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Article

The Effect of Ethanol Solvent Concentration on Antimicrobial Activities The Extract of Andalas Endophytic Bacteria (*Morus Macroura* Miq.) Fermentation Product

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Larasati Arum Utami [1], Dwi Hilda Putri[1*]

Department of Biology, Faculty of Mathematics and Natural Science (FMIPA), Universitas Negeri Padang, Indonesia

Abstract. Anti-Biotic resistence is a health problem globally. According to the data of WHO, there is an increasing resistance of *Staphylococcus* sp. bacterium toward anti-biotic to 80% in 2013. It is able to overcome with a new anti-microbial compound which can be produced from Andalas endophytic bacteria. This compound can be obtained through fermentation process. In order to separate this active-compound, it is needed to use extraction method. In this method, the solvent is functioned as the extractor. One of the solvent which is commonly used is ethanol. This research is aimed to know the effect ethanol concentration toward antibacterial activity from extracted bacterial fermentation products of Andalas endophytic bacteria isolate JDT 1B. The fermented products are extracted by using maceration method. The concentrations are 100%, 80%, 70%. A test of anti-microbial activity is used disk diffusion method. The extracted concentrations tested for each solvent are 50%, 25%, 12,5%, and 6.25%. Anti-bacterial activity is analyzed by using factorial design. The factorial result showed there is no significant contrast between ethanol concentration mentioned toward anti-bacterial activity from extracted bacterial fermentation products of Andalas endophytic bacteria Isolate JDT 1B. The concetration of extracted fermentation product using 70% ethanol has the same inhibition zone as control is 6,25%.

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Corresponding Author :
Dwi Hilda Putri
Department of Biology, Faculty of Mathematics and Natural Science (FMIPA),
Universitas Negeri Padang, Indonesia
Email : dwihildaputri.08@gmail.com

1. Introduction

The use of anti-biotic exaggeratedly triggers the case of bacterial resistence appeared in health problem globally. Based on the data of *World Health Organization* (WHO) (2014) [1], there was an increasing resistance of *Staphylococcus* sp. bacterium toward anti-biotic from 63% in 2009 up to 80% in 2013. The increasing of that percentage become the encouragement for the scientists to find a better anti-microbial active compound [2].

Anti-microbial compound can be obtained from herbs, one of them is Andalas (*Morus macroura* Miq.). There are various active compound can be found in Andalas. Such as, *lunularin, oksiresveratrol, Andalasin* A*, morasin* M, *coumarin, umliferon,* and *β-resolsilaldehid* [3,4]. A direct exploration from herb needs plenty biomass [5]. In order to make it efficient, it need endophytic bacteria.

Endophytic bateria is bacteria that make living plant tissue as its host without harming and infecting other plants [6]. This bacterium is also able to produce the same bioactive as its host [7]. According to Putri (2018) [8], she is manage to isolate endophytic bacteria from Andalas (*Morus macroura* Miq.) which has better anti-bacterial activity. Anti-bacterial compound can be produced by the process of fermentation. In an effort to separate the active compound, it can be used the extraction method where a solvent is functioned as an extractor [9].

Each type of solvent is a factor which affects the concentration of compound from the extraction [10]. As the concept *like dissolve like*, a polar compound will dissolve in polar solvents while non-polar compounds will dissolve in non-polar solvents. One of the solvent which is commonly used is polar solvents (Ethanol). Ethanol is the universal solvent with polarity index 5,2. It is able to dissolve almost entire all secondary metabolite compounds such as *alkaloid*, *flavonoid*, *saponin*, *tannin*, *steroid*, and *terpenoid* [11]. Beside the type of polarity, the concentration of the solvent is also neccesary in affecting the extraction. According to Fathurrachman (2014) [12], he opined that there is a different polarity in a same solvent for each different concentrations (96%, 70%, 50%). As the result, it affect the result of secondary metabolite which is managed to filter in the extraction process.

The aim of this research is to know the effect of ethanol concentrations toward anti-bacterial activity from extracted endophytic bacterial fermentation product of Andalas isolate JDT 1B (*Morus macroura* Miq.).

