Effectiveness of Leaves Extract Fractions of *Archidendron jiringa* (Jack) I.C Nielsen Against Microbes

"*"Oom Komala[1], Sri Wardatun[2] and Lia Puspita Sari[3]

[1)]Department of Biology, [2)&3)]Department of Farmacy,
Faculty of Mathematics and Natural Sciences, Pakuan University, Bogor, Indonesia
Jalan Pakuan P.O Box 452 Telp/Fax. (0251) 8375547 Bogor
"* Email: oom.komala@unpak.ac.id.

Abstract

Archidendron jiringa (Jack) I.C Nielsen) contains a lot of essential oils, saponins, alkaloids, terpenoids, steroids, tannins, glycosides, and flavonoids. Flavonoid is a secondary metabolite compound in a plant as antimicrobial. This study aims to determine the antimicrobial activity of fractions of ethanol 96% , n-hexan , ethyl acetate , and ethanol-water leaves extract. *A. jiringa* leaves extract were tested against *Streptococus mutans*, *Pseudomonas aeruginosa*, and *Candida albicans*. The research using agar disc diffusion technique for antimicrobial activity and colorimetric method to know the total of flavonoid. Chloramphenicol and ketoconazole were used as a reference standard. The result of this study shows fractions of ethanol 96% , n-hexane , ethyl acetate , and ethanol-water *A. jiringa* leaves extract can inhibit microbial growth of *S. mutans*, *P. aeruginosa*, and *C. albicans*. Total of flavonoids on the extract respectively are 1,13%, 0,494%, 2,337%, and 0,549%. The higher percent of flavonoids would cause greater inhibitory zone bacteria but only 0.090 for pearson correlation value. Conclusions ethyl acetate leaves extract most effective inhibit microbial growth from the other fractions.

Keywords: Antimicrobial, *Archidendron jiringa*, , Extract Fractions

Abstrak

Archidendron jiringa (Jack) I.C Nielsen) mengandung senyawa minyak atsiri, saponin, alkaloid, terpenoid, steroid, tanin, glikosida, dan flavonoid. Flavonoid adalah senyawa metabolit sekunder dalam tanaman sebagai antimikroba. Penelitian ini bertujuan untuk mengetahui aktivitas antimikroba dari fraksi etanol 96%, n-heksan, etil asetat, dan ekstrak daun etanol-air. Ekstrak daun *A. jiringa* diuji terhadap *Streptococus mutans*, *Pseudomonas aeruginosa*, dan *Candida albicans*. Penelitian ini menggunakan metode difusi cakram untuk aktivitas antimikroba dan metode kolorimetri untuk mengetahui persentase kadar

flavonoid. Kloramfenikol dan ketokonazol digunakan sebagai standar aktivitas anti mikroba. Hasil penelitian ini menunjukkan fraksi etanol 96%, n-heksana, etil asetat, dan ekstrak etanol-air daun *A. jiringa* dapat menghambat pertumbuhan mikroba *S. mutans*, *P. aeruginosa*, dan *C. albicans*. Total flavonoid pada ekstrak masing-masing adalah 1,13%, 0,494%, 2.337%, dan 0,549%. Persentase flavonoid yang lebih tinggi akan menyebabkan bakteri zona hambat yang lebih besar tetapi hanya 0,090 untuk nilai korelasi pearson. Kesimpulan fraksi ekstrak daun etil asetat paling efektif menghambat pertumbuhan mikroba dari fraksi lainnya.
Kata kunci: Antimikroba, *Archidendron jiringa*, Fraksi Ekstrak

Introduction

The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives (Ventola, 2015). Herbal medicine has been used especially in developing countries for many years. It is used for the traditional treatment of health problems. *A. jiringa* (Jack) I.C Nielsen is native to tropic countries of Southeast Asia; such as Malaysia, Bangladesh, Myanmar, South Thailand and parts of Indonesia. This plants contain substances protein, calcium, phosphorus, djenkolic acid (Bunawan, Dusik, Bunawan, and Amin, 2013), vitamins A and B, carbohydrates, essential oils, saponins, alkolids, terpenoids, steroids, tannins, and glycosides. Purified *A.Jiringa* seed lectin have inhibitory effect against growth of plant-pathogenic bacteria and fungi (Charungchitrak, Petsom, Sangvanich, and Karnchanatat, 2011). Flavonoids are secondary metabolites in plants as antimicrobial. Flavonoids can be used as antimicrobials because they can form complex compounds with extracellular and dissolved proteins that can damage microbial cell membranes. Xie, Yang, Tang, Chen, and Ren (2015) explain antibacterial mechanism of flavonoid are mainly as follows : nucleic acid synthesis inhibition, alteration in cytoplasmic membrane function, energy metabolism inhibition, reduction in cell attachment and biofilm formation, inhibition of the porin on the cell membrane, changing of the membrane permeability, and attenuation of the pathogenicity. Flavonoids are large class of natural compounds, flavonoids showed up to sixfold stronger antibacterial activities than standard drugs in the market (Farhadi, Khamemeh, Iranshahi, and Milad, 2019). Biofilm formation of *Candida albicans* can inhibitory and disruption by flavonoids were extracted from *Moringa oleifera* seed coat (Onsare & Arora, 2015). This study aims to determine

