NUSANTARA BIOSCIENCE
Vol. 8, No. 1, pp. 111-116
May 2016

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n080119

Phytochemicals analysis and antibacterial activity of the leaf of red meranti, *Shorea leprosula* (Dipterocarpaceae)

SUDRAJAT[1,♥], DWI SUSANTO[1,♥♥], RUDI KARTIKA[2,♥♥♥]

[1,2]Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University. Jl. Barong Tongkok Kampus Gunung Kelua, Samarinda 75123, East Kalimantan, Indonesia, Tel./fax. +62-0541-749152, ♥email: sudrajat.fmipa@gmail.com ; ♥♥ susantodwiki@yahoo.com
[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University. Kampus Gunung Kelua, Samarinda 75123, East Kalimantan, Indonesia. email: ♥♥♥ rudi_biokimia@yahoo.com

Manuscript received: 7 March 2016. Revision accepted: 24 May 2016.

Abstract. *Sudrajat, Susanto D, Kartika R. 2016. Phytochemicals analysis and antibacterial activity of the leaf of red meranti,* Shorea leprosula *(Dipterocarpaceae). Nusantara Bioscience 8: 111-116.* This study aims to investigate the antibacterial activity of the total ethanolic extract and its fractions of red meranti (*Shorea leprosula*) leaf against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. The ethanolic extract and its fractions were subjected to antibacterial assay using well diffusion technique. Column chromatography was used to purify the active compounds from the mixture, while GC/MS was used to identify the phytocomponents of the fractions. The GC/MS study demonstrated the presence of different types of compounds were 1,2-benzenedicarboxylic acid (65.77%), eicosanoic acid (9.82%), 2-pentadecenone (6.53%), tricosane (5.86%) and hexanedioic acid (4.13%). The result showed that the inhibition zone diameters of the ethanolic extracts ranged from 15.56-16.44 mm against *S. aureus*, and 15.37-16.56 mm for *E. coli*, respectively. The total ethanolic extract from red meranti leaf showed a strong category of antibacterial properties against both types of bacteria which were equivalent to antibiotic chloramphenicol. While, the fractions extracts showed that antibacterial activity was lower than total ethanolic extract against Gram-positive bacteria *S. aureus* and Gram-negative bacteria *E. coli.* The results obtained from this study justify the use of this plant in traditional medicine and provide leads which could be further exploited for the development of new and potent antibacterial.

Keywords: Antibacterial activity, *Escherichia coli*, phytocomponent, *Shorea leprosula*, *Staphylococcus aureus*

INTRODUCTION

Shorea is one genus of the family of Dipterocarpaceae which spread throughout tropical rain forests lowland of Indonesia, mainly on the island of Borneo. The three main genuses are *Shorea* with members of 150 species, *Hopea* consists of 100 species and *Dipterocarpus* consists of 75 species. In Indonesia, they are known as Meranti (*Shorea*), Merawan (*Hopea*) and Keruing (*Dipterocarpus*). Dipterocarpaceae is a plant family that is well known for its property as resistant to biological attack. The active substance contained in very diverse members of this family includes phenolic groups, such as; oligostilbenoid, flavonoids, phenyl propanoid and phenolic acid derivatives, as well as non-phenolic groups, i.e. triterpenoids (Sotheeswaran and Pasuparthy 1993; Hakim 2002).

Based on phytochemical research that has been done on Dipterocarpaceae, it is found that in addition to being a source of wood and resin, this plant family is also a source of compounds of stilbenoid (Newman et al. 1999), monomers and oligomers of resveratrol (Atun 2006; Rosyidah et al. 2007; Sahidin et al. 2007). Some stilbenoid compounds and resveratrol, that can be isolated from Dipterocarpaceae or other families, are known to have an interesting biological activity, such as cytotoxic to cancer cells, block the acetylcholinesterase, anti-HIV, anti-fungal, anti-inflammatory, antioxidant, and anti-bacterial (Sultanbawa et al. 1980; Seo et al. 1999; Murthy et al. 2011; Norizan et al. 2012; Muhammad et al. 2012; Mulyono et al. 2013; Nainwal et al. 2013; Subramanian et al. 2013; Manjang et al. 2015).

