P-ISSN: 2305-6622; E-ISSN: 2306-3599
International Journal of Agriculture and Biosciences
www.ijagbio.com; editor@ijagbio.com

Research Article

Antimicrobial Activity of Quercetin Rich Methanol Fraction of *Manilkarazapota* Bark

Swira Ekalina[1*], Purwantiningsih Sugita[1*], and Irma Herawati Suparto[1,2]

[1]Department of Chemistry, [2]Primate Research Center, Bogor Agricultural University, Bogor 16680
***Corresponding author:** atiek_ps@yahoo.com

Article History: Received: February 23, 2017 Revised: May 26, 2017 Accepted: May 28, 2017

ABSTRACT

Manilkarazapota bark have been reported to have antimicrobial activities. However, the specific active compound other than triterpenoid that contribute to the antimicrobial activity is not known yet. Therefore, this study was aimed to identify antimicrobial active compound in the selected fraction of the selected solvent extract of *Manilkarazapota* bark. *Manilkarazapota* bark was macerated with water, methanol, ethanol and acetone. Furthermore, the yield of each extract was calculated and evaluated for antimicrobial activities employing disc diffusion and micro-dilution method. Extract of the selected solvent fractionated gradually with n-hexane, ethyl acetate, and dichloromethane, then its tannins were removed. The fraction that showed the best antimicrobial activities proceed to the stage of isolation using preparative thin layer chromatography. Lastly, the result of isolation was characterized using Fourier Transform Infrared Spectroscopy and Liquid Chromatography with Mass Spectrometer. There was a significant difference between the ability of antimicrobial activities for water, methanol, ethanol and acetone extract of *Manilkarazapota* bark. Based on the amount of yield and antimicrobial test results, methanol was the selected solvent to extract the *Manilkarazapota* bark of the four solvents. The fractionation of the methanol extract showed an increase of antimicrobial activity. Methanol tannins free fraction at concentration of 0.05 mg/mL inhibit *Bacillus cereus, Staphylococcus aureus* and *Shigellaflexneri*. This fraction at concentration of 0.2 mg / mL was able toeliminat *B. cereus* and *S. aureus*. Based on the results of the analysis and characterization, the most important antimicrobial compounds from the *Manilkarazapota* bark was 3',4',5,7-tetramethylquercetin.

Key words: Antimicrobial, Bark, *Manilkarazapota*, Quercetin

INTRODUCTION

Microorganisme can grow anywhere in a quite short time. Infections caused by microorganisms such as bacteria are the main cause of contagious and deadly disease. New infections with bacterial resistance to many antibiotics were emerging (Kong and Ryu 2015). This problem has become the focus of serious action by the World Health Organization to find ways to solve it including exploring new treatment (WHO) in 2016.

Manilkarazapota (sapodilla) is a plant rich in biological activities, one of them as antimicrobials. The extract of roots, flowers, leaves and bark of *Manilkarazapota* were proven to be active antimicrobials. *Manilkarazapota*has been proven to be very effective antimicrobials for various species of gram-positive and negative bacteria (Bhargavi *et al*., 2013; Islam *et al*., 2013; Priya *et al*., 2014) including fungi (Abu-Osman *et al*. 2011; Kaneria and Chanda 2012). However, the previous studies were only limited to crude extract.

Based on the research of ethyl acetate extract which was reported by Abu-Osman *et al*. (2011) and ethanol extract by Islam *et al*. (2013), it was known that the extract of the bark of *Manilkarazapota* had antimicrobial activities better than the leaves. In addition, it was known that ethanol was better than ethyl acetate. The concentration of 30 mg / mL of ethyl acetate extract from leaves of *Manilkarazapota* showed no antimicrobial activity. *Manilkarazapota* bark extract at concentration of 30 mg / mL obtained the inhibition zone 8-9 mm. The concentration of 60 mg / mL of the extract obtained the inhibition zone of up to 13 mm (Abu-Osman *et al*., 2011). The concentration of 40 mg / mL ethanol extract of leaves of *Manilkarazapota* showed an inhibition zone up to 9 mm, while the bark extract showed an inhibition zone of up to 13.5 mm (Islam *et al*. 2013). Studies reported that the steps of leaf extraction used a solvent with a graded polarities, petroleum ether, toluene, ethyl acetate, acetone, and water (Kaneria and Chanda 2012). They reported in the ethyl acetate extract all microbial had no response (5