antimicrobial activity of fractions extract of *A. jiringa* leaves, and determine total flavonoid. Anze, Lamsing, Ugwoke, and Ezugwu (2017) reported fractionation make antimicrobial activity as well as a brood spectrum activity.

Materials and Methods

1. Making Dry Extract

*A. jiring*a leaves were collected from Lampung, South Sumatra. Extract is made with cold maceration method. *A. jiringa* leaves powder 400 g is extracted by maceration. Solvent ethanol 96% 4000 mL (1:10) and leaves powder for maceration is protected from light at room temperature while repeatedly stirred (every 6 hours). Filtrat is filtered. The extracts added with enough fluid and stirred and then filtered again. The extract is stored in a place protected from light for 2 days, the precipitate wicth formed separated and then filtered. The filtrate was evaporated with evaporator vacuum so get dry extract (Anonim, 2013).

2. Fractionation of Ethanol Extract of *A. jiringa* Leaves

The fractionation is done by Fractionation Liquid-Liquid (FLL) method with n-hexane, ethyl acetate and ethanol solvent continuously with different solvent polarity properties. Fractionation is done (Yanti, Irnawati, Vivian, and Wulandari, 2015) as follows:

a. Ethanol 96% extract dissolved in water with a ratio of 1: 1 as much as 200 mL.
b. Subsequently incorporated into the separated flask, 200 ml of n-hexane was added, mixed, until separation between the n-hexane fraction and ethanol 96%.
c. The n-hexane fraction is separated, then repeated several times until clear colored.
d. The fractionation is continued with ethyl acetate in the same process as n-hexane.
e. Fractions of liquid n-hexane, liquid ethylacetate and ethanol-water evaporated on rotary evaporator for obtain a fraction extract.
f. All fractions obtained were tested for antibacterial activity.

3. Phytochemical Test of *A. jiringa* Leaves Extract

Phytochemical test were conducted qualitatively on *A. jiringa* leaves extract to determine the presence of flavonoids, saponins, tannins, and alkaloids in extracts that may act as antimicrobials**.** Flavonoid total was determined by complementary colorimetry to each fractions of *A. jiringa* leaves extract by alumunium chloride method.

4. Antimicrobial Test

4.1 Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration by using agar dilution technique against microorganism. The concentration of extract were made 50mg/mL, 100mg/mL, 200mg/mL, 400mg/mL. Sterile media cooled and included in each petri dish 20 mL. Each petri dish was inserted 1 mL of extract concentration, 0.2 mL of microbial suspension then homogenized and allowed to harden (Mazzola, Jozala, Novaes, Moriel, and Penna, 2009). Incubated for 24 hours at 37ºC, and observed the existence of colony growth or not. The lowest concentration of antimicrobials that do not occur microbial growth in petri dishes is the Minimum Inhibitory Concentration (MIC).

4.2 Antimicrobial effectiveness test

Testing the effectiveness of *A. jiringa* leaves extract by using agar disc diffusion technique. In this method are seen the clear zones around the paper disc. A total of 0.2 mL bacterial inoculum of 10^6 and mushrooms inoculum of 10^4 were added into petri containing 20 mL of nutrient agar medium then homogenized and allowed solid. Paper disc containing *A. jiringa* leaves extract, chloramphenicol 10 ppm and ketoconazole 50 ppm as a reference standard were placed on agar medium. Petri cup was sealed and incubated in at 37ºC. it was observed and measured the diameter of the inhibitory area using a ruler after 24 hours incubation, so inhibitory zone of the *A. jiringa* leaves extract was known (Balouiri, Sadiki, and Ibnsouda, 2016).