Currently, there is a rising interest towards emerging antibacterial species in biological systems and its functions. One type of meranti that can be found in Borneo is *S. leprosula* or red meranti. Until now, there is no detailed information about the chemical content, especially of chemical compounds that are bioactive against bacteria. Therefore we need a study of the types of any bioactive compounds that exist in this plant and how its properties can be used as an anti-bacterial compound. This study was designed to evaluate the phytochemical analysis and the antibiotic activities of the leaf extract of *S. leprosula*.

MATERIALS AND METHODS

Plant material

The leaf of *S. leprosula* was collected in bulk (mixed from the different trees) from the Botanical Gardens of Mulawarman University at Samarinda, East Kalimantan, Indonesia (0°30′00″ S, 117°09′00″ E). Plant species was identified by a taxonomist in Laboratory of Biodiversity, Faculty of Mathematics and Natural Sciences, Mulawarman University. Plant samples collected from field was cut into pieces and dried at room temperature in the laboratory for two weeks. Then leaves were milled and refined with a

sieve sized of 40-60 mesh. The powder was kept in a constant room until the moisture content is approximately 15%.

Extraction

Powdered leaf (200 g, dry weight basis) were weighed and then were macerated using 75% ethanol in a glass container with a ratio of 1:1 for one night and then filtered using filter paper No. 1. The maceration was repeated until the filtrate was clear. The obtained extract was concentrated by rotary evaporator and put in vacuum oven to yield 15.618 g (7.809%) of solid paste.

The ethanol extract was fractionated successively with *n*-hexane and ethyl acetate to give the respective soluble fractions. The last one was collected and extract of ethyl acetate was evaporated, and then in extract ethanol, antimicrobial was tested by agar diffusion method. Further, in the extract, content of bioactive compounds were analyzed using column chromatography. Then, it was fractionated using chromatographic column techniques with a fixed phase Silica gel. A total of 0.5 g of sample was stirred with celite until homogeneous. Then, it made compact of silica gel 60 column using the mobile phase of chloroform, methanol and water with ratio 7:3:1. After the solid coated filter paper was placed as a cover and the mixture celite was put and the sample was then eluted. Purification is done to the fraction of ethanol and the results of chromatography column against potential fraction that has the same stain pattern combined and coded separately. The isolates obtained is then washed continuously with pure methanol to obtain a colorless and analyzed by GC/MS Shimadzu QP2010S.

Gas chromatography/mass spectrometry (GC/MS analysis)

Since the ethyl acetate fractions obtained from TLC showed almost different Rf values, both fractions was subjected to GC/MS analysis. The active fractions (fraction A) was dissolved in HPLC grade solvent and subjected to GC/MS a Shimadzu QP2010 GC/MS apparatus (Shimadzu Corp. Japan) equipped with Ri-5MS column Rastex capillary (30 m, 0.25 mm, ionization: EI 70 Ev), heated at a temperature of 80°C, with pressure of 16.5 kPa, and total flow of 20.0 ml/min, column flow of 0.50 ml/min, linear velocity of 26.1 cm.sec-1, purge flow of 3.0 ml.min-1, injector and detector temperature was 3100C, using helium as the carrier gas, at a flow rate of 3.0 ml.min-1. Interpretation on mass spectrum of GC/MS was done using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The mass spectrum of the unknown component was compared to the spectrum of the known components stored in the NIST08 and Wiley08 library. The name, molecular weight and structure of the components of the test materials were ascertained.

