RESEARCH ARTICLE | DECEMBER 14 2018

Antibacterial activities of *Syzygium polyanthum* wight leaves

Nurina Rizka Ramadhania; Adi Setyo Purnomo; Sri Fatmawati

AIP Conf. Proc. 2049, 020024 (2018)
https://doi.org/10.1063/1.5082429

Antibacterial Activities of *Syzygium polyanthum* Wight Leaves

Nurina Rizka Ramadhania, Adi Setyo Purnomo, and Sri Fatmawati*

Department of Chemistry, Faculty of Science, Institut Teknologi Sepuluh Nopember (ITS), Kampus ITS Sukolilo, Surabaya 60111, Indonesia

*Corresponding author: fatma@chem.its.ac.id

Abstract

Public health is one of the main problems for the people of Indonesia. One of the causes of health problems is pathogenic bacteria from the environment that easily enter the body and multiply to produce various diseases and infections. Plants extracts are one of the most important natural resources and alternative in the treatment effort as well as improving public health. This research was conducted to determine the antibacterial activities of methanol extract from *Syzygium polyanthum* Wight leaves as well as its fractions against bacteria Gram-positive *Staphylococcus aureus* and bacteria Gram-negative *Eschericia coli.* Ampicillin as positive control showed antibacterial activities against *S. aureus* and E. coli with IC_{50} value of 37.82 µg/mL and 10.28 µg/mL, respectively. Methanol extract showed antibacterial activities against *S. aureus* and E. coli with IC_{50} value of 23.16 µg/mL and 35.01 µg/mL, respectively. The *n*-hexane fraction had the highest antibacterial activity against *S. aureus* and *E. coli* with IC_{50} value of 49.25 µg/mL and 27.54 µg/mL, respectively, compared with aqueous and ethyl acetate fractions. The results demonstrated of methanol extract of *S. polyanthum* Wight leaves and its fractions as antibacterial sources.

INTRODUCTION

Public health is one of the main issues for the people of Indonesia. One of the causes of health problems is pathogenic bacteria. Microorganisms such as pathogenic bacteria that come from the environment, can easily enter into the human body through food, air, direct contact, injury, blood transfusion and needle syringe. These bacteria can be easy to breed in the body and cause various infections as well asvarious health problems caused by diarrhea, typhoid, dysentery, cholera and food poisoning. This is the impact of the spread of bacteria and if not treated immediately and it can result in death. Many diseasesand infections can cause damage to cells and tissues. The presence of damage to cells and tissues can cause the immune system or immune system decreased, thus the body's metabolism is disrupted.

Indonesia has been known as a country that has biodiversity and potential in the development of herbal medicine based on native tropical plants. Plants are one of the most important natural resources in the treatment effort and improving people's health. The main source of drug synthesis is the use of potentially derived compounds from plant or plant insulation. Since 2005, 25% of plant isolation compounds used as a drug and more than 80% of people depend on the use of the drug [1]. In Indonesia, there are about 70,000 species of plants including 7,000 species of medicinal plants that have potential as a medicine. The number of medicinal plants utilized by the community is about 1,000 to 1,200 species and is used by traditional medicine industry around 300 species [2].

The compounds from the isolated plant either pure compounds or standard plant extracts can be developed into new drugs that suit the needs of the community. Important secondary metabolites componentsin plant contents are used as drugs with therapeutic purposes [1], including alkaloids, flavonoids, tannins and phenolic compounds [3]. This phenolic compound has been used medicinally to treat various diseases such as antibacterial [4], anti-inflammatory, antidiabetes and antioxidant [5]. Extracts of medicinal plant also contain many nutrients and have no side or low effects, so that it is safe to be consumed regularly as a prevention and treatment of various diseases. Various studies of the pharmacological activity of medicinal plant have been widely reported. Some of the currently