5. Analyzed Method

Data of Inhibitory zone was analyzed by using experimental method of Randomized Complete Design (RAL) of factorial pattern using SPSS 17 with 4 treatment (3 treatment) with various concentration of *A. jiringa* leaves extract, 1 treatment chloramphenicol 10 ppm or ketoconazole 50 ppm were used as a reference standard, is repeated 2 times. The analysis was continued by Duncan test to compare the antimicrobial power between each treatment.

Results and Discussion

As many as 400 grams of *A. jiringa* leaves powder dissolved in 4 liters of ethanol 96% solvent. Dry extract obtained as much as 100.1 g. The etanol 96% extract is fractionated

stepwise with the aim of simplifying the extracted secondary metabolite compound. Fractionation is done based on the nature of polarity. The fractionation process is used with 3 different solvents, n-hexane, ethyl acetate, and ethanol-water. The use of solvents during the fractionation process is intended to get secondary metabolite compounds such as oil to completely separate or dissolve the fat contained in the extract. The fractionation is continued by using an ethyl acetate solvent to attract all semi-polar secondary metabolite compounds. The final fractionation using a ethanol-water solvent is intended to attract polar compounds. The three fractions obtained were evaporated to get dry extract .

1. Phytochemical test

The results of phytochemical tests showed that *A. jiringa* leaves contains saponins, flavonoids, tannins and alkaloids compounds. These simplicia results are consistent with the results of phytochemical screening of *A. jiringa* leaves by Hussin, Osman, Harun, and Daud (2018). n-hexane extract are not have flavonoids, tannins and saponins. Phytochemical test results can be seen in Table 1.

Table 1. Phytochemical results of *A. jiringa* Leaves Extract

Identification of Compounds	Sample				
	Simplicia	ethanol 96% extract	n-hexane extract	ethyl acetate extract	ethanol-water extract
flavonoids	+	+	-	+	+
alkaloids	+	+	+	+	+
tannins	+	+	-	+	+
saponins	+	+	-	+	+

Note: + = There are compounds

2. Flavonoids Total

Flavonoids content is in ethanol 96% extract is 1,13%, in n-hexane fractions extract is 0,494%, in ethyl acetate extract is 2,337%, and ethanol-water extract is 0,549%. Flavonoid flavones and flavonols are more easily soluble in semipolar solvents so that the total flavonoid content in the ethyl acetate extract is greater. Farhadi, Khamemeh, Iranshahi, and Milad (2019) argued total flavonoid affect the strongest of antibacterial activity. The greater total flavonoid content be effect the higher the antibacterial activity.

Table 2. Percentage of Total Flavonoid of *A. jiringa* Leaves Extract

Extract	Total Flavonoid (%)
Ethanol 96%	1,13
n-hexane fractions	0,494
Ethyl acetate fractions	2,337
Ethanol-water fractions	0,549

3. Minimum Inhibitory Concentrations

The results of Minimum Inhibitory Concentrations (MIC) leaves extract of *A. jiringa* can be seen in Table 3. Extract of n-hexane fractions did not could inhibit to *P. aeruginosa* , *S. mutans* and *C. albicans* fungus because it is not contain flavonoids, tannins and saponins. Yanti et al. (2015) state that minimum inhibitory concentrations (MIC) of fractions is different for each microbe. Leaves, pods and seeds of *P. jiringa* were extracted using methanol showed the antimicrobial and antifungal activities against the test organisms. Minimum inhibitory consentration in previous research showed that the leaves extract of *A. jiringa* was most active against *S. aureus*, *S. epidermidis* and *Microsporum gypseum* (100 mg/mL) (Bakar, Ahmad, and Sulaiman, 2012).

Table 3. Minimum Inhibitory Concentrations

Microbial	Extract	MIC (%)	MIC mg/mL
P. aeruginosa	Ethanol 96%	10	100
	n-hexane fractions	-	-
	Ethyl acetate fractions	5	50
	Ethanol-water fractions	5	50
S.mutans	Ethanol 96%	20	200
	n-hexane fractions	-	-
	Ethyl acetate fractions	20	200
	Ethanol-water fractions	5	50
C. albicans	Ethanol 96%	5	50
	n-hexane fractions	-	-
	Ethyl acetate fractions	40	400
	Ethanol-water fractions	5	50