Antibacterial assay

The extracts and isolated compounds were tested for their antibacterial activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacterial strains using agar well diffusion method. The Mueller Hinton Agar No 2 medium for the assay microbial growth was determined by measuring the diameter of the zone of inhibition. Inoculating of the bacterial used a sterile cotton swab which was dipped into the adjusted suspension and was used to streak all over the dried surface of a sterile Mueller Hinton Agar plate. This streaking process was repeated 2-3 times to ensure that the test organisms were evenly distributed. The inoculum was allowed to diffuse into the agar for about 10 min. Four wells (6 mm) were aseptically made using sterile cork borer equidistance to each other, fixed volume 0.1ml (100µl) of the plant extract at different concentrations were carefully placed into each holes while the fourth hole contained a broad spectrum antibiotic (chloramphenicol) as control.

The plates were prepared in triplicates and then incubated at 37^0C for 18-24 hours. The zone of inhibition of each well was obtained by measuring the underside of the plate in two planes with a ruler calibrated in millimeter. The antibacterial activity extract of *S. leprosula* parts was examined against Gram-negative and Gram-positive bacteria and their inhibitory activity was quantitatively assessed by the presence or absence of inhibition zones and zone diameters.

Data analysis

Observation of the inhibitory activity of bacteria is done by measuring the diameter of inhibitory regions (DDH) formed around the wells.

RESULTS AND DISCUSSION

Phytochemical analysis

The result of preliminary phytochemical test revealed that the ethanol leaf extracts of *S. leprosula* had contents of alkaloids, phenolics, triterpenoids and flavonoids. The gas chromatography profile of fraction A was displayed in Figure 1 and Table 1. The GC/MS study demonstrated that the presence of different types of compounds were 1,2 benzenedicarboxylic acid (65.77%), eicosanoic acid (9.82%), 2-pentadecenone (6.53%), tricosane (5.86%), hexanedioic acid (4.13%), 2 (3H)-furanone (2.81%) (Table 1).

Antibacterial activities

The result of the susceptibility profile of the test organisms is shown in Table 2. Leaf extract of *S. leprosula* was able to inhibit most of the bacterial test organisms with measurable zones of inhibitions (Figure 2 and Figure 3). The standard (chloramphenicol) showed an average inhibition diameter of 20.31 to 22.19 mm. The average inhibition zone is highest at concentrations of 11.25% against *S. aureus* with an average of 16.25 mm zone of inhibition while against *E. coli*, it showed strength as antibacterial at a concentration of 3.75% with an average of 16.43 mm. Based on data on Table 2, it is shows that the leaf extract of *S. leprosula* were categorized as strong antibacterial against *S. aureus* and *E. coli*. From this data, the leaf extract of *S. leprosula* has antibacterial properties

against *S. aureus* and the *E. coli.* The average value which followed by the same letter in the same line showed no significant difference in Duncan Multi Range Test (DMRT) at confidence level 95%.

The four sub fractions obtained by column chromatography of A-D were evaluated their antibacterial activity against *S. aureus* and the *E. coli.* The results are presented in Table 3. Based on Table 3, it can be argued that the anti-bacterial activity of leaf extracts fractions (fractions A, B, C, and D) against Gram-positive bacteria is higher than against Gram-negative bacteria. Fraction A has moderate category antibacterial properties against *S. aureus* bacteria.

Table 1. Result of identified compounds in fraction A of the leaf extract of the *S. leprosula*

No peak	Retention time (min)	Compounds	Area under peak (%)
1	21.082	2-Pentadecenone	6.53
2	21.931	Eicosanoic acid	9.82
3	23.625	2 (3H)-furanone	2.81
4	23.800	Tricosane	5.86
6	26.488	Hexanedioic acid	4.13
8	29.541	1,2-Benzenedicarboxylic acid	65.77

Table 2. Antibacterial activities leaf extract of the total extract *S. leprosula* at different concentrations