The 3rd International Seminar on Chemistry
AIP Conf. Proc. 2049, 020024-1–020024-6; https://doi.org/10.1063/1.5082429
Published by AIP Publishing. 978-0-7354-1775-5/$30.00

available methods for knowing the biological activity of natural compounds from more than 25,000 plant species [6] are antibacterial activity. The bacteria can be found in places such as soil, water and air. Activity carried the bacteria can be controlled by the inhibition or killing physically and chemically. Antibacterial are substances or compounds that can inhibit or kill and control the bacterial cell growth and metabolism of bacteria that are harmful [7]. Control of bacterial growth aims to prevent the spread of disease and infection. It also prevented the decay and destruction of material by bacteria [8].

Natural compounds in plant extracts or pure compounds can provide unlimited opportunities for use in drug therapy due to unmatched chemical diversity. Several methods are currently available to detect their antibacterial activity and since not all of them are based on the same principles, the results obtained are influenced not only by the method selected, but also by the microorganism used, and by the extraction method or the degree of solubility of each test-compound [2]. Medicinal plant are referred to as traditional medicines or herbal remedies derived from nature and have beneficial physiological effects in the human body and the manufacturing process is conventional by using the minimal equipment. Although using conventional methods, traditional medicines is a well known globally as a health supplement or herbal medicine (in Indonesia) in the treatment of disease [9]. In addition to being well known in Indonesia, over 80% of traditional medicines is also well known in other developing countries in Asia and Africa.

In the development of medicine in the medical field, indigenous plants of Indonesia that can be utilized as a traditional herb is *S. polyanthum* Wight leaves. *S. polyanthum* Wight leaves are believed to have properties that can be used as medications for the treatment of hypertension, diabetes mellitus, gout, diarrhea, ulcers, toothache and bacterial wound infections [10]. *S. polyanthum* Wight leaves contains secondary metabolite compounds and cause synergistic effects between compounds so that it has many pharmacological activities. The content of the plant's secondary metabolite compounds has a polyvalent activity, making it possible to overcome various diseases. The phytochemical review of methanol extract of *S. polyanthum* Wight leaves shows the compound of saponin, tannin, alkaloid, triterpenoid, flavonoid, carbohydrate [11], polyphenols [12], and essential oils [13] thus it is also potential as an antioxidant and antibacterial agent [10]. Based on phytochemical results of various extracts and *S. polyanthum* Wight fractions in previous studies, this research was carried out the methanol extracts and its fractions from *S. polyanthum* Wight that have biological activity as antibacterial.

EXPERIMENTAL

S. polyanthum Wight leaves was obtained from Probolinggo, East Java. The organic solvent were methanol, water, ethyl acetate, *n*-hexane, and dimethyl sulfoxide (DMSO). The antibacterial assay support materials were nutrient broth (NB), nutrient agar (NA), ampicillin, and stocks of bacteria (*S.aureus*, *B.subtilis*, *E. coli* and *P. aeruginosa*) from Microorganism Chemistry Laboratory ITS. Instrumentations were spatula, glass beaker, erlenmeyer, wooden rack, test tubes, vial, micro pipette, tip, parafilm, falcon tubes, eppendorf tubes, filter paper, vortex, macerator, rotary vacuum, autoclave, laminary flow, incubator shaker, rotary evaporator and 96-microwell plate reader.

Extraction of *S. polyanthum* Wight Leaves

Subject used in this study was *S. polyanthum* Wight leaves obtained from Probolinggo, East Java, was shown in Fig.1. The leaves were cut into small size (60 mesh) that were then dried and blended to be dried powder of *S. polyanthum* Wight leaves. The dried powder of *S. polyanthum* Wight leaves (3.5 kg) was extracted by maceration for 24 hours with 21 L of methanol. The result of maceration was then concentrated with a rotary evaporator to get concentrated extract (245.2 g).

