 2011).

Phenolics compounds are free radical eliminators and metal chelators, and cause inhibiting various bacterial physiological activities (Takó *et al.* 2020). Another mechanism of phenolic as antibacterials are denaturing cell proteins and inhibiting bacterial nucleic acid synthesis (Bachtiar *et al.* 2012). The antibacterial mechanisms of flavonoids are inhibition of microbial enzymes, inhibition of energy metabolism, inhibition of cytoplasmic membrane function, inhibition of porins in cell membranes, alteration of membrane permeability, inhibition of nucleic acid synthesis, inhibition of biofilm attachment and formation, and attenuation of pathogenicity (Miklasińska-Majdanik *et al.* 2018; Xie *el al.* 2015).

Triterpenoid compounds inhibiting bacterial growth through protein synthesis inhibition (Siregar *et al*. 2012) and disrupt the cytoplasmic membrane (de Leon *et al.* 2010). The antibacterial mechanisms of steroid compounds are inhibiting the cell membrane function and forming a complex compound of extracelluler protein. Steroid compounds cause damage of the cell membrane, follow and release the intracellular compound (Maesyaroh *et al.* 2017). Glycoside compounds have inhibition the bacterial growth by penetrating into the cell wall, damaging the components of the bacterial cell wall, inhibiting the cell membrane functions and bind extracellular proteins to form complex compounds. The complex compounds cause cell membranes damage, and follow and release the intracellular compounds (Sunawan *et al.* 2018; Supriatno & Rini 2018).

The mechanism of phytochemical compounds playing a role in antibacterial activity. Phytochemical compounds are metal chelators and efflux pump inhibitors that will inhibit the bacteria growth using the mechanism of quorum sensing inhibitory by preventing the formation and destroying the biofilm formed by pathogenic bacteria. Besides, phytochemical compounds can disturb the regulation of quorum sensing and gene expression that is responsible for the biofilm formation and the virulence of pathogenic bacteria (Borges *et al.* 2016). Thus, phytochemical compounds naturally have a mode of distinctive multi-target.

Antibacterial Activities

The average of zone of inhibition of bacterial growth *E. coli, Sh. dysenteriae, S. aureus,* dan *P. aeruginosa* shown in Figure 1. The zone of inhibition is shown by the formation of a clear zone around the well indicating no bacteria growth is shown. This is caused by the toxicity of antibacterial activity from the ethanolic extract of senggani leaves that is bacteriocide, indicating that it can kill the test microorganisms to form the zone of inhibition (Sulistiyowati & Siswati 2016). The potency of a clear zone to be formed can be compared to the CLSI (2016), if the zone of inhibition diameter is ≤ 14 mm, the bacteria is considered resistant. If the zone of inhibition diameter is 15-19 mm, the bacteria are considered intermediate, and if the zone of inhibition is ≥ 20 mm, the bacteria are considered sensitive.

With such a result, it can be said that *E. coli* and *Sh. dysenteriae* did not show sensitivity to the ethanolic extract of senggani leaves that had been given. *S. aureus* is sensitive to the ethanolic extract of senggani leaves at the following concentrations: 50%, 75%, and 100% with a zone of inhibition diameter of 23.30mm, 23.60mm, and 24.50 mm consecutively. *P. aeruginosa* is sensitive to the ethanolic extract of senggani leaves with a concentration starting from 75% (Table 2).

In the positive control of 10 µg amoxicillin, the zone of inhibition diameter with an average of 19 mm in *E. coli* can be formed, so it is considered intermediate. The other test bacteria are also sensitive to 10 µg amoxicillin because they have an inhibition zone of more than 20 mm. The average zone of inhibition diameter shown by *Sh. dysenteriae* of 27.60 mm, *S. aureus* of 47.30 mm, and *P. aeruginosa of* 29.43 mm. In the negative control of distilled water, the zone of inhibition is not formed because distilled water does not