Microorganisms	Zone of inhibition (mm)				
	3.75% (w/v)	7.5% (w/v)	11.25% (w/v)	15% (w/v)	Chloramphenicol
S. aureus	13.12^{a}±4.493	14.75^{a}±1.554	16.25^{a}±1.670	15.56^{a}±2.321	22.19^{b}±0.898
E. coli	16.43^{a}±0.986	16.43^{a}±2.672	15.37^{a}±1.010	16.56^{a}±1.983	20.31^{b}±1.434

Note: Data represent mean ± SEM of zonal inhibition of bacteria

Table 3. Activity spectrum of fraction A-D extract of leaf *S. leprosula* against gram-positive and gram-negative bacteria

Indicator organisms	Zone of inhibition (mm)				
	Fraction A	Fraction B	Fraction C	Fraction D	Chloramphenicol
Gram-positive bacteria					
S. aureus	6.725	0.000	5.562	3.850	18.500
Gram-negative bacteria					
E. coli	2.162	0.000	0.816	0.000	19.750

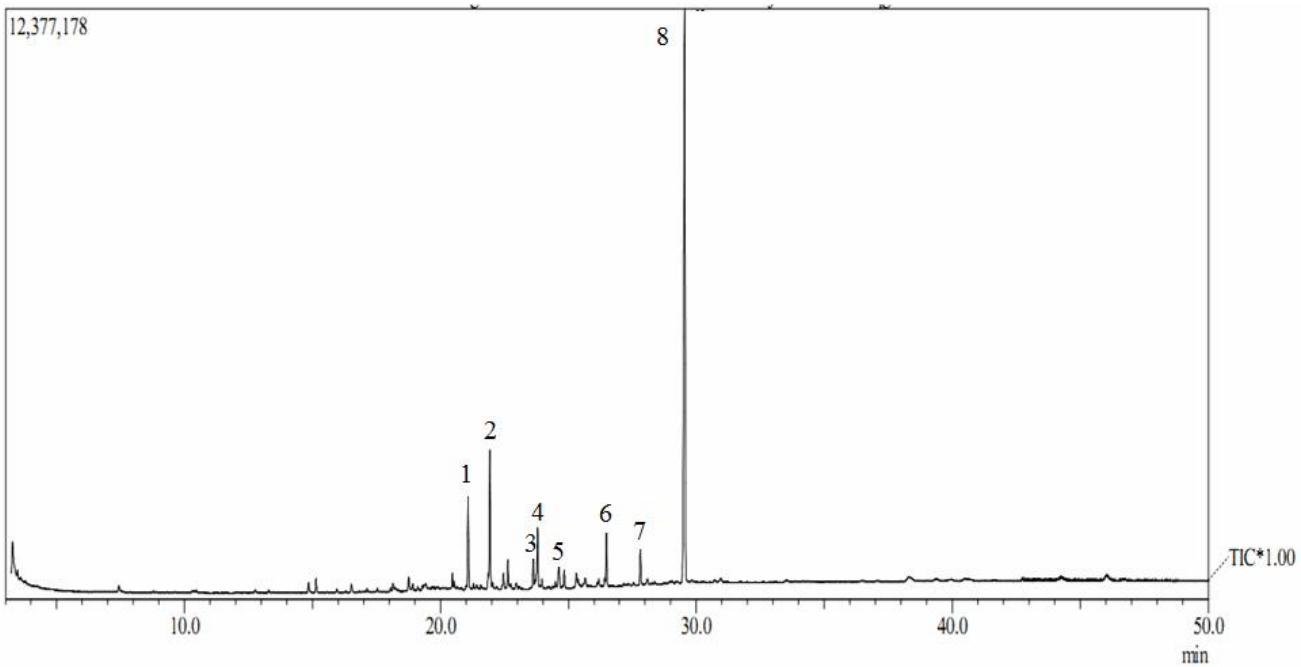

Figure 1. Chromatogram of compounds composition in fraction A of the leaf extract of the *S. leprosula*