Cite This Article as: Ekalina S, Suparto IH and Sugita P, 2017. Antimicrobial activity of quercetin rich methanol fraction of *Manilkarazapota* bark. Inter J Agri Biosci, 6(2): 114-119. www.ijagbio.com

types of gram-positive bacteria, gram-negative bacteria and fungi). Toluene extract was active on only one species and petroleum ether inhibits three species of microbes. The use of acetone and water gave better antimicrobial effects when compared to other solvents. The root extraction used water as solvent agent (Bhargavi *et al.*, 2013). Good antimicrobial results were also found in the flower extraction using methanol (Priya *et al.*, 2014). Nevertheless, among ethanol, water, acetone and methanol it was still unknown which is the best solvent to extract the stem bark of *Manilkarazapota* as the antimicrobial.

A total of 12 compounds of triterpenoids had been isolated from the bark of *Manilkarazapota*. The compound consisted of seven compounds of taraksastana triterpenoids and 5 lupana triterpenoid compounds. A total of two lupana triterpenoids compounds were new pentacyclic triterpenoids compound (3-acetyltaraxer-14-en-12-one and 3-hydroxy-7-oxolup-20(29)-en-28-oic acid). The antimicrobial activities of these compounds had been tested by Toze *et al*. (2015). Microbial samples used by Toze *et al*. (2015) were a gram positive and negative bacteria, fungi and plant pathogenic oomycetes. The results showed no antimicrobial activities in some of the compounds. In addition, the pure compounds which had antimicrobial activities, the value of the minimum inhibitory concentration (MIC) obtained Toze *et al*. (2015) was higher than 1 mg / mL. At the same genus, namely *Manilkarasubsericea*, the extracts of leaves, bark and fruit has been proven to be capable of inhibiting *Staphylococcus aureus.* The results of the analysis showed that ester triterpenoid compounds were antimicrobial active compounds of *Manilkarasubsericea* (Fernandes *et al*. 2013). Nevertheless, no other studies has reported on the compounds of *Manilkarazapota* as active antimicrobial. Therefore, this study aimed to characterize the active antimicrobial compound on the selected fraction of the selected solvent agent of *Manilkarazapota* bark.

MATERIALS AND METHODS

Samples preparation

The bark of *Manilkarazapota* was obtained from the village of East Pemenang, district Pemenang, North Lombok regency, West Nusa Tenggara. The researcher used old, scaly, brown, dried up bark with a thickness of 0.35 to 1.25 cm. The bark was cleaned and dried using dry air oven at a temperature of 37-40 °C. The water content was determined by AOAC (2005).

Extraction and fractionation

The dried powder of *Manilkarazapota* bark was macerated using each acetone, ethanol, methanol and water. Sample-solvent ratio was 1: 3. This process was carried out in room temperature for 36 hours (Hossain *et al*. 2012) for two times. The crude extract was calculated by using antimicrobial and phytochemicals assays (Harborn, 1987). Methods for antimicrobial assay was disc diffusion and micro-dilution methods then further analyzed statistically. This analysis, used one-way analysis of variance (ANOVA) method, with the significance of 1% and 5%. Micro-dilution method was used for determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against the bacteria samples.

The fractionation was performed by dissolving the selected solvent agent (methanol) using a solvent with a graded polarity (Harborn, 1987). Solvents used were n-hexane, ethyl acetate and then dichloromethane (DCM). Then the fraction of n-hexane (n-Hex), the fraction of ethyl acetate (EtOAc), the fraction of DCM and methanol fraction were obtained. The methanol fraction was insoluble methanol extract in n-hexane, ethyl acetate and DCM.

Fractionating was followed by the phase of separating the tannins from the methanol fraction. This phase was carried out by dissolving the methanol fraction with methanol, then added with 50 mL of acetone. Precipitation of tannins (tannins fraction) were separated by the filter paper. The addition of acetone was repeated until no tannin precipitates (Harborn, 1987). Tannin-free filtrate was concentrated and tannin-free methanol fraction was obtained.

On the fraction n-Hex, EtOAc, DCM, methanol, and tannin-free methanol fraction the antimicrobial test was applied. Then, the selected fractions were conducted for compound characterization analysis.