Note : - not inhibit

4. Diameter of Inhibition zone

Concentration of 50mg/mL, 100mg/mL, 200mg/mL fractions of *A. jiringa* leaves extract and Chloramphenicol/Ketoconazole have effect significant on microbial growth. Fractions of *A. jiringa* leaves extract have different effects in inhibiting microbial growth against *P. aeruginosa, S. mutans and C. albicans* (Table 4 & Figure 1). As for the interaction between the concentration and microbes obtained that ethyl acetate extract concentration of 50mg/mL, 100mg/mL, and 200mg/mL have different effect from control (+) in inhibiting microbial growth. Interaction between concentration of extract and inhibitory zone of microbes in this research show that the concentration of 50mg/mL, 100mg/mL, and 200mg/mL ethanol 96%, ethyl acetate and etanol-water extract did not give the same effect with reference standard for inhibit of microbial growth. *A. jiringa* leaves extracts have antibacterial and antifungal activity against the tested organisms in this research i.e. *P. aeruginosa*, *S. mutans* and *C. albicans*. On this research fraksions of *A.*

jiringa leaves extract which showed the strongest barriers is fraksions of ethyl acetate against *S. mutans*. This is because flavonoids compounds contained in the extract. Hussin et al. (2018) state that tannins, flavonoids, terpenoids and saponin were the main metabolit found in phytochemical screenings from *P. jiringa* stem bark. Effect of *A. jiringa* pericarp extract (inhibition zone = 13,35 ±0,45 mm) showed the highest growth inhibitory against *E.coli* and *S. aureus*. Ramli (2013) state that extract of *A. jiringa* pericarp were contributed tentatively from flavonoids glycoside and proanthocyanidins.

Table 4. Results of Diameter of Inhibition zone *A. jiringa* leaves extract (mm) against *P. aeruginosa*, *S. mutans* and *C. albicans*

Extract	Microbial	Concentration			Chloram phenicol/ Ketoconazole (reference standard)
		50mg/mL	100mg/mL	200mg/mL	
Ethanol 96%	*P.aeruginosa*	7 ± 0.02^{f}	8 ± 0.02^{e}	9.5 ± 0.02^{cd}	30 ± 0.02^{a}
	S. mutans	8 ± 0.01^{e}	9 ± 0.01^{d}	10 ± 0.02^{c}	30 ± 0.02^{a}
	C. albicans	7.5 ± 0.02^{ef}	9 ± 0.02^{d}	11 ± 0.01^{b}	30 ± 0.03^{a}
Ethyl acetate	*P.aeruginosa*	8 ± 0.02^{g}	9 ± 0.03^{f}	10 ± 0.03^{e}	32 ± 0.02^{a}
	S. mutans	9 ± 0.02^{f}	10 ± 0.02^{e}	12 ± 0.02^{d}	31.5 ± 0.02^{b}
	C. albicans	8 ± 0.01^{g}	9 ± 0.02^{f}	12 ± 0.02^{d}	30 ± 0.03^{a}
Ethanol-water	*P.aeruginosa*	7 ± 0.02^{e}	8 ± 0.03^{de}	9 ± 0.01^{de}	27.5 ± 0.02^{b}
	S. mutans	7.5 ± 0.01^{de}	8.5 ± 0.02^{de}	10 ± 0.02^{d}	30 ± 0.02^{a}
	C. albicans	7 ± 0.01^{e}	9 ± 0.02^{de}	10 ± 0.02^{d}	25 ± 0.02^{c}

Note : Reference standard : Chloramphenicol for *P. aeruginosa* and *S. mutans*, Ketoconazole for *C. albicans*

Zore, Thakre, Jadhad, and Karuppayil (2011) state that terpenoids tested (linalool, benzyl benzoate, eugenol, citral, linalyl acetate and citronellal) exhibited excellent activity against *C. albicans* yeast and hyphal form growth at the concentrations that are non toxic to HeLa cells at ≤0,064% ($^{v}/_{v}$). There were not correlation between the total flavonoid with the size of the inhibitory diameter in this research using pearson correlation method. The value of significance (sig (2-tailed) was 0.454 ($p > 0.05$). Retrieved a correlation value of 0.090. Based on these results that antimicrobial activity is only 9% influenced by the content of flavonoids while the remaining 91% is the contribution of other compounds that also have the potential as an antimicrobial. Norulaini, Zaidul, Azizi, Zhari, Noranin, Sahena, and Omar (2011) state that results of analysis chromatography time of flight mass spectrometry djenkolic acid has been found in *A. jiringa* bean. The volatile oil *A. jiringa* seeds using supercritical carbon dioxide with fast gas chromatography time of flight mass spectrometry revealed 55 metabolites. Pods of *A. jiringa* contain active phenolic compound as methyl gallate that has high antioxidant activity (Lubis, Marpaung, Siburian, and Nasution, 2018). The metabolites identified were generally found to be fatty