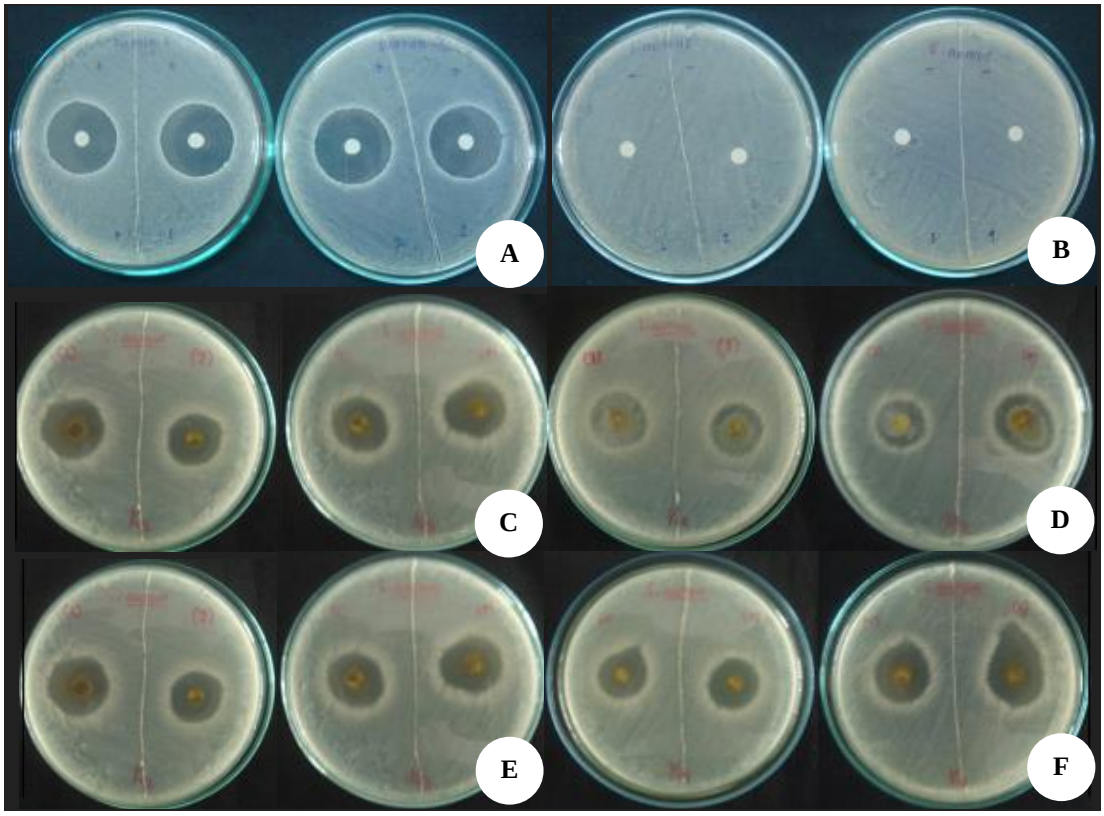

Figure 2. Zone of inhibition treatment extract leaves *S. leprosula* against the bacterium *S. aureus* at concentration: A. K + (chloramphenicol), B. 0% (sterile distilled water), C. 3.75% D. 7.5%, E. 11.25%, and F. 15%