Fractionation of *S. polyanthum* Wight Leaves Extract

The methanol extract of *S. polyanthum* Wight leaves was dissolved in methanol: H_2O (2: 1) of 3 L and then liquid-liquid partitioned with *n*-hexane and ethyl acetate solvents, respectively. Each fractionation result was concentrated by rotary evaporator to obtain the fraction of methanol extracts. *n*-hexane fraction (73.34 g), ethyl acetate fraction (47.58 g) and aqueous fraction (47.56 g).

FIGURE 1. *S. polyanthum* Wight leaves

Antibacterial Test with Broth Dilution Method

Testing of antibacterial activity by liquid dilution method based on research method [8] with slight modifications. The methanol extract of *S. polyanthum* Wight leaves (10 mg) was used to antibacterial test by dissolving in 1 mL DMSO. Fifty microliters suspension of bacteria (*S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa,* 10^4 CFU/mL) were mixed with 5 µL sample with concentration of 100 mg/mL into the falcon tube containing 445 µL NB media. Ampicillin (100 mg/mL) was used as positive control, whereas solvent DMSO was used as negative control. The mixture was homogenized using a vortex. Samples incubated at 37ºC for 18 hours with incubator shaker. The results was measured at a wavelength of 630 nm (OD_{630}) with repeatability of measurements (n=3) using 96-microwell plate reader. Blank used at each variation was a mixture of 5 µL sample and 495 µL NB media. The antibacterial activity was calculatedby formula % inhibition of bacteria [14] as follows:

$$\%\ \text{Inhibition} = \frac{\text{OD}_{630}\ \text{Negative Control} - \text{OD}_{630}\ \text{Samples}}{\text{OD}_{630}\ \text{Negative Control}} \times 100\% \quad (1)$$

RESULT AND DISCUSSION

Extraction of *S. polyanthum* Wight Leaves

The dried powder of *S. polyanthum* Wight leaves (3.5 kg) was macerated with methanol solvent. The maceration method is an effective method to extract large quantities of a sample at room temperature by regulating the length of the sample immersion process in a particular solvent. Methanol solvent is a polar solvent and is very efficiently used for extraction. Methanol solvent is able to take the content of more secondary metabolite compounds that are polar and non-polar in *S. polyanthum* Wight leaves than other solvents, which have more non-polar properties that only take non-polar compounds [15]. Based on previous research, has proven that in the methanol extract of *S. polyanthum* Wight leaves contains metabolite compounds such as saponins, tannins, flavonoids, alkaloids, triterpenoids and reducing sugars, whereas in the use of more non-polar solvents such as ethyl acetate and *n*-hexane contains only flavonoids, alkaloids and terpenoids [11]. This suggested that the use of methanol solvents was very effective in leaves extraction methods.

Fractionation of *S. polyanthum* Wight Leaves Extract

Methanol extract of *S. polyanthum* Wight leaveswas 245.2 g. The methanol extract of 196.5 g of *S. polyanthum* Wight leaf extract was dissolved in MeOH: H_2O (2: 1), that further subsequently partitioned with ethyl acetate and *n*-hexane solvents. This treatment produced water, ethyl acetate and *n*-hexane fractions according to its polarity. Each partition result was concentrated with a rotary evaporator to obtain the fraction of *n*-hexane (73.34 g), ethyl acetate (47.58 g) and aqueous (47.56 g) fractions.Based on previous research, ithave proved that phytochemical results in water fraction and fraction of ethyl acetate of *S. polyanthum* Wight leaves contain saponin, tannins, flavonoid, alkaloid, triterpenoid, and reducing sugar, while in *n*-hexane fraction only contains flavonoids, alkaloids and terpenoids [11].

Antibacterial Test with Broth Dilution Method

In this work, the antibacterial assay was conducted using broth dilution method. Broth dilution method was applied to determine the lowest concentration of antibacterial that inhibits the visible growth of bacteria. Compared with the other methods, broth dilution method can decrease much labor and time [3]. Broth dilution method is more sensitive than screening agar or agar diffusion methods, so it most appropriate for the determination of the antibacterial activity of plant extracts quantitatively and quickly. This potent broth dilution method is more economical than the cost of source bacteria and plant extracts [8].