Test of antimicrobial activity

The disc diffusion method (Parhusip *et al*., 2008)

Sterile discs containing the sample (crude extract various solvents, fractions and kanamycin 25 mg/mL) was placed on a nutrient agar containing the bacteria that have been tested previously. Bacteria samples used was *Bacillus cereus* (ATCC: 10876), *Staphylococcus aureus* (ATCC: 6538) and *Shigellaflexneri* (ATCC: 12022). Furthermore, they were incubated at 37°C for 18-24 hours for microbial growth. Then the diameter of inhibition zone around the disc was observed. All assays were done in duplicate.

Micro-Dilution Method (Batubara *et al*., 2009)

Each sample (crude extract various solvents and fractions) was given the dilution in dimethyl sulfoxide into various concentrations (50-4000 ppm). On each well was added nutrient broth (NB) 100 mL, 100 mL bacteria (with 0.6-0.8 McFarland turbidity) and 20 mL sample. Incubated at 37°C for 24 hours. The minimum concentration that remained clear is the MIC. In the sample determined as MIC, then further added 100 mL of NB. Incubated at 37°C for 24 hours. If in the minimum concentration samples remained clear, it is determined as the MBC.

Characterization

The characterization of antimicrobial compounds from the *Manilkarazapota* bark was performed by using the instrumentation of liquid chromatography mass spectrometry (LC-MS). The detector used was photodiede-array detector connected to a quadrupole detector. The source of ionization was electrospray ionization (ESI) operated using positive ionization mode (+1) at a temperature of 150^{o}C. Column separation used Water AccQ. Ultra Tag (2.1 mm × 100 mm, 1.7 m) with temperature at 55°C. The mobile phase (A) used the mixture of aquabidest, formic acid and acetonitrile (B).

The injection volume in 1 mL at a flow rate of 0.7 mL/min. Analyses were performed using Waters Mass Lynx software and Quan Lynx also supported with Massbank and Chems draw for compound determination.

RESULTS

The water content, yield and phytochemicals of *Manilkarazapota* bark extract with Various Solvents

The water content of dried samples was of 1:46%±0:41. Based on the yield of the extract (Figure 1) it can be seen that the ability of the solvent to extract the compound from the *Manilkarazapota* bark arranged in following order: methanol> ethanol> water> acetone.

Phytochemical test results showed that methanol, ethanol and acetone could extract the class of flavonoids, alkaloids, saponins, tannins, steroids and terpenoids. It did not apply to the water solvent. The phytochemical test results of water extracts showed the negative result to terpenoids and steroids.

The antimicrobial activities of Extract of *Manilkarazapota* Bark with Various Solvents

Statistical analysis of antimicrobial activity in disc diffusion showed that there was a significant difference in the antimicrobial activities between water, methanol, ethanol and acetone extract of the *Manilkarazapota* bark. Based on Table 1 it could be seen that antimicrobial activity of methanol extract> ethanol> acetone> water against *B. cereus* and *S. aureus*. It was also shown that the entire extract to a concentration of 40.0 mg/mL could not inhibit the growth of *S. flexneri*.

The Antimicrobial activity of methanol extract fractions of *Manilkarazapota* bark

Fractionating was performed by dissolving the methanol extract using solvent n-hexane, ethyl acetate and then dichloromethane (DCM) gradually. From the process of fractionation, obtained of n-hexane fraction (n-Hex), the fraction of ethyl acetate (EtOAc), the fraction of DCM and methanol fraction. Fractionating followed by the separation phase of tannins with the methanol fraction. Then, obtained tannin and tannin-free (TF) methanol fraction. The fractionation of 10 g of methanol extract generated 2.59 g n-Hex fraction (25.9%), 2.18 g EtOAc fraction (21.8%), 0:14 g DCM fraction (1.4%), 5:09 g methanol fraction (50.9%), 4.63 g tannins (46.3%), and 0.56 g BT methanol fraction (5.6%). Based on Table 2, TF methanol fraction showed the highest antimicrobial activity among the entire fractions.