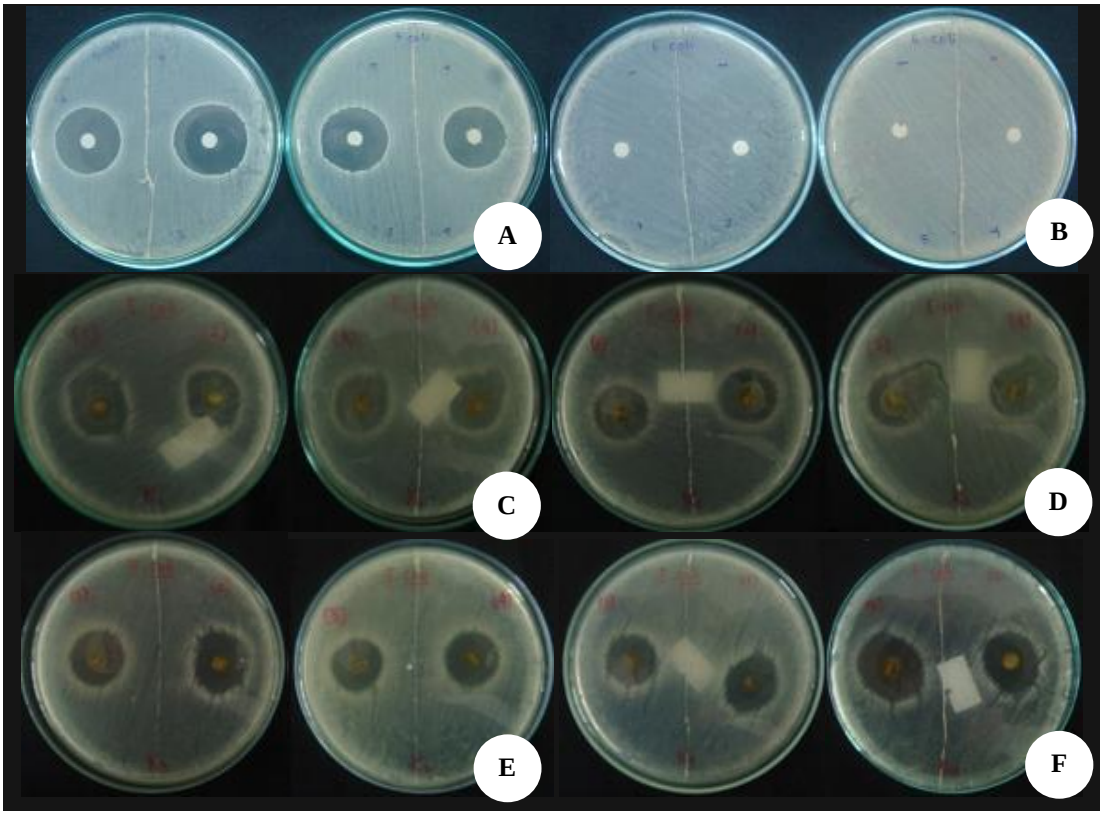

Figure 3. Zone of inhibition treatment extract of leaves *S. leprosula* against the bacteria *E. coli* at a concentration of A. K + (chloramphenicol), B. 0% (sterile distilled water), C. 3.75%, D. 7.5%, E. 11.25%, and F. 15%

Discussion

Discovery of antibacterial from plant extracts has been recently emphasized as a potential method for developing traditional healing. Traditional healers have long used plants to prevent or cure infectious conditions. The development of antibacterial resistance to available antibiotics has informed the need to explore natural disease control options which has led to further investigation of antimicrobial activity of local medicinal plants. Studies have been carried out to discover useful antibacterial of *S. leprosula*. This extract showed an inhibition of the growth of *S. aureus* bacteria and *E. coli* with varying effects.