2. Experimental Section

2.1. Extracting Active Compound from Endophytic Bacterial Fermentation Product of Andalas Isolate JDT 1B

The extraction is done through maceration method. The result of fermented Andalas endophytic bacterial isolat JDT 1B is macerated with ethanol (1:1 v/v). After that, the solvent is stirred speed (100 rpm) using *shaker incubator* for 3 days. Ethanol concentration used are 100%, 80%, and 70%. Furthermore, each of maceration result is evaporated in *rotary vacum evaporator* at 50°C with speed 60 rpm until a solvent become a thick extract.

2.2. A Test of Anti-bacterial Activity of an Extract Fermentation Product from Andalas Endophytic Bacteria

The anti-bacterial activity test of Andalas endophytic bacteria fermentation product is carried out by disk diffusion method. Extracts which are obtained from each concentration of the solvent are made diluted with concentrations of 6,25%, 12,5%, 25%, 50%. Tests are carried out on the bacterium *Staphylococcus aureus* by dripping 30 µl extracted dilution on a paper disc. Next, paper disc is placed

on NA medium which has been inoculated with *S. Aureus* suspension. The culture is incubated at 37°C for 24 hours. The inhibition zone measurements are made using a caliper to determine the anti-microbial activity produced. Anti-bacterial activity is statistically analyzed using the ANOVA test and factorial design if significantly different followed by DMRT at 5% level.

3. Results and Discussion

The extract of Andalas endophytic bacterial fermentation products is obtained from JDT 1B isolates using maceration method. Maceration method is usually used organic solvent. One of the example is ethanol. The advantage of it is the ability to dissolve almost all secondary metabolite compounds. Because of that reason, ethanol is considered as universal solvent. Ethanol is also more durable. It also has a lower toxicity value than other organic solvents and it is volatile (compared to non-organic solvents) [13]. In addition, ethanol has a good absorption ability and it is not easily overgrown with molds and germs in concentration ≥20% [14]. Beside the type of solvent, the concentration is one of the factor in affecting the substance of the active compounds produced by the extraction. The higher the concentration is able to expand the contact between solvent and extracted material. As the result, this case is followed by higher ability of solvent to attract bio-active compounds within extracted material [15].

In the study of Mubarak et al., (2018) [16], there are differences anti-bacterial activity produced using different concentrations of solvent. The result shows that, Bligo extract (*B. Hispida* Thunb) used 70% ethanol has a good inhibitory zone activity of 25,223 mm compared with 96% and 50% concentration. Moreover, according to Noviyanty et al., (2018) [17], she argues that the highest number of total phenolic content and the best anti-oxidant activity of extracts from husk of Cocoa beans are obtained with a concentration of 95% ethanol. This study shows different condition where there is no significant differences between the concentration of 100%, 80%, 70% ethanol on the anti-bacterial activity produced. The avarage yield of inhibition zone extracts of Andalas endophytic bacterial fermentation products at each concentration which can be seen in Table 1.

Table 1. A Diameter of Inhibition Zone Extract Fermentation Product of Andalas Endophytic Baterial for Each Ethanol Concentration

No	Extract Treatment (B)	Ethanol Concetration (A)			Main Average B
		100%	80%	70%	
1	6,25%	1,379	1,267	1,157	$1,268^{D}$
2	12,5%	1,889	1,720	1,808	$1,806^{C}$
3	25%	2,332	2,180	2,351	$2,288^{B}$
4	50%	2,803	2,929	3,122	$2,952^{A}$
	Main Average A	2,101	2,024	2,110	

According the data in table 1, avarage diameter of the largest inhibitory zone is produced by the 70% ethanol extract. However, this result has no significant different with the 100% and 80%. It indicates that the anti-bacterial compound from Andalas JDT 1B isolates are polar. The three ethanol concentrations used are still able to attract the active compound from the fermentation product. Given that ethanol is polar with a polarity index 5,2, because of that reason, ethanol is efficient in pulling component which is also polar [18]. The lack of this research is that it does not use any concentrations which are below 70%. It because there is a possibility for the concentrations lower than 70% to be affected by anti-bacterial activity. It is relevant with Luginda et al., (2018) [19] which indicates the effect of ethanol on the total *flavonoid* content of Beluntas (*Pluchea Indica*