Inhibition of bacterial activity in the body can be done with the addition of antibacterial substances contained in the plant. It can be tested with one of the effective methods of liquid dilution. This inhibitory activity was measured by adding methanol extracts and its fractions of *S. polyanthum* Wight leaves into bacterial suspension (*S. aureus, E. coli, P. aeruginosa* and *B. subtilis*) which was then compared with positive control (Ampicillin) and negative control (DMSO). The antibacterial activity measured optical density at a wavelength of 630 nm using 96-microwell plate reader.

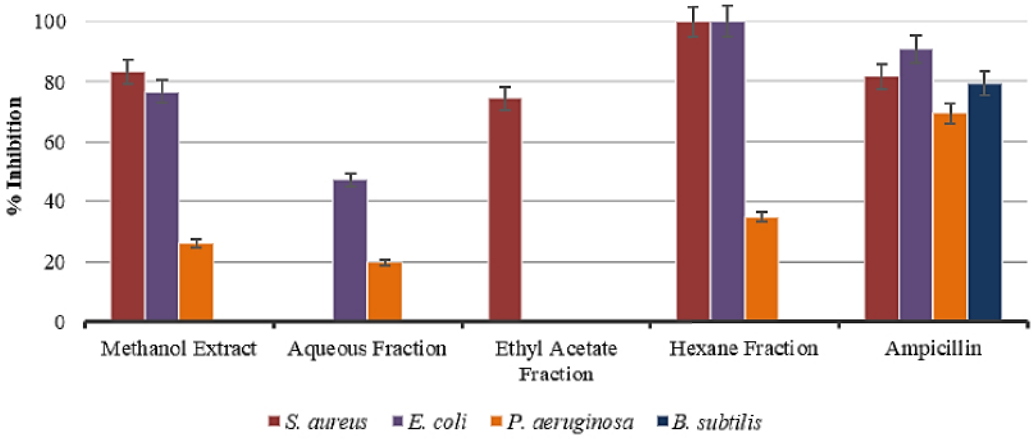

FIGURE 2. Antibacterial activity of *S. polyanthum* Wight leaves against bacteria

The antibacterial activity of the methanol extract and its fractions of *S. polyanthum* Wight leaves against 4 pathogenic bacteria was shown in Fig.2. The 4 pathogenic bacteria was used in the antibacterial test were bacteria Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *P. aeruginosa*). The methanol extract and *n*-hexane fraction showed higher antibacterial activity than positive control ampicillin against bacteria *S. aureus* but ethyl acetate fraction inhibition was slightly lower one. The *n*-hexane fraction showed higher antibacterial activity than ampicillin against bacteria *E. coli* but methanol extract and aqueous fraction inhibition was lower one. The methanol extract, aqueous fraction and *n*-hexane fraction showed lower antibacterial activity than ampicillin against bacteria *P. aeruginosa*. All samples had no antibacterial activity against bacteria *B. subtilis* excepted ampicillin. Based on these results, the *n*-hexane fraction showed completely inhibited bacteriaandhad the highest antibacterial activity. The negative control (DMSO) had no effect on inhibiting the growth of bacteria, which indicated that DMSO did not effect on the assay. The extract and fractions showed high antibacterial activity against *S. aureus* and *E. coli* only.