Characterization of active antimicrobial compound from Tannin-Free Methanol Fraction

Based on the results of LC-MS analysis of TF methanol fraction (Figure 2), showing a lot of the peaks until the retention time (Rt) 35 min. Peak with the highest intensity (nearly 100%) on Rt 16.83 minutes was the focus of the study. Peak at Rt 0.92, 3.43, 11.8, 14.99, 18.33, 21.68 and 30.72 minutes intensity of up to 11% and the peak at 5.27 and the Rt 15.99 minutes intensity close to 20%. The fragmentation results of mass spectrum peaked at Rt 16.83 minutes had an 81% similarity with 3',4',5,7-tetramethylquercetin on the database ID TY000124. The compound had the molecular formula $C_{19}H_{18}O_7$ and molecular mass (m/z) was equal to

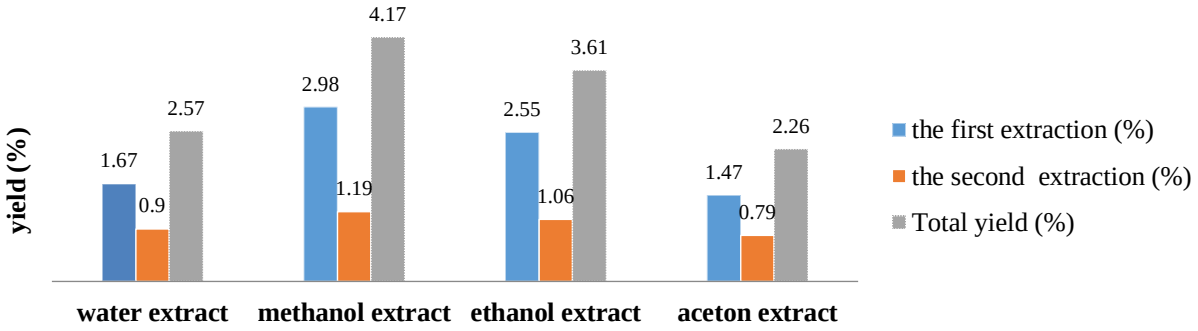

Fig. 1: The yield of the first extraction, the second and the total yield from the *Manilkarazapota* bark with water, methanol, ethanol and acetone as the solvent agents.

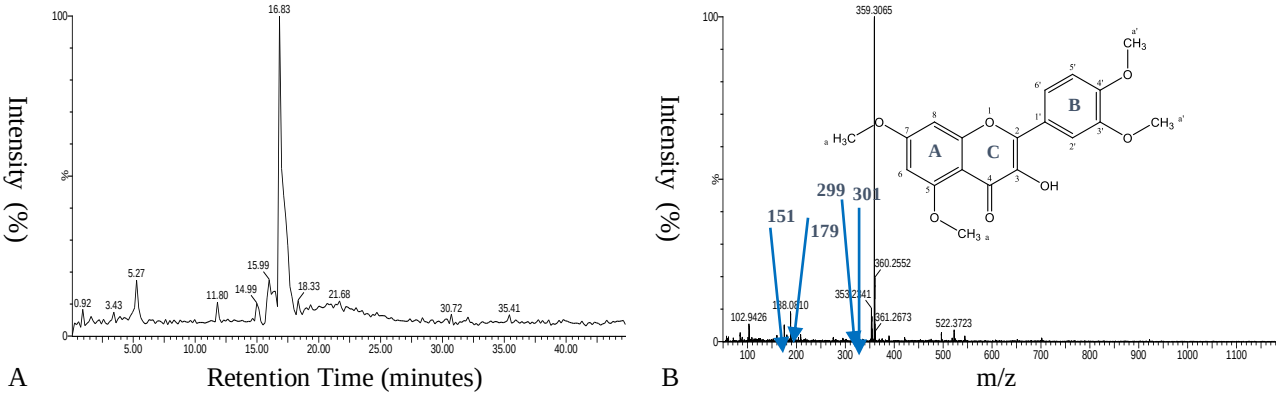

Fig. 2: The chromatogram of TF methanol fraction (A) and the peak of mass sprectum compound on minute 16.83 in chromatogram of TF methanol fraction (B).