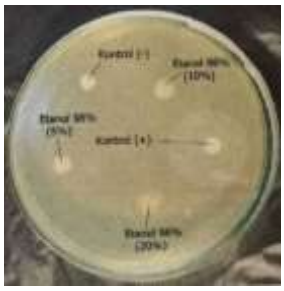
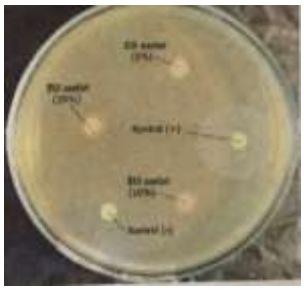
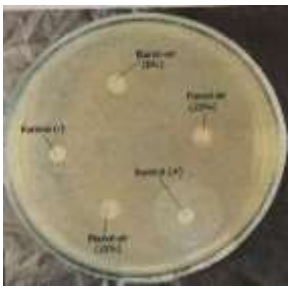
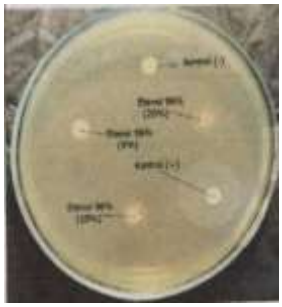
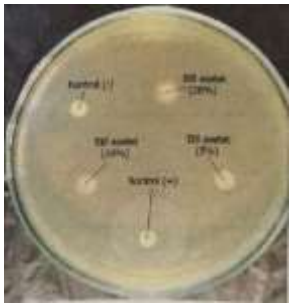
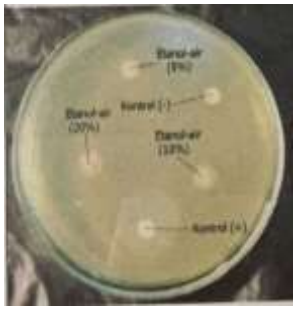
Microbial	Etanol 96%	Etil Asetat	Etanol – Air
S. mutans			
P.aeruginosa			
C. albicans			

Figure 1. Inhibitory Zone of *A. jiringa* (Jack) I.C Nielsen Leaves extract based on difference of fractions Against microbe

acids, terpenoids, ally sulphur, vitamin E and alkaloids. In the disc paper diffusion method, extracts of *A. jiringa* pericarp make inhibitory zone = 13,35 ± 0,45 mm showed the highest growth inhibitory againt *S. aureus*. Extracts of *A. jiringa* pericarp showed the highest inhibitory in the in vitro to tyrosinase enzyme inhibitory property with L-Tyrosine as substrates. Hasan (2013) state that the biological activities of *A. jiringa* pericarp ethanolic extracts was contributed by the tentatively characterized flavonoids glycoside and proantocyanidins from the extracts. Pods examination of *A. jiringa* afforded tree proantocyanidins known as procyanidinds B-3 and B-4 and prodelphinidin B-1, as well as

flavan 3-ols. Proanthocyanidins have a good inhibitory effect against some isolate except high concentrations 500mg/mL to *E. coli* and *Salmonella typhimurium* was more inhibitory activity. Results in research, extract of *A. jiringa* leaves ethanol 96%, ethyl acetate fractions and ethanol-water fractions can inhibit microbes. The greater the content of flavonoids in extracts, the greater the antimicrobial activity, but there are not correlation using pearson correlation method.

Conclusion

1. Extract of *A. jiringa* leaves fractions can inhibit the growth of microbes *S. mutans*, *P. aeruginosa*, and *C. albicans*.
2. Fractions of ethyl acetate leaves extract most effective from the other fractions.

Acknowledgements

Thank to the Pharmacy Laboratory, Faculty of Mathematics and Natural Sciences, Pakuan University and Bogor Agricultural University.

References

Anze, S.P.G., Lamsing, N., Ugwoke, C.E.C., & Ezugwu,C.O. (2017). Antimicrobial Activity of Methanol Extracts and Fractions of the Leaf and Stem Bark of Vitex Doniana Sweet (Lamiaceae). *International Journal of Physical and Human Geography, 5*(1),14-21.

Anonim. (2013). *Farmakope Herbal Indonesia* (First ed.) (pp106-107). Dep. Of Health RI, Indonesia: Jakarta.

Balouiri,M., Sadiki,M., & Ibnsouda, S.K. (2016). Methods in vitro evaluating antimicrobial activity : A review. *Journal of Pharmaceutical Analysis, 6*(2),71-79.