Based on Tables 2 and 3, it can be seen that the bacterial activity of plant extracts of red meranti determined by the origin of parts of plants, the concentration, fraction and the type of bacteria tested. Of the two types of bacteria tested, it appears that *S. aureus* bacteria (Gram-positive) are more susceptible (sensitive) compared to *E. coli* bacteria (Gram-negative). *E. coli* bacteria are more resistant to treatment plant extract compared to *S. aureus* bacteria. Antibiotics are the main drug against microbial infection. However, the genetic variability of microbial pathogens that can develop resistance to the antibiotic properties requires a continuous effort to develop it into drug. Based on this, the development of material resources of antibiotic from plant material strongly supports the provision of new drugs that are environmentally friendly. The red meranti shows one of the prospective plants as a source of anti-bacterial material. The results informed that the ethanol of extracted leaf at a concentration of 3.75% showed strong antibacterial activity against bacteria in the category of *S. aureus* and *E. coli.* It may be concluded that leaf of *S. leprosula* have a stronger and broader spectrum of antibacterial activity against a number of pathogenic microorganisms. This result is supported by Murthy et al. (2011), who reported that methanolic extracts from oleoresin of *Shorea robusta* found to be most effective against the bacterial organisms tested. Norizan et al. (2012) reported that extract methanol of *Shorea resinosa* displayed moderate inhibition against Gram-negative *E. coli*, Gram-positive *S. aureus* and *S. pyogenes*. Mulyono et al. (2013) also reported that flesh dammar (*S. leprosula*) had antibacterial activity but not cat eye dammar (*S. leprosula*). The obtained extract could inhibit *C. violaceum, Streptococcus* sp., *S. aureus, S. epidermidis,* and *B. cereus.* Their MICs were 32 ppm for *C. violaceum* and 256 ppm for other bacteria tested. Zuraida et al. (2011) also reported that α-viniferin, resveratrol trimer from *Dipterocarpus verrucosus* gave moderate activity towards antibacterial (*E. coli, Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus, Salmonella paratyphi* and *Pseudomonas aeruginosa*). A study conducted by Wibowo et al. (2013) in Selangor Malaysia showed that oligostilbenoid derivatives from the stem bark of *Dryobalanops lanceolata* exhibited promising antibacterial property as they inhibited the cell growth of three Gram-positive strains, *Staphylococcus epidermidis, S. aureus* and *S. xylosus* with MIC 25/75, 50/100 and 25/75 µM, respectively.

Plants are rich in a wide variety of secondary metabolites, such as terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Muhammad et al. (2012) reported that the acetone extract of the stem bark of *Shorea acuminata* contained a new resveratrol dimer, acuminatol. Extract of *S. leprosula* which was tested for antibacterial activity was found to have a broad spectrum effect on growth of common pathogenic bacterial, *S. aureus* and *E. coli.* Our study indicates that the extract of *S. leprosula* leaf can be an alternative of new antibacterial for cases of infectious diseases in an isolated area. Having potent as an antibacterial, the results shows that this extract is more effective on Gram-positive bacteria (*S. aureus*). Antibacterial activity of extracts is allegedly associated with the presence of the active compound which allegedly can interfere the metabolism of bacteria so that bacteria growth is inhibited or it died, and also because of the secondary metabolites of the compounds contained in the leaf that have potential as an antibacterial.

Result of this study shows that the leaves of *S. leprosula* possessed potential to be developed as natural antibacterial agents against pathogenic bacteria. GC/MS analysis indicates that methanol fraction was dominated by 2-benzenedicarboxylic acid (65.77%), eicosanoic acid (9.82%), 2-pentadecenone (6.53%), tricosane (5.86%) and hexanedioic acid (4.13%) in leaves. This result supports the facts of the use of this plant in traditional medicine for treatment of different diseases. The antibacterial bioassay tests of the total extracts ethanolic of *S. leprosula* shows no significant different activity on both *S. aureus,* and *E. coli*. The plant can be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made via simple processes of maceration or infusion. Thus, it should be explored further for pharmaceutical uses as this is particularly important in supporting the recent observation in inhibiting the emergency of drug resistant bacteria at inland areas.

ACKNOWLEDGEMENTS

The author would like thank to the research supported by Indonesian Directorate General of Higher Education (DIKTI) has funded this research and we are grateful to our students Jamransyah, Aulia, Sadani Noor, and Singkir for help and contributions during field work and in the laboratory.

REFERENCES

Atun S. 2006. Oligoresveratrol activity from stem bark of the *Hopea mengarawan* (Dipterocarpaceae) as hydroxylradical scavenger. Jurnal Hayati 13 (2): 65-68.