Table 1: Antimicrobial activity of extract of *Manilkarazapota* bark with water, methanol, ethanol and acetone solvent by disc diffusion method

Bacteria tester	Various solvent extract	The average diameter of inhibition zone (mm) on concentration (mg/mL)						
		2.5	5.0	7.5	10.0	12.5	40.0	kanamycin (25.0)
B. cereus	water	-	-	-	-	-	8.5	31
	methanol	6	6.75	7.75	8	8.25	13.05	
	ethanol	5.5	6	6.5	8.2	8.25	12.25	
	acetone	-	-	5.5	6	6.5	12.25	
S. aureus	water	-	-	-	-	-	8.5	20
	methanol	-	7.5	8	8	8.5	10	
	ethanol	-	5.5	7	7.75	8.25	9	
	acetone	-	-	6	6	6	9	
S. flexneri	water	-	-	-	-	-	-	30
	methanol	-	-	-	-	-	-	
	ethanol	-	-	-	-	-	-	
	acetone	-	-	-	-	-	-	

Note: undetected, kanamycin (25.0) = positive control (kanamycin) on concentration 25.0 mg/ml

Table 2: The antimicrobial Activities of the methanol extract fractions from the *Manilkarazapota* bark by disc diffusion method and micro-dilution method

Bacteria tester	Fractions	Disc-difusion method		micro-dillusion method	
		The average of inhibition zone (mm) on the concentration (mg/mL)		Antimicrobial activity (mg/mL)	
		kanamycin (25.0)	Fractions (40.0)	KHM	KBM
B. cereus	n-Hex	31	-	-	-
	EtOAc		-	-	-
	DCM		-	-	-
	Methanol		11	0.05	1.0
	Tannin		12	0.05	1.0
	TF Methanol		14.5	0.05	0.2
S. aureus	n-Hex	20	-	-	-
	EtOAc		-	-	-
	DCM		-	-	-
	Methanol		10	0.05	2.0
	Tannin		11	0.05	1.0
	TF Methanol		13.5	0.05	0.2
S. flexneri	n-Hex	30	-	-	-
	EtOAc		-	-	-
	DCM		-	-	-
	Methanol		11	0.05	3.0
	Tannin		11	0.05	3.0
	TF Methanol		12	0.05	2.0

Note: - = undetected, kanamycin (25.0) = positive control (kanamycin) on concentration 25.0 mg/mL, Fractions (40.0) = each of the methanol extract fractions on concentration 40.0 mg/mL.

358.3065. Alleged fragmentation compound 3',4',5,7-tetramethylquercetin formed fragment m / z 301, 299, 179 and 151 shown in Figure 3.

DISCUSSION

The water content of dried samples was 1.46% ± 0.41. This value indicated that the sample is resistant to microbes and can be stored within 1 to 3 years at room temperature (Herawati, 2008), make it worth as a raw material of herbal medicines. Based on the yield of the extract (Figure 1) it could be seen that the ability of the solvent to extract the compound from the *Manilkarazapota* bark arranged in following order: methanol> ethanol> water> acetone. Moreover, methanol was the selected solvent in extracting the *Manilkarazapota* bark. Extraction with methanol produced the highest yield amount (4.17%) compared to the three other solvents. This was because methanol is a polar organic solvent, capable of dissolving almost all the components of polar, semipolar or nonpolar (Al-Ashary *et al*., 2010).

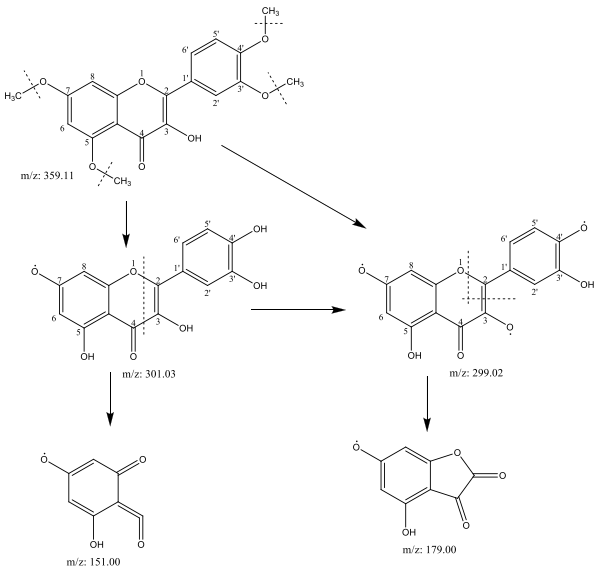

Fig. 3: Alleged fragmentation compound 3',4',5,7-tetramethylquercetin formed fragment m/z 301, 299, 179 and 151.