Barceloux, D.G. (2009). Djenkol Bean [*Archidendron jiringa* (Jack) I.C Nielsen]. *Disease-a-Mouth, 55*(6), 361-364.

Bakar RA, Ahmad, I., & Sulaiman, S.F. (2012). Effect of *Pithecellobium jiringa* as antimicrobial agen. *Bangladesh Journal of Pharmacology, 7*(2), 131-134.

Bunawan, H., Dusik, L., Bunawan. S.N., & Amin, M.N. (2013). Botany, Traditional Uses, Phytochemistry and Pharmacology of Archidendron jiringa: A Review. *Global J. Pharmacol, 7* (4), 474-478.

Charungchitrak, S., Petsom, A., Sangvanich,P., & Karnchanatat, A. (2011). Antifungal and antibacterial activities of lectin from the seeds of *Archidendron jiringa* Nielsen. *Food Chemistry*, *126*(3),1025-1032.

Farhadi, F., Khamemeh, B., Iranshahi, M., & Milad I. (2019). Antibacterial activity of flavonoids and their structure-activity relationship : An update review. *Phytotherapy Research*, (33), 13-40. Doi : 10.1002/ptr.6208

Hasan, A.M. (2013). In-Vitro Antibacterial Activity Of Proanthocyanidins Against some of Pathogenic Bacterial Isolates. *Al-Mustansiriyah J.Sci*., *24*(1),53-60

Hussin, Z.M., Osman, N.A., Harun, A., & Daud S. (2018). Phytochemical and Antimicrobial Evaluation of *Pithecellobium jiringa* Stem Bark Extracts. *Malaysian Journal of Analytical Sciences*, *22*(1), 123-127. DOI: https://doi.org/10.17576/mjas-2018-2201-15

Lubis, M.Y., Marpaung, L., Siburian, R., & Nasution, M.P. (2018). Methyl Gallate from *Jiringa* (*Archidendron jiringa*) and Antioxidant Activity. *Asian Journal of Pharmaceutical and Clinical Research, 11*(1), 346.

Mazzola, P.G., Jozala, A.F., Novaes, L.C.L., Moriel, P. & Penna, T.C.V. (2009). Minimal inhibitory concentration (MIC) determination of disinfectant and/or sterilizing agents. *BJPS*, *45*(2), 241-248. Retrieved from www.scielo.br/pdf/bjps/v45n2/v45n2a08.pdf

Norulaini, N.A.N., Zaidul, I.S.M., Azizi, C.Y.M., Zhari, I., Noranin, M.N., Sahena, F., & Omar, A.K.M. (2011). Supercritical Carbon dioxide fractionation of *Pithecellobium jiringa* Jack seed compositions using fast gas chomatography time of flight mass spectrometry. *Journal of food Process Engineering*, *34*(5), 1746-1758.

Onsare, J.G., & Arora, D.S. (2015). Antibiofilm potential of flavonoids extracted from *Moringa oleifera* seed coat against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. *J Appl Microbiol*, *118*(2), 313-25.

Ramli, S.(2013). Bioactivity evaluations and phytochemical characterizations of ethanolic extracts from selected mimosaceous plants endemic to Thailand. *Global Journal of Pharmacology*, *7* (4), 474-478.

Ventola, C.L. (2015). The Antibiotic Resistance Crisis. Part 1 : Causes and Threats. *PT*, *40*(4), 277-283.

Xie ,Y, Yang W, Tang, Chen X & Ren I. (2015). Antibacterial activities of flavonoids: structure – activity relationship and mechanism. *Current Medicinal Chemistry*, *22*(1), 132-149.

Yanti, Irnawati, F., Vivian, M., & Wulandari, Y.R.E. (2015). Extraction yield and antioxidant activity of biomolecule and bioactive fractions from seed and peel parts of *Pithecellobium jiringa*. *Sch.Acad J.Biosci*., *3*(9), 790-795. Retrieved from https://www.researchgate.net/publication/327136893_Extraction_yield_and_antioxidant_activity_of_biomolecule_and_bioactive_fractions_from_seed_and_peel_parts_of_Pithecellobium_jiringa

Zore, G.B., Thakre, A.D., Jadhad, S., & Karuppayil, S.M. (2011). Terpenoids inhibit *Candida albicans* growth by affecting membrane integrity and arrest of Cell Cycle. *Phytomedicine*, *18*(13), 1181-90.