JURNAL KARTIKA KIMIA

J. Kartika Kimia, Nov 2018, 1, (1), 1-4
Journal homepage: http://jkk.unjani.ac.id/index.php/jkk

Phytochemical Screening and Antibacterial Activity of Ethanolic Extract of *Syzygium samarangense* Leaves

Nur Amalia Choironi*, Muhamad Salman Fareza
Department of Pharmacy, Faculty of Health Sciences, Universitas Jenderal Soedirman
Jl. Dr. Soeparno, Karangwangkal, Purwokerto Utara, 53123
*E-mail: n.a.choironi@gmail.com

Abstract

The ethanolic extracts of *S. samarangense* leaves were examined for antibacterial activity in vitro using broth microdillution method. The extracts showed effective against *Bacillus cereus, Salmonella enterica* with MIC (minimum inhibition concentration) value 78 µg/ml and against multi strains of *Escherichia coli, Enterobacter aerogenes, Kocuria rhizophila* with MIC value 2500 µg/mL, 2500 µg/mL and 156 µg/mL. Phytochemical screening of *S. samarangense* was observed for the presence of tannins, alkaloids, flavonoids, and terpenoids.

Keywords: antibacterial, microdillution, *Syzygium samarangense*, MIC

1. Introduction

S. samarangense (Bloom) Merr. and L.M. Perry (Myrtaceae) is wide distribution in Asia, is commonly known as wax jambu, java apple, samarang apple. The plant is wide distribution in Malaysia, Indonesia, Thailand, Cambodia, Laos, Vietnam, India, Australia, and Taiwan [1], [2]. The tree of this plant is a small tree with pink edible fruit. The yellowish to dark bluis green leaves, opposite, elliptical with short stalks and aromatic when crushed [3]. Traditionally, the leaves of this plant were used as astringent, treat fever and halt diarrhoea [4]. As a potential plant, many publication had reported that the leaves extract of this plant had pharmacological activity like antibacterial, antidiabetic, immunomodulatory, anticancer [3], [5], [6], [7]. The previous phytochemical study of the leaves of *S. samarangense* showed the presence of triterpenoids and flavonoids [5], [8], [9]. For antibacterial properties, the in vitro study of the leaves extract of *S. samarangense* from India showed strong antibacterial activity against both Gram (+) and Gram (-) bacteria using diffusion agar method with inhibition zone value are 20-25 mm/50µL [7].

However, some publications about antibacterial activity of the leaves of *S. samarangense* from Indonesia has not been reported yet. The aim of this research are to know the chemical constituent and antibacterial properties of *S. samarangense* leaves. The antibacterial activity was carried out with microdillution methods to get the value of minimal inhibitory concentration (MIC).

2. Methodology

2.1. Plant material

Leaves of *S. samarangense* used in this research were collected from Faculty of Biology, Universitas Jenderal Soedirman. The specimen (424e/FB.Unsoed/TaksTumbXI/2016) was identified at Laboratory of Plant Taxonomy, Faculty of Biology, Universitas Jenderal Soedirman. The collected leaves was shade dried and ground with a blender.

2.2. Chemical materials

Ethanol 70% and 96% (Bratachem), sodium hypochloride, methanol (Merck), Mueller Hinton Broth, Potato Dextrose Agar (HiMedia), Potato Dextrose Broth (HiMedia), Mueller Hinton Agar, DMSO, Mc Farlant solutions, aquadest, bacterial strains (*Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 11778, *Enterobacter aerogenes* ATCC 13048, *Salmonella enterica* ATCC 14028, *Kocuria rhizophila* ATCC 533).

2.3. Extraction

Dried leaves (500 g) were extracted by maceration using ethanol 96% (in a 1:10 ratio) for 24 hours, subsequently filtered. Residue was re-extracted twice with the same method and solvent.

Ethanol extract were concentrated using rotary vacuum evaporator at 60°C and followed by using waterbath.

2.4. Phytochemical screening

Phytochemical screening of the ethanol extract of *S. samarangense* was carried out in order to ascertain the presence of major phytochemical group by using standard methods [10], [11], [12].

2.5. Antibacterial assay

The minimum inhibition concentration (MIC) was determined by broth microdilution method according to the Clinical and Lamatory Standars Institute (CLSI) [13]. The samples were tested against *E. coli* ATCC 25922, *B. cereus* ATCC 11778, *Enterobacter aerogenes* ATCC 13048, *Salmonella enterica* ATCC 14028, *Kocuria rhizophila* ATCC 533. Chloramphenicol was used as positive control. The sample was dissolved in dimethylsulfoxide (DMSO) to achieve 10000 µg/mL in the first well. Two fold serial dilutions of sample was performed in a 96-wells microplate. The lowest concentration of extract that inhibited visible growth was recorded as the MIC value.

3. Result and Discussion

In our research we used microdillution method to determine antibacterial properties from the leaves of *S. samarangense*. Exploration of extract to know antibacterial properties usually using difusion or dilution methods. According to Klancnik et al. [14], the dilution method was recommended for studying polar and non-polar substances as well as all types of complex extract than diffusion methods. The dilution methods have sencitivity 30 times better than diffusion methods with the limit sample [15].