The IC_{50} values of antibacterial activity of the methanol extract and its fractions of *S. polyanthum* Wight leaves against *S. aureus* and *E. coli* was determined, was shown in Table.1. The IC_{50} values of methanol extract against *S. aureus* was indicating fine inhibition value where at low concentration 23.16 µg/mL can inhibit 50% effectively than positive control ampicillin. The IC_{50} values of ampicillin against *E. coli* was indicating fine inhibition value, where at concentration 10.28 µg/mL can inhibit 50% effectively than methanol extract and its fractions of *S. polyanthum* Wight leaves. Extracts and fractions of *S. polyanthum* Wight leaves had a low inhibition and/or can not inhibited the bacteria *P. aeruginosa* and *B. subtilis*. This shows the great potential of methanol extract and fractions of *S. polyanthum* Wight leaves as an antibacterial source.

TABLE 1. The IC_{50} values of antibacterial activity of the methanol extract and its fractions of *S. polyanthum* Wight leaves against *S. aureus* and *E. coli*

Samples	The IC_{50} values against bacteria (μg/mL)	
	S. aureus	***E. coli***
Methanol extract	23.16	35.01
Aqueous fraction	-	75.70
Ethyl acetate fraction	80.82	-
n-Hexane fraction	49.25	27.54
Ampicillin	37.82	10.28

The *n*-hexane extracts induced the highest antibacterial activity against bacteria *S. aureus* and *E. coli*. This can be explained with the active compounds which are responsible for the antibacterial activity of the extract reside in the non-polar fractions in relatively higher concentration [16]. Both extracts were observed to inhibit Gram-positive and Gram-negative bacteria tested *in vitro* to suggest that *S. polyanthu*m Wight leavesextract and fractions has a broad-spectrum antibacterial activity. Studies previous research, showed the similar antibacterial potentials have been observed in the culture extracts of *Irpex lacteus* [17].

Variations solvents used might be due to the differential solubility of the leaves constituents. Due to the presence of various bioactive compounds in *S. polyanthu*m Wight leaveshad different antibacterial potentialfrom previous research such as can inhibited bacteria *Salmonella thypi* (1-1.25 mm), *Tricophyton mentagrophytes* (0.25-0.41 mm), *B. cereus* (0, 31-0.62 mm) [17], *E. coli*, *S. aureus*, *S. typhimurium*, *Vibrio cholera* (MIC> 1000 μg/mL), *B. subtilis* (MIC 31.25 μg/mL) [19], *S. typhimurium* (MIC 31.25 μg/mL) a *B. subtilis* (MIC 62.5 μg/mL) [20]. The antibacterial activity of the extracts depends on the concentration/dose and type of bacteria used as a chemical component in the extracts. The higher concentration of an antibacterial compound, the content of antibacterial compounds will be more and more powerful antibacterial activity [6].

The results showed that antibacterial activity had greater inhibition against Gram-negative bacteria (*E. coli*) than Gram-positive bacteria (*S. aureus*). Generally, Gram-positive bacteria more easily inhibited compared with Gram-negative bacteria, not the other way around. This is due to the difference in sensitivity of Gram-positive and Gram-negative bacteria is the morphological constitutional differences between these microorganisms. Gram-negative bacteria have three layers of thick membrane and there is a phospholipid membrane in the outer portion that can carry the structural lipo-polysaccharide components. This makes the cell wall impermeable to antibacterial chemical substances so that the antibacterial compound cannot enter and penetrate the cell. The Gram-negative bacteria cell wall is more complex than the Gram-positive bacteria cell walls [7]. Gram-positive bacteria tend to be more susceptible because they have a single layer of external peptidoglycan which is not an effective permeability barrier. This is due to the simpler Gram-positive bacteria cell walls structure, making it easier for the antibacterial compounds to enter the cells by penetrating the bacterial cell wall.

CONCLUSION

This research was conducted to determine the antibacterial activities of methanol extract and its fractionsfrom *S. polyanthum* Wight leaves against bacteria Gram-positive *S. aureus* and bacteria Gram-negative *E. coli*. The n-hexane fraction and ampicillin had fine antibacterial activity against *S. aureus* and *E. coli* compared to aqueous and ethyl acetate fractions. The results demonstrated great potential of methanol extract and its fractions of *S. polyanthum* Wight leaves as antibacterial sources.