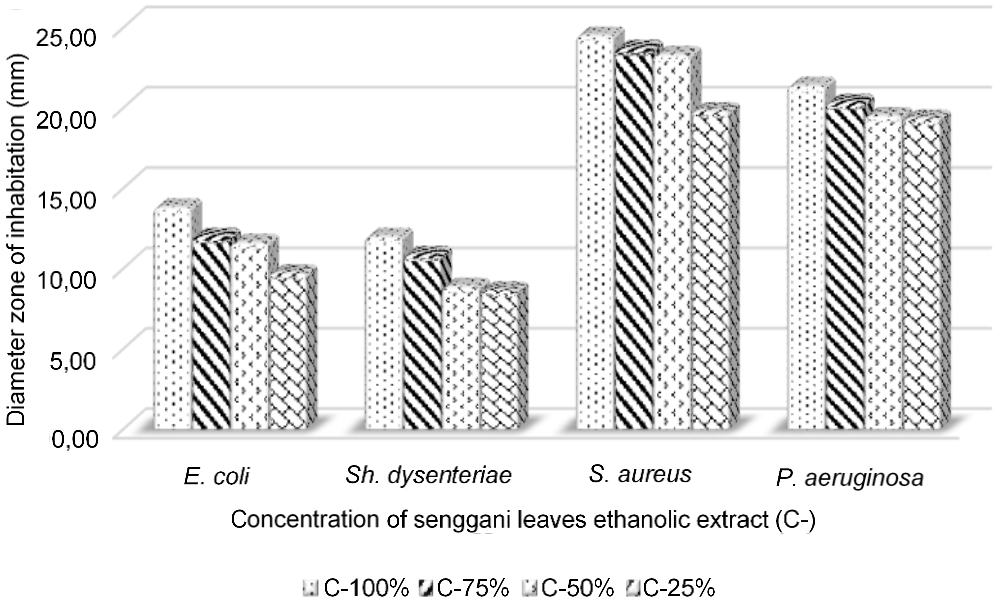

Figure 1 Average diameter zone of inhibition

Table 2 Post-hoc test results using Tukey method

Senggani leaves ethanolic extract concentration	Zone of inhibition diameter of bacterial growth (mm)			
	E. coli	*Sh. dysenteriae*	*S. aureus*	*P. aeruginosa*
25%	9.70[a]	8.63[a]	19.80[a]	19.33[a]
50%	11.60[b]	8.86[a]	23.30[b]	19.50[a]
75%	11.96[b]	10.83[b]	23.66[b]	20.30[ab]
100%	13.80[c]	11.96[b]	24.50[b]	21.36[b]
Control +	19.03[d]	27.66[c]	47.30[c]	29.43[c]
Control -	6.00[e]	6.00[e]	6.00[e]	6.00[e]

Different letters behind the numbers indicate significant differences based on the post-hoc test conducted using Tukey method with $p < 0.05$ for each type of bacteria, at each concentration, positive control, and negative control

contain antibacterial compounds. Thus, adding distilled water does not affect the concentration variation of the ethanolic extract of senggani leaves (Table 2).

The results of post-hoc test analysis using the Tukey method obtained the zone of inhibition diameter as an antibacterial form for each senggani leaves ethanolic extract concentration, positive control, and negative control on each type of test bacteria, are shown in Table 2. The data analysis results using one-way ANOVA test regarding the antibacterial activities of the ethanolic extract of senggani leaves obtained a significance value of $p < 0.05$. This indicates that the ethanolic extract of senggani leaves has the anti-bacterial activities on *E. coli, Sh. dysenteriae, S. aureus*, and *P. aeruginosa*. The post-hoc test was further conducted using the Tukey method to determine the concentrations that had significant differences. Different results were obtained from the antibacterial testing of the senggani leaves ethanolic extract on the growth of the test bacteria at each concentration level, in which the greater the concentration, the greater the zone of inhibition. It indicates that ethanolic extract of senggani leaves at a concentration of 100% is the best concentration as an antibacterial *E. coli*. Ethanolic extract of senggani leaves at a concentration of 75% and 100% are the same activities ability as antibacterial *Sh. dysenteriae*, and the recommended concentration is 75% which is not significantly different from 100% concentration.

Ethanolic extract of senggani leaves at concentrations of 50%, 75% and 100% had same anti-bacterial ability against *S. aureus*, and the recommend-ed concentration is 50% which is not significantly different from 75% and 100%. The ethanolic extract of senggani leaves with a concentration of 75% and 100% had same antibacterial ability against *P. aeruginosa*, and the recommended concentration is 75% which is not significantly different from the concentration of 100%.

The ethanolic extract of senggani leaves had antibacterial activites on all bacteria, both Gram positive (*S. aureus*) and Gram negative (*Sh. dysenteriae, E. coli, P. aeruginosa*). Antibacterial activeties in the ethanolic extract of senggani leaves are combination of various types of active substances contained. The results of the previous supporting research indicated that each of these active sub-stances from the phytochemical screening had antibacterial abilities. Alkaloids have inhibition ability against both of bacteria Gram negative, and Gram positive including *Sh. dysenteriae* and *E. coli* (Karou *et al*. 2005) and *S. aureus* (Wang et al. 2013). Saponins have ability to inhibit the growth of *E. coli* isolated from hospitals (Arabski *et al.* 2012) and multidrugs resistant

strains Gram-negative bacteria (Karou *et al.* 2005). Tannins have antibacterial activities to pathogenic bacteria Gram negative and Gram positive (Kurhekar 2016).

The antibacterial effect of phenolic compounds is very heterogeneous in both of bacteria Gram negative and Gram positive (Bouarab-Chibane *et al.* 2019). Flavonoids have antibacterial activities on bacteria Gram negative and Gram positive (Cushnie & Lamb 2011). Triterpenoids showed antibacterial activities against *E. coli* and *S. aureus* (Angeh *et al.* 2007), and methicillin-resistant *S. aureus* (MRSA3) (Nzogong *et al.* 2018). Glycosides extracts indicated an inhibition effect on *S. aureus* strain ATCC23 and *P. aeruginosa* strain ATCC53, but had no effect on *E. coli* strain ATCC22 (Soulef *et al*. 2014).

Therefore, the discussion explained above revealed that ethanolic extract of senggani leaves have shown their antibacterial ability which is to inhibit the growth of the test bacteria *E. coli* (ATCC 8739), *Sh. dysenteriae* (UnasCC 310), *S. aureus* (ATCC 6539), and *P. aeruginosa* (ATCC 9027). This is supported by the ability of the antibacterial activitis of eight active substances that have been successfully screened.

CONCLUSIONS

Senggani leaves ethanolic extract contain eight active substances, including alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides. These active substances have the inhibition ability against the growth of all the tested bacteria.

The best ability of senggani leaves ethanolic extract in inhibiting *E. coli* bacteria was at a concentration of 100%, *Sh. dysenteriae* and *P. aeruginosa* starting at the concentration of 75%, and *S. aureus* starting at the concentration of 50%.

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