Our results (Table I) showed that the ethanolic extract of *S. samarangense* leaves possessed various antibacterial activity from strong to weak activity against the five tested bacteria with MIC value between 78-2500 µg/mL. The MIC value were determined as the lowest concentration of the extract capable to inhibit microorganism growth. According to Abreu [16], phytochemical products that produce minimum inhibitory concentrations (MIC) in the range 100-1000 µg/mL in in vitro susceptibility test can be classified as a antibacterials. The extract which has antibacterial activity with the concentration below 100 µg/mL is a potentially as antibacterial agent [17]. The present study reveals that the leaves extract of *S. samarangense* was effective against *B. cereus* and *S. enterica* than the other strains tested with the MIC value 78 µg/mL. The previous study showed that the fruit extract of *S. samarangense* has weak antibacterial activity against *Staphylococcus aureus* (MIC 500 µg/ml), *Bacillus cereus* (MIC 125 µg/mL) and *Escherichia coli* (MIC 250 µg/mL) [18].

Table I. Minimum inhibitory concentration of *S. samarangense* leaves

Strains	MIC (µg/mL)	
	S.samarangense	Chloramphenicol
E. coli	2500	15.6
B. cereus	78	3.9
E. aerogenes	2500	3.9
S. enterica	78	3.9
K. rhizophila	156	3.9

According phytochemical screening, the extract was observed for the presence of tannins, alkaloids, flavonoids, and terpenoids (Table II). Flavonoid and tannin, are phenolic compound which have important function as defense against plant pathogens, animal herbivore aggression or biotic stress conditions [19]. Previous study reported that the phytoconstituents of leaves are tannin, steroid, triterpenoid, flavonoid, sitosterol anthocyanidins, proanthocyanidins and carotenoid [4], [5], [8], [9], [20]. Nair et al. [8] and Hanshella et al. [3] reported that flavonoid has been isolated from leaves *S. samarangense* are flavonol mearnsitrin; 2'-methyl-5'-O-galloylmyricetin-3-O-rhamnopyranoside; 2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone; 5-O-methyl-4'-desmethoxymatteucinol and 2',4'-dihydroxy-6'-methoxy-3'-methylcalcone. Tannins are inhibit digestive enzyme and affect the absorption of vitamins and minerals. However, some kinds of tannins can reduce the mutagenicity of a number mutagens and display anticarcinogenic, antimicrobial, and antioxidant activities [21], [22]. Flavonoid, which is one of the largest and broadly antibacterial activities with hydroxyl or prenylated substitutions of these structure [22]. According Chusnie and Lamb [19], antibacterial mechanism of flavonoid are inhibition of nucleic acid synthesis, inhibition of sytoplasmic membrane function and inhibition of energy metabolism in bacteria. Terpenoids are known for antimicrobial

properties, 60% of terpenoids inhibit fungi while 30% inhibit bacteria. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by these lipophilic compounds [23].

Table II. Phytochemical screening of *S. samarangense* leaves

Phytochemicals	*S. samarangense*
Saponin	-
Tannin	+
Flavonoid	+
Alkaloid	+
Steroid/triterpenoid	+

Further phytochemical investigations of bioactive compound from *S. samarangense* should be continued to treat infectious disease. The potential of *S. samarangense* against *B. cereus* and *S. enterica* could be explored to be used for food preservatives. *B. cereus and S. enterica* have been incriminated as the causative agents of food-borne illness [24], [25].

4. Conclusion

From our study we found that the ethanolic extract of *S. samarangense* leaves from contains tannin, flavonoid, alkaloid and terpenoid as chemical constitutent. Besides that, it's ethanolic extract posses potential antibacterial properties against *B. cereus* and *S. enterica*.

Acknowledgements

Authors are grateful for the financial support from BLU Research Grant 2017 from Universitas Jenderal Soedirman.

References

[1] Simirgiotis, M.J., Adachi, S., To, S., Yang, H., Reynertson, K.A., Basile, M.J., Gil, R.R., Weistein, I.B. and Kennelly, E.J., 2007, Cytotoxic chalcones and antioxidants from the fruits of *S. samarangense* (Wax Jambu), *Food Chemistry*, 107: 813-819.

[2] Lim, T.K. 2003. *Edible Medicinal and Non-Edible Medicinal, Fruits*. Springer Dordrecht Heidelberg London New York. 3: 778-786.

[3] Hanshella, C., Magno, R., Villasenor, I.M., Harada, N. and Monde, K., 2005, Antihyperglycaemic Flavonoids from *Syzygium samarangense* (Blume) Maerr and Perry, *Phytotherapy Research*, 19: 246-251.

[4] Peter, T., Padmavathi, D., Sajini, R.J., Sarala, A., 2011, Syzygium samarangense: A Review on Morphology, Phytochemistry and Pharmacological Aspects, *Asian Journal of Biochemical and Pharmaceutical Research*, 4 (1): 155-163.