ACKNOWLEDGMENTS

This work was supported by a grant from research project from the Directorate of Research and Community Service, Directorate General of Strengthening Research and Development, Ministry of Research, Technology and Higher Education, Indonesia. The financial support for Nurina R.R. from Fresh Graduate Scholarship - Institut Teknologi Sepuluh Nopember was also acknowledged.

REFERENCES

1. World Health Organization. *WHO Technical Report Series* **641**, 1-44 (1979).
2. D. P. Kisangau, K. M. Hosea, C. C. Joseph, and H. V. M. Lyaruu. Afr. J. Tradit. Complement. Altern. Med. **4** (4), 510-523 (2007).
3. A. Hassan, S. Rahman, F. Deeba, and S. Mahmud. J. Med. Plants Res. **3** (1), 20-23 (2009).
4. T. Ersam, S. Fatmawati, and D. N. Fauzia. Indones. J.Chem. **16** (2), 151-155 (2016).
5. W. D. Fitriana, T. Ersam, K. Shimizu, and S. Fatmawati. Indones. J. Chem. **16** (3), 297-301 (2016).
6. M. A. Calvo, E. L. Arosemena, C. Shiva, and C. Adelantado. *Sciences against microbial pathogens; communicating current research and technological advances*, 1179-1185 (2011).
7. A. Nostro, M. P. Germano, V. D'Angelo, A. Marino, and M. A. N. Cannatelli. Lett. in Appl. Microbiol. **30**, 379-384 (2000).
8. M. E. Arias, J. D. Gomez, N. M. Cudmani, M. A. Vattuone, and M. I. Isla. Life Sci. J. **75**,191-202 (2004).
9. S. Fatmawati, K. Kurashiki, S. Takeno, Y. Kim, K. Shimizu, M. Sato, K. Imaizumi, K. Takahashi, S. Kamiya, S. Kaneko, and R. Kondo. Phyto. Res. **23**, 28-32 (2009).
10. A. Evendi. *Mahakam Medical Laboratory Technology Journal* **2** (1), 1-9 (2017).
11. M. D. Hidayati, T. Ersam, K. Shimizu, and S. Fatmawati. Indones. J. Chem. **17** (1), 49-53 (2017).
12. R. A. A. Lelono, S. Tachibana, and K. Itoh. Pakistan J. Biol. Sci. **12**, 1564-1570 (2009).
13. R. Noveriza and Miftakhurohmah. J Littri **16** (1), 6-11 (2010).
14. C. L. Quave, L. R. W. Plano, T. Pantuso, and B. C. Bennet. Journal Ethnopharmacology **118** (3), 418-428 (2008).
15. J. Azmir, I. S. M. Zaidul, M. M. Rahman, K. M. Sharif, A. Mohamed, F. Sahena, M. H. A. Jahurul, K. Ghafoor, N. A. N. Norulaini, and A. K. M. Omar. J. Food Engineering **117**, 426–436 (2013).
16. H. Tadeg. Pharmaceutics Thesis, School of Pharmacy, Addis Ababa University. 1-148 (2004).
17. L. H. Rosa, K. M. G. Machado, C. C. Jacob, M. Capelkari, C. A. Rosa, and C. L. Zani. Mem. Inst. Oswaldo Cruz. **98**, 1–19 (2003).
18. I. W. Kusuma, H. Kuspradini, E. T. Arung, F. Aryani, Y. Min, J. Kim, and Y. Kim. J. Acupunct. Meridian Stud. **4** (1), 75-79 (2011).
19. H. Alwani, M. P. M. Gigih, Istifah, and H. Dwi. J. Food Pharm. Sci. **4**, 1-5 (2016).
20. A. Hamad, M. G. P. Mahardika, I. Yuliani, and D. Hartanti. Rasayan J. Chem. **10** (2), 564-569 (2017).