Hakim EH. 2002. Oligostilbenoid from the Dipterocarpaceae trees. Bul Indon Soc Nat Prod Chem 2 (1):1-19.

Manjang YY, Abdi D, Djaswir D, Edison M. 2015. Steroids from N-Hexane fraction of the stem bark of *Shorea singkawang* Mig and anticancer activity as tested with murin leukemia P-388 Cells. Res J Pharm Biol Cem Sci 6 (2): 1315-1320.

Muhammad M, Din LB, Sahidin I, HashimSF, Ibrahim N, Zakaria Z, Yaacob WA. 2012. Acuminatol and other antioxidative resveratrol oligomers from the stem bark of *Shorea acuminata*. Molecules 17 (8): 9043-9055.

Mulyono M, Lay BW, Wahyuningjati M. 2013. Exploration of flesh Dammar (*Shorea leprosula*) and Cat Eye Dammar (*Shorea Javanica*) as antibacterial agent. J Pharmacy 3 (1): 1-3

Murthy KSR, Lakshmi N, Ramulu DR. 2011. Biological activity and phytochemical screening of the oleoresin of *Shorea robusta* Gaertn. J Trop Subtrop Agroecosyst 14 (3): 787-791.

Nainwal P, Bath R, Nanda D, Saini P. 2013. Aqueous extract of leaves of *Shorea robusta*. Int J Pharmacol Screening Meth 3 (2): 43-45

Newman MF, Burgess PF, Whitemore TC. 1999. Key of identification to Dipterocarpaceae trees from Sumatra Island. Prosea, Bogor.

Norizan N, Ahmad N, Mohamad SAS, Nasri NAAM, Ramli SSA, Kasim SNM, Zain WZWM. 2012. Total phenolic content, total flavonoid content, antioxidant ant antibacterial activities of Malaysian *Shorea*. Res J Med Plant 6 (7): 489-499.

Rosyidah KL, Juliawati D, Syah YM, Hakim EH, Achmad SA, Makmur L, Bin Din L, Latip J. 2007. Thimer resveratrol from the stem bark of *Shorea parvifolia* Dyer. Sains dan Terapan Kimia 1 (1): 47-52. [Indonesian]

Sahidin S,Hakim EH, Syah YM, Juliawaty LD, Achmad SA, Lajis N. 2007. Oligomer resveratrol from the stem bark of *Shorea assamica* Dyer (Dipterocarpaceae) and its citotoxicity. Jurnal Matematika dan Sains 12 (3):115-118. [Indonesian]

Seo EK, Chai, H, Constant HL, Santisuk T, Reutrakul V, Beecher CWW, Farnsworth NR, Cordell GA, Pezzuto JM, Kinghorn AD. 1999. Resveratrol tetramers from Vatica diospyroides. J Org Chem 64: 6976-6983.

Sotheeswaran S, Pasuparthy V. 1993. Distribution of resveratrol oligomers in plants. Phytochemistry 32: 1083-1092.

Subramanian P, Raj V. 2013. Antioxidant activity of the stem bark of *Shorea roxburghii* and its silver reducing power. Springer Plus 2:28

Sultanbawa MUS, Surendrakumar S, Bladon P. 1980. New antibacterial polyphenol copalliferol A from *Vateria copallifera* (Retz) Alston (Dipterocarpaceae). J Chem Soc Chem Commun 13: 619-620.

Wibowo A, Ahmad N, Hamzah AS, Low ALM. 2013. Antibacterial Activity of Oligostilbenoid Derivatives from the Stem Bark of *Dryobalanops lanceolata.* Open Conf Proc J 4:116

Zuraida WM, Ahmad N, Norizan NH, Nasri NAAM. 2011. The evaluation of antioxidant, antibacterial and structural identification activity of trimer resveratrol from Malaysia's Dipterocarpaceae. Austr J Basic Appl Sci 5 (5): 926-929.