Based on the of phytochemical test results known that methanol, ethanol and acetone could extract the class of flavonoids, alkaloids, saponins, tannins, steroids and terpenoids. It did not apply to the water solvent. The phytochemical test results of water extracts was adversely negative to terpenoids and steroids. Islam *et al*. (2013) have done phytochemical screening of ethanol extract from the *Manilkarazapota* bark. The bark positively contained alkaloids, flavonoids, saponins and tannins. *Manilkarazapota* bark samples were taken from Rajshahi, Bangladesh. The phytochemical screening of methanol extracts of *Manilkarazapota* bark samples from Khulna, Bangladesh, positively contained contain tannins, saponins and flavonoids. The test result was negative for alkaloids and steroids (Hossain *et al*., 2012). Differences in the content of secondary metabolites were caused by differences where the plants grow.

Screening for antimicrobial activity has been conducted with aagar medium (Abd-Allah *et al*., 2015). The inhibition zone was large due to the minimum concentrations of antimicrobial samples demonstrated the effectiveness of the sample against a microorganisme. The test result of antimicrobial water extract from the *Manilkarazapota* bark against bacteria test results indicate the lowest compared to other extracts. Antimicrobial effect of water extract was merely shown at a concentration of 40.0 mg/mL against *Bacillus cereus* and *Staphylococcus aureus*. Acetone extract provided antimicrobial effect is better than the water extract (Table 1). This was possible because the water had a low ability to extract the antimicrobial compound found in the *Manilkarazapota* bark, while acetone was better in this case.

Methanol was the selected solvent in extracting the antimicrobial compounds from the bark of *Manilkarazapota*. Besides, methanol was able to produce the highest yield compared to other solvent extract (Figure 1), the extract also provided the highest antimicrobial effect at any concentration used (Table 1). *Shigellaflexneri* gram-negative bacteria were more resistant than gram-positive bacteria. This condition is due to the plasma membrane in gram-negative bacteria are protected by peptidoglycan layer and outer membrane consists of lipoproteins, phospholipids and lipopolysaccharide (Poeloengan and Praptiwi 2010).

The research sample from the village of East Pemenang, district Pemenang, North Lombok regency, West Nusa Tenggara showed the different antimicrobial activity with samples from other places. The research sample Islam *et al*. (2013) obtained from Rajshahi, Bangladesh. The ethanol extract of *Manilkarazapota* bark at a concentration of 40 mg/mL showed different results. It obtained the inhibition zone on *B. cereus* by 9 mm, *S. aureus* by 9 mm and *S. flexneri* by 13 mm. Differences in results were possible due to different compounds and concentrations of antimicrobial active compounds in the study sample. In addition, it was likely due to differences of bacterial sources.

Table 2 was based on the n-Hex, EtOAc or DCM fractions showed no antimicrobial activity. The tannin-free (TF) methanol fraction demonstrated the highest results of antimicrobial activity compared to all factions, including the methanol fraction and tannins. Antimicrobial compounds in the TF methanol fraction worked better if separated from the tannins. The unification of tannins and antimicrobial compounds in the TF methanol fraction caused the compound did not work optimally in the methanol fraction, which could decrease antimicrobial activity. Tannin separation was important because tannins could bind chemical compounds through hydrogen bonding, so it could disrupt the process of separation of the compound. Presence of tannins cause a thin layer chromatography stain to be trailing thereby disrupting the separation of the chemical components (Rahmawati, 2013).

The antimicrobial activity of TF methanol fraction was higher than ethyl acetate fraction (EAF) of ethanol extract of *Manilkarazapota* leaves on research Rahman *et al*. (2015). At a concentration of 30 mg/mL EAF produced 9-10 mm zone of inhibition against microbial testing. Microbial samples used Rahman *et al*. (2015) was positive and gram negative bacteria and fungi. *S. aureus, B. cereus*and some bacteria from genus *Shigella* among microbial testing. In addition, the antimicrobial activity of TF methanol fraction was better than some pure triterpenoid compounds which were tested with antimicrobial by Toze *et al*. (2015). The test used bacteria such as *Bacillus subtilis*, *S. aureus*, and *Escherichia coli*. *Escherichia coli* is a bacterium that is one family with *Shigella.* In pure active antimicrobial compound, MIC obtained Toze *et al*. (2015) greater than 1 mg / mL. The MIC and MBC TF methanol fraction lower (Table 2) compared to the MIC value obtained Toze *et al*. (2015).

