

[JM] Article Review Request

From: Amin Fatoni (j.molekul@gmail.com)

To: haryunsri@yahoo.com

Date: Sunday, August 4, 2019 at 05:39 AM GMT+7

Dr. Hary Widjajanti:

I believe that you would serve as an excellent reviewer of the manuscript, "Effectiveness of Leaves Extract Fractions of Archidendron jiringa (Jack) I.C Nielsen Against Microbes," which has been submitted to Molekul. The submission's abstract is inserted below, and I hope that you will consider undertaking this important task for us.

Please log into the journal web site by 2019-08-10 to indicate whether you will undertake the review or not, as well as to access the submission and to record your review and recommendation.

The review itself is due 2019-08-23.

Submission URL:

<https://ojs.jmolekul.com/ojs/index.php/jm/reviewer/submission/96?key=34yc4fDB>

Thank you for considering this request.

Amin Fatoni
Molekul

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

{AbstractTermIfEnabled}

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 Streptococcus

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

Editor in Chief of Journal Molekul

<http://ojs.jmolekul.com/ojs/index.php/jm>

Re: [JM] Article Review Request

From: Hary Widjajanti (haryunsri@yahoo.com)

To: j.molekul@gmail.com

Date: Friday, August 23, 2019 at 11:02 PM GMT+7

Editor in Chief of Journal Molekul,

I hereby submit the result of the article review 538-1339-1-RV

Hary Widjajanti

On Sunday, August 4, 2019, 05:39:56 AM GMT+7, Amin Fatoni <j.molekul@gmail.com> wrote:

Dr. Hary Widjajanti:

I believe that you would serve as an excellent reviewer of the manuscript, "Effectiveness of Leaves Extract Fractions of Archidendron jiringa (Jack) I.C Nielsen Against Microbes," which has been submitted to Molekul. The submission's abstract is inserted below, and I hope that you will consider undertaking this important task for us.

Please log into the journal web site by 2019-08-10 to indicate whether you will undertake the review or not, as well as to access the submission and to record your review and recommendation.

The review itself is due 2019-08-23.

Submission URL:

<https://ojs.jmolekul.com/ojs/index.php/jm/reviewer/submission/96?key=34yc4fDB>

Thank you for considering this request.

Amin Fatoni
Molekul

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

{AbstractTermIfEnabled}

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 Streptococcus 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

Editor in Chief of Journal Molekul

<http://ojs.jmolekul.com/ojs/index.php/jm>



538-1339-1-RV.docx

651.4kB

Re: [JM] Article Review Request

From: Jurnal Molekul (j.molekul@gmail.com)

To: haryunsri@yahoo.com

Date: Tuesday, August 27, 2019 at 09:42 AM GMT+7

Dear **Dr. Hary Widjajanti**,

Thank you for your referee's report on the previous requested article. Your very knowledgeable and constructive comments are greatly appreciated. Peer review such as this is an essential element in our efforts to attain and maintain the high academic standards that we would like our journal to become renowned for.

On behalf of the Journal of MOLEKUL, may I thank you once again for your kind assistance. I hope that we will be privileged to avail ourselves of your help again, if we may, on a future occasion.

Yours sincerely,
Amin Fatoni
Editor

On Fri, Aug 23, 2019 at 11:02 PM Hary Widjajanti <haryunsri@yahoo.com> wrote:

Editor in Chief of Journal Molekul,

I hereby submit the result of the article review 538-1339-1-RV

Hary Widjajanti

On Sunday, August 4, 2019, 05:39:56 AM GMT+7, Amin Fatoni <j.molekul@gmail.com> wrote:

Dr. Hary Widjajanti:

I believe that you would serve as an excellent reviewer of the manuscript, "Effectiveness of Leaves Extract Fractions of Archidendron jiringa (Jack) I.C Nielsen Against Microbes," which has been submitted to Molekul. The submission's abstract is inserted below, and I hope that you will consider undertaking this important task for us.

Please log into the journal web site by 2019-08-10 to indicate whether you will undertake the review or not, as well as to access the submission and to record your review and recommendation.

The review itself is due 2019-08-23.

Submission URL:

<https://ojs.jmolekul.com/ojs/index.php/jm/reviewer/submission/96?key=34yc4fDB>

Thank you for considering this request.