[5] Srivastava, R., Arun, K., Shaw and Kulshreshtha, K., 1994, Triterpenoids and Chalcone from *Syzygium samarangense*, *Phytochemistry*, 38 (3): 687-689. 62: 1-5.

[6] Kuo, Y.C., Yang, L.M., Lin, L.C., 2004, Isolation and Immunomodulatory Effect of Flavonoids from *Syzygium samarangense*, *Planta Medica*, 70 (12): 1237-1239.

[7] Reddy, L.J. and Rose, B., 2011, Chemical Composition and Antibacterial Activity of The Volatile Oil from The Leave of *Syzygium samarangense* (Blum) Merr. & L.M. Perry, *Asian Journal of Biochemical and Pharmaceutical Research*, 3 (1): 263-269.

[8] Nair, A.G.R., Krishnan, S., Ravikrishna, C. and Madhusudanan, K.P., 1998, New and Rare Flavonol Glycosides from Leaves of *Syzygium samarangense*, *Fitoterapia*, 70: 148-151.

[9] Ragasa, C.Y., Franco, F.C., Raga, D.D. and Shen, C., 2014, Chemical Constituen of Syzygium samarangense, *Deer Pharma Chemica*, 6 (3): 256-260.

[10] Farnsworth, N.R., 1966, Biological and Phytochemical Screening of Plants, *Journal of Pharmaceutical Sciences*, 55 (3): 225-276.

[11] Zuhrotun A., Suganda AG., Sukandar EY., 2017, Toxicity of Selected Apocynaceae, Magnoliaceae and Simaroubaceae of Indonesian Plants Using Brine Shrimp Lethality Bioassay, *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* 8: 10-15.

[12] Muthoharoh A., Zainab, 2015, Phytochemical Screening, Determination of Napthoquinone Content and Antifungal Activity of Insoluble Ethyl Acetate Fraction of Ethanolic Extract of *Lawsonia inermis* L. Leave Against Candida Albicans ATCC 10231, *Pharmaciana*, 5 (2): 199-208.

[13] Cockerill, F. R., Wikler, M. A., Alder, J., Dudley, M. N., Elipoulos, G. M. and Ferraro, M. J., 2012, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard-Ninth Edition*, Clinical and Laboratory Standars Institute, USA. 32 (2): 52-53.

[14] Klancnik, A., Piskernik, S., Jersek, B., Mozina, SS., 2010, Evaluation of Diffusion

and Dilution Methods to Determine The Antibacterial Activity of Plant Extracts, *Journal of Microbiological methods*, 81 (2): 121-126.

[15] Eloff, J. N., 1998, A Sensitive and Quick Microplate Method to Determine the Minimal Inhibitory Concentration of Plant Extracts for Bacteria, *Planta Medica*, 64: 711-713.

[16] Abreu, A.C., McBain, A.J. and Simoes, M., 2012, Plants as Sources of Nes Antimicrobials and Resistance-modifying Agents, *Natural Product Rep*, 29: 1007-1021.

[17] Rios, J.L. and Recio, M.C., 2005, Medicinal Plants and Antimicrobial Activity, *Journal of Ethnopharmacology*, 100: 80-84.

[18] Ratnam, K.V. and Raju, R.R.V., 2008, In Vitro Antimicrobial Screening of The Fruit Extracts of Two Syzygium Species (Myrtaceae), *Advances in Biological Research*, 2 (1-2): 17-20.

[19] Cushnie, T.P.T., Lamb, A.J., 2005, Antimicrobial activity of Flavonoids, *International Journal of Antimicrobial Agents*, 26: 343-356.

[20] Pawar, M.M., Patil, S.D., Jadhav, A.P., Kadam, V.J., 2015, In vitro Antimicrobial Activity and Phytochemical Screening of Selected Indian Medicinal Plant, *Indo American Journal of Pharmaceutical Research*, 5 (1): 593-600.

[21] Oliveira, G.F., Furtado, N.A.J.C., Filho, A.A.S., Martins, C.H.G., Bastos, J.K., Cunha, W.R. and Silva, M.L.A., 2007, Antimicrobial activity of *Syzygium cumini* (Myrtaceae) Leaves Extract, *Brazillian Journal of Microbiology*, 38: 381-384.

[22] Silva, N.L., Zimmer, K.R., Macedo, A.J. and Trentin, D.S., 2016, Plant Natural Products Targeting Bacterial Virulences Factors, *Chemical Reviews*, 116: 9162-9236.

[23] Saleem, M., Nazir, M., Ali, M.S., Hussain, H., Lee, Y.S., Riaz, N., Jabbar, A., 2009, *Natural Product Report,*. 27: 238-254.

[24] Threlfall, E.J., Ward, L.R., Frost, J.A., Willshaw, 2000, The Emergence and Spread of Antibiotic Resistance in Food-borne Bacteria, *International Journal of Food Microbiology*, 768-789.

[25] Alzoreky, N.S. and Altaher, A.A., 2014, Antibacterial Activity of Spathe from *Phoenix dactylifera* Against Food-borne Pathogens, *Industrial Crops and Products*, 65: 241-246.