The peak of mass spectrum fragmentation (Figure 2 (B)) on Rt 16.83 minutes were m/z 361.3, 360.3, 359.3, 353.2, 301, 299, 188.0, 179.0, 151 and 102.9. Each mass spectrum was the molecular mass plus one. Fragment with m/z 301, 299, 179 and 151 had in common with some of the fragmentation pattern of the compound quercetin which had been reported by Falcao *et al*. (2013) and Chen *et al*. (2015).

Quercetin and quercetin-3-O-neohesperidoside had been reported to have fragments of m/z 179 and 151. The fragment m/z 179 was formed as a result of the termination of H or CH_3 on the ring A ring followed by cleavage 1,2 B ring. The fragment m/z 151 was formed due to the termination of H or CH_3 on the ring A ring followed by cleavage 1,3 B ring (Chen *et al*. 2015). The spectrum of m/z 361.3 and 360.3 deduced as isotopes of $[C_{19}H_{18}O_7]\cdot^+$. Radical cation $C_{19}H_{18}O_7$ m/z 359.3 were the mass of the original molecule was found as the highest peak in the mass spectrum. Fragmentation patterns of compounds 3',4',5,7-tetramethylquercetin with m/z 301, 299, 179 and 151 have in common with some of the compound quercetin fragmentation patterns that have been reported are shown in Figure 3

Flavonoids and quercetin has been widely reported as the antimicrobial agents. Species of bacteria in the mouth has been proven sensitive to quercetin. This sensitivity associated with low MIC and MBC compound quercetin against bacteria (Shu *et al*., 2011). Quercetin showed antifungal and antibacterial properties were very strong (Abd-Allah *et al.* 2015). The compound 3- (Quercetin-8-yl) -2,3-epoxyflavanone has proven the capability of inhibiting the growth of gram positive and negative bacteria (Ramos *et al*. 2006).

Conclusion

The results of compound characterization by LC-MS in tannin-free methanol fraction from selected solvent extract (methanol) of *Manilkarazapota* bark had mass spectroscopy chromatogram with an intensity of close to 100% on Rt 16.83 minutes. Compound was suspected has 3',4',5,7-tetramethylquercetin as the important antimicrobial compound from the *Manilkarazapota* bark.

REFERENCES

Abd-Allah WA, Awad HMand Abdel-Mohsen MM, 2015. HPLC Analysis of Quercetin and Antimicrobial Activity of Comparative Metanol Extracts of *Shinusmolle* L. Int J Curr Microbiol App Sci, 4: 550-558.

Abu-Osman M, Abdul-Aziz M, Rowshanul-Habib M and Rezaul-Karim M, 2011. Antimicrobial investigation on *Manilkarazapota* (L) Proyen. IJDDR, 3: 185-190.

Al-Ashary MN, Supriyanti FMT and Zackiyah, 2010. Penentuanpelarutterbaikdalammengekstraksisenyawa bioaktifdarikulitbatang *Artocarpusheterophyllus*. J Sains Teknol Kim, 1:150-158.

[AOAC] Association of Official Analytical Chemists, 2005. Official Methods of Analysis of AOAC International. AOAC, Maryland.

Batubara I, Mitsunaga T and Ohasi H, 2009. Screening antiacne potency of Indonesian medicinal plants; antibacterial, lipase inhibition, and antioxidant activities. J Wood Sci, 55: 230-235.

Bhargavi S, Kanakaiah B, Sowmya DK, Ravi B and Nama S, 2013. An evaluation of the antibacterial activity of root extracts of *Manilkarazapota* against *Staphylococcus aureus* and *Escherichia coli.* IJP, 4:171-173.

Chen Y, Yu H, Wu H, Pan Y, Wang K, Jin Y and Zhang C, 2015. Characterization and Quantification by LC-MS/MS of the Chemical Components of the Heating Products of the Flavonoids Extract in Pollen Typhae for Transformation Rule Exploration. Molecules, 20: 18352-18366.