Amin Fatoni
Molekul

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

{AbstractTermIfEnabled}

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 Streptococcus 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

Editor in Chief of Journal Molekul

<http://ojs.jmolekul.com/ojs/index.php/jm>



Reviewer Acknowledgement - HW.pdf

190.8kB

Re: [JM] Article Review Request

From: Hary Widjajanti (haryunsri@yahoo.com)

To: j.molekul@gmail.com

Date: Sunday, September 22, 2019 at 04:45 PM GMT+7

Dear Dr.Amin Fatoni

Thank you very much for your appreciations. Please forgive me if what I have done doesn't satisfy you. I hope I can help you on future occasion.

Best regards,
Hary Widjajanti

On Tuesday, August 27, 2019, 09:42:51 AM GMT+7, Jurnal Molekul <j.molekul@gmail.com> wrote:

Dear **Dr. Hary Widjajanti**,

Thank you for your referee's report on the previous requested article. Your very knowledgeable and constructive comments are greatly appreciated. Peer review such as this is an essential element in our efforts to attain and maintain the high academic standards that we would like our journal to become renowned for.

On behalf of the Journal of MOLEKUL, may I thank you once again for your kind assistance. I hope that we will be privileged to avail ourselves of your help again, if we may, on a future occasion.

Yours sincerely,
Amin Fatoni
Editor

On Fri, Aug 23, 2019 at 11:02 PM Hary Widjajanti <haryunsri@yahoo.com> wrote:

Editor in Chief of Journal Molekul,

I hereby submit the result of the article review 538-1339-1-RV

Hary Widjajanti

On Sunday, August 4, 2019, 05:39:56 AM GMT+7, Amin Fatoni <j.molekul@gmail.com> wrote:

Dr. Hary Widjajanti:

I believe that you would serve as an excellent reviewer of the manuscript, "Effectiveness of Leaves Extract Fractions of Archidendron jiringa (Jack) I.C Nielsen Against Microbes," which has been submitted to Molekul. The submission's abstract is inserted below, and I hope that you will consider undertaking this important task for us.

Please log into the journal web site by 2019-08-10 to indicate whether you will undertake the review or not, as well as to access the submission and to record your review and recommendation.

The review itself is due 2019-08-23.

Submission URL:

<https://ojs.jmolekul.com/ojs/index.php/jm/reviewer/submission/96?key=34yc4fDB>

Thank you for considering this request.

Amin Fatoni
Molekul

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

{`$abstractTermIfEnabled`}

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 Streptococcus

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

Editor in Chief of Journal Molekul

<http://ojs.jmolekul.com/ojs/index.php/jm>

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

Oom Komala^{1*}, Sri Wardatun² and Lia Puspita Sari³

¹Department of Biology, ²&³Department of Pharmacy,
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 *Streptococcus 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 *Streptococcus mutans*, *Pseudomonas aeruginosa*, dan *Candida albicans*. Penelitian ini menggunakan metode difusi cakram untuk aktivitas antimikroba dan metode kolorimetri untuk mengetahui persentase kadar

Commented [A1]: 1 spacing

Commented [A2]: Writing number in English

Commented [A3]: bacteria inhibitory zone

Commented [A4]: 1 spasi

Commented [A5]: Apakah untuk mengetahui kadar flavonoid bukan tujuan?

Commented [A6]: Ekstrak etanol-air daun A.jiringa

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, C.L., 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, et al., 2013), vitamins A and B, carbohydrates, essential oils, saponins, alkaloids, terpenoids, steroids, tannins, and glycosides. Purified *A. Jiringa* seed lectin have inhibitory effect against growth of plant-pathogenic bacteria and fungi (Charungchittrak et al., 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 et al. (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 et al., 2019). Biofilm formation of *Candida albicans* can inhibitory and disruption by flavonoids were extracted from *Moringa oleifera* seed coat (Onsare and Arora, 2015). This study aims to determine antimicrobial activity of fractions extract of *A. jiringa* leaves, and determine total

Commented [A7]: Yang benar 2.337 atau 2,337?

Commented [A8]: zona hambat bakteri

Commented [A9]: ekstrak etil asetat daun....

Commented [A10]: !

Commented [A11]: A.jiringa

Commented [A12]: idem

Commented [A13]: inhibited and disrupted by....

flavonoid. Anze et al.,(2017) reported fractionation make antimicrobial activity as well as a brood spectrum activity.