Falcão SI, Vale N, Gomes P, Domingues MRM, Freire C, Cardoso SM and Vilas-Boas M, 2013. Phenolic Profiling of Portuguese Propolis by LC–MS Spectrometry: Uncommon Propolis Rich in Flavonoid Glycosides. Phytochem Anal, 24: 309-18.

Fernandes CP, Corrêa AL, Lobo JFR, Caramel OP, Almeida BF, Castro ES, Souza KFCS, Burth P, Amorim LMF, Santos MG, Ferreira JLP, Falcão DQ, Carvalho JCT and Rocha L, 2013 Triterpene Esters and Biological Activities from Edible Fruits of *Manilkarasubsericea* (Mart.) Dubard, Sapotaceae. BioMed, 2013: 1-7.

Harborn JB. 1987. MetodeFitokimia: Penuntun Cara Modern Menganalisa Tumbuhan. Padmawinata K, Soediro I, translater. ITB, Indonesia.

Herawati H, 2008. Penentuan Umur Simpanpada Produk Pangan. J Lit Pert, 27: 124-130.

Hossain MH, Jahan F, Islam MS, Howlader, Kanti-Dey S, Hira A, Ahmed A and Sarkar RP, 2012. Evaluation of Anti-inflammatory Activity and Total Flavonoids Content of *Manilkarazapota* (Linn) Bark. IJPPR, 2: 35-39.

Islam MR, Parvin MS, Banu MR, Jahan N, Das N and Islam ME, 2013. Antibacterial and phytochemical screening of ethanol extracts of *Manilkarazapota* leaves and bark. IJPS, 3: 394-397.

Kaneria M and Chanda S, 2012. Evaluation of antioxidant and antimicrobial properties of *Manilkarazapota* L (Chiku) leaves by sequential soxhlet extraction method. APJTB, S1526-S1533.

Kong M and Ryu S, 2015. Bacteriophage PBC1 and Its Endolysin as an Antimicrobial Agent against *Bacillus cereus.* AEM, 81: 2274-2283.

Parhusip AJN, Handayani R and Vilona, 2008. Kajianaktivitasantimikrobekstrakkulitbuahmanggis (*Garcinia mangostana L.*) sebagaipengaw *et al*amipada mi basah. JITP, 6: 25-43.

Poeloengan M and Praptiwi, 2010. Antibacterial activity test of mangosteen (*Garcinia mangostana* Linn) peel. Med Lit Kes, 2: 65-69.

Priya P, Shoba FG, Parimala M and Sathya J, 2014. Antioxidant and antibacterial properties of *Manilkarazapota* (L) royen flower. IJPCR, 6: 174-178.

Rahman SMA and Ganguly A, 2015. Evaluation of The Cytotoxic, Antimicrobial, Antioxidant, Anthelmintic and CNS Depressant Activities of *Manilkarazapota* Leaf (Sapotaceae). WJPR, 4: 272-283**.**

Rahmawati S, 2013. Metil*p*-Hidroksibenzoatdari Fraksi Nonpolar Ekstrak Metanol Daun Ketepeng (*Cassia alata*). Institute Pertanianbogor, Indonesia.

Ramos FA, Takaishi Y, Shirotori M, Kawaguchi Y, Tsuchiya K, Shibata H, Higuti T, Tadokoro T and Takeuchi M, 2006. Antibacterial and Antioxidant Activities of Quercetin Oxidation Products from Yellow Onion (*Allium cepa*) Skin. J Agric Food Chem, 54: 3551-3557.

Shu Y, Liu Y, Li L, Feng J, Lou B, Zhou X and Wu H, 2011. Antibacterial activity of quercetin on oral infectious Pathogens. AJMR, 5:5358-5361.

Toze FAA, Fomani M, Nouga AB, Chouna JR, Kouam, Waffo AFK and Wansi JD, 2015. Taraxastane and Lupane Triterpenoids from the Bark of *Manilkarazapota.* IRJPAC,7: 157-164.

World Health Organization. 2016. Antibiotic resistance. Downloadedfromhttp://www.who.int/mediacentre/ factsheets/antibiotic-resistance/en/at 11-01-2017.