Commented [A14]: Antimicrobial activity and flavonoid contain of extract fraction.....

Commented [A15]: idem

Commented [A16]: broad

Commented [A17]: what do you mean?

Commented [A18]: This is extraction, isn't it?

Materials and Methods

1. Making Dry Extract

A. jiringa 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).

Commented [A19]: Is this one sentence?

Commented [A20]: What do you mean fluid?

Commented [A21]: ?

Commented [A22]: To get an extract

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 et al., 2015) as follows:

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

Commented [A23]: make in a paragraph form

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 et al., 2009). Incubated for 24 hours at 37°C, 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).

Commented [A24]: ?

Commented [A25]: make true the sentence

Commented [A26]: how much the bacterial density?

Commented [A27]: the growth of bacteria and fungi

Commented [A28]: make true your sentence

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 37°C. 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 et al., 2016).

Commented [A29]: Please added Experimental Design that used in this experiment → Factorial Randomized Complete Design. Explain the treatment.

Commented [A30]: what unit? cells/mL ?

Commented [A31]: what unit?

Commented [A32]: in separate petri dishes?

Commented [A33]: what medium for *C.albicans*?

Commented [A34]: ??

Commented [A35]: Inhibitory area or inhibitory zone or clear zone?

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.

Commented [A36]: Data analysis

Commented [A37]: Analysis of Variance (ANOVA)

Commented [A38]: This is experimental design Experimental Design move to point 4.2

Commented [A39]: What do you mean?

Commented [A40]: two replication

Commented [A41]:

Commented [A42]: Data analysis using SPSS-17 Program

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 ethanol 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 an ethanol-water solvent is intended to attract polar compounds. The three fractions obtained were evaporated to get dry extract.

Commented [A43]: This is material and methods ! not result and discussion

6. Results of 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 et al. (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	Simplicia	Sample			
		ethanol 96% extract	n-hexane extract	ethyl acetate extract	ethanol-water extract
flavonoids	+	+	-	+	+
alkaloids	+	+	+	+	+
tannins	+	+	-	+	+
saponins	+	+	-	+	+

Note: + = There are compounds

Commented [A44]: The minus (-) ?

7. Results of 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 et al. (2019) argued total flavonoid affect the strongest of antibacterial activity. The greater total flavonoid content be effect the higher the antibacterial activity.

Commented [A45]: Writing number in English : 1.13 not 1,13 etc

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

Commented [A46]: idem

8. 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.

Table 3. Minimum Inhibitory Concentrations

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

Note : - not inhibit

Commented [A47]: Minimum Inhibitory Concentrations of.....

Commented [A48]: Microbes

9. 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.

Commented [A49]: Significant effect

Commented [A50]: of

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

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

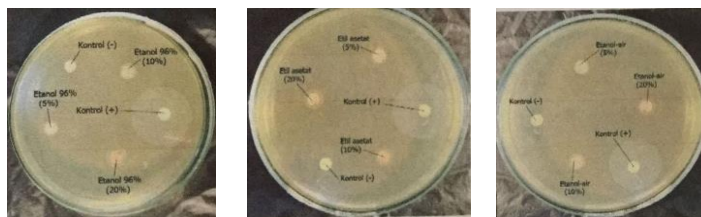
Note : Reference standard : Chloramphenicol for *P. aeruginosa* and *S. mutans*, Ketoconazole for *C. albicans*

Commented [A51]: microbes

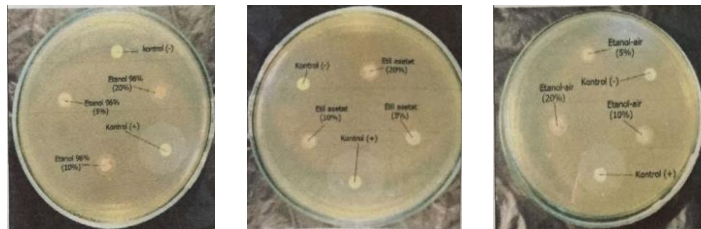
Commented [A52]: Please recheck again each notation for standard
Ex : 32 → a
31.5 → b
27.5 → b

Microbial	Etanol 96%	Etil Asetat	Etanol – Air
-----------	------------	-------------	--------------

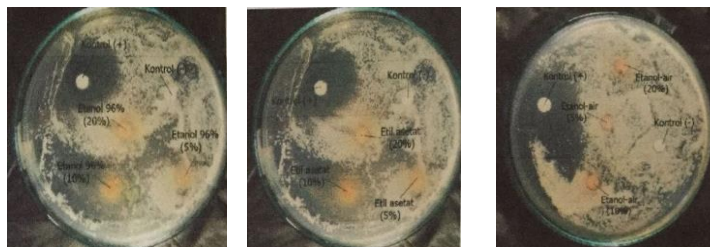
S. mutans



P.aeruginosa



C. albicans



Commented [A53]: microbes

Commented [A54]: In English!

Commented [A55]: In English !

Commented [A56]: In English!

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

Commented [A57]: Inhibition zone not so clear in figure 1

10. Discussion

A. jiringa leaves extract ethanol 96%, ethyl acetate fractions and ethanol-water fractions can inhibit microbes. 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 (Yanti et al., 2015). 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*. Minimum inhibitory concentration in previous research showed that the leaves extract of *A. jiringa* was most active against *S. aureus*, *S. epidermidis* and *Microsporium gypseum* (100 mg/mL) (Bakar et al., 2012). 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. tannins, flavonoids, terpenoids and saponin were the main metabolit found in phytochemical screenings from *P. jiringa* stem bark (Hussin et al., 2018). 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 $\leq 0,064\%$ ($\%_v$) (Zore et al., 2011). Effect of *A. jiringa* pericarp extract (inhibition zone = $13,35 \pm 0,45$ mm) showed the highest growth inhibitory against *E.coli* and *S. aureus*. Extract of *A. jiringa* pericarp were contributed tentatively from flavonoids glycoside and proanthocyanidins (Ramli et al., 2013). 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. Results of analysis chromatography time of flight mass spectrometry djenkolic acid has been found in *A. jiringa* bean. Norulaini et.al. (2011) reported on 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 et al., 2018). The metabolites identified were generally found to be fatty acids, terpenoids, ally sulphur, vitamin E and alkaloids. In the disc paper diffusion method, extracts of *A. jiringa* pericarp make inhibitory zone = $13,35 \pm 0,45$ 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. The biological activities of *A. jiringa* pericarp ethanolic extracts was

Commented [A58]: Antibacterial on *P. aeruginosa* and *S. mutans*, antifungal on *C. albicans*

Commented [A59]: After this statement you must explain the reason of this result, you must explain the mechanisms of antibacterial and antifungal

Commented [A60]: idem

Commented [A61]: ?

Commented [A62]: *A. jiringa* leaves extract fractions

Commented [A63]: What doyou mean?

Commented [A64]: idem

Commented [A65]: secondary metabolites

Commented [A66]: ?

contributed by the tentatively characterized flavonoids glycoside and proanthocyanidins from the extracts. Pods examination of *A. jiringa* afforded three proanthocyanidins known as procyanidins B-3 and B-4 and prodelfinidin 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 (Hasan, 2013). 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.

Commented [A67]: Write the conclusion completely

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. *Dep. Of Health RI*, Indonesia: Jakarta, pp106-107.
- Balouiri, M., Sadiki, M., & Ibsouda, 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, R.A., 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.

Commented [A68]: Write in correct form and see the author guidance to write references

See the alphabet of author name

- 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. 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 chromatography 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, Imawati, 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
<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., Jadhav, S., & Karuppayil, S.M. (2011). Terpenoids inhibit *Candida albicans* growth by affecting membrane integrity and arrest of Cell Cycle. *Phytomedicine*, **18**(13), 1181-90.

MOLEKUL

p-ISSN: 1907-9761 e-ISSN : 2503-0310

Accredited by RISTEKDIKTI No: 2/E/KPT/2015

Indexed in: SCOPUS, ACI, DOAJ, CAS, Sinta, Google Scholar, Crossref

Certificate of Excellent in Reviewing

Awarded to

Dr. Hary Widjajanti

in recognition of an outstanding contribution to the
quality of the journal

Purwokerto, August 2019



Amin Fatoni, Ph.D
Editor-in-Chief



Jurnal Ilmiah Kimia MOLEKUL
Department of Chemistry, Faculty of Mathematics and Natural Sciences
Universitas Jenderal Soedirman
website: www.jmolekul.com
email: j.molekul@gmail.com