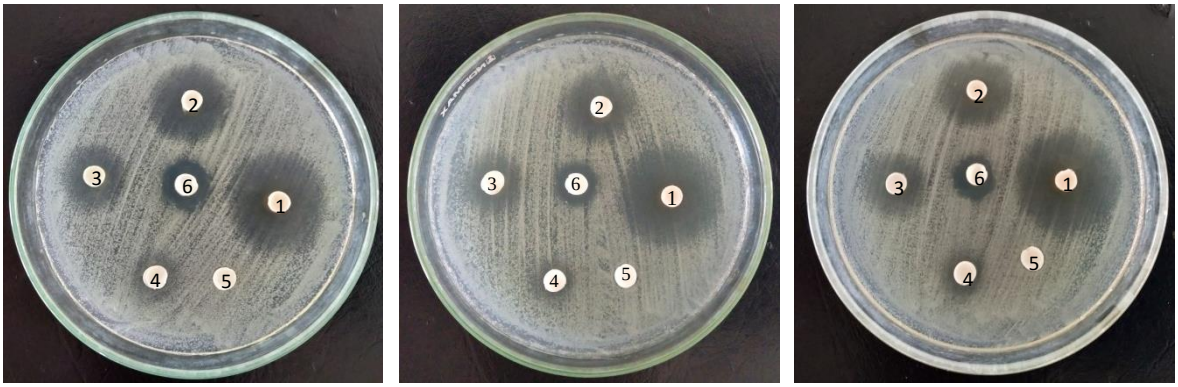
(L.)Less) on 60% concentration with a *flavonoid* avarage level of 2.8087%. Whereas, In case of 70% and 80% concentrations are on the same subset. It means, they do not give any different effect significantly. Similarly in case of 90% concentration, it has significant different. However, it has a smaller *flavonoid* avarage of 1,9143%.

Since there is no significant differences in anti-bacterial activity shown by the three concentrations, 70% concentration is definitely favorable to be chosen. This concentration is considered to be more efficient. It because the smaller concentrations inccures the smaller cost. Because of this reason, it is needed to do research on 70% ethanol concentration of the Andalas fermentation anti-bacterial product. The avarage diameter of inhibitory zone of extracted Andalas product can be seen in table 2.

Table 2. Diameter of Inhibition Zone Extract Fermentation Product of Andalas Endophytic Bacterial Using 70% Ethanol

No	Treatment	Diameter of Inhibition Zone (cm)			Total	Average
		1	2	3		
1	6,25%	1,053	1,235	1,183	3,470	1,157[d]
2	12,5%	1,760	2,005	1,660	5,425	1,808[c]
3	25%	2,303	2,528	2,223	7,053	2,351[b]
4	50%	3,028	2,918	3,420	9,365	3,122[a]
5	Control +	1,300	1,360	0,998	3,658	1,219[d]

Based on the data in table 2, the avarage diameter of inhibitory zones of Andalas JDT 1B extraction on *S. Aureus* shows different result for each different treatment. At 6,25% concentration, the avarage of inhibition zone produced has the smallest value of 1,157 cm. The increasing of the concentration is followed by the increasing value of avarage diameter of inhibitory zone. Based of further test of DMRT, extracts of Andalas fermentation product JDT 1B using 70% of ethanol concentration has best treatment at 6,25% concentration. Because it is able to inhibit the growth of *S. Aureus* approaching to positive control of anti-biotic *ampicilin*.



A B C

Fig 1. Clear Zone of Ethanol Extract Against S. aureus. A) Ethanol Concentration 100%, B) Ethanol Concentration 80%, and C) Ethanol Concentration 70% on. 1) Extract Concentration 50%. 2) Extract Concentration 25%. 3) Extract Concentration 12,5%. 4) Extract Concentration 6,25%. 5) Internal Control. And 6) Positive Control

4. Conclusion

The difference in the concentration of ethanol does not show any siginificant difference on the anti-bacterial activity of Andalas fermentation products of JDT 1B. As the result, the more efficient ethanol concentration is at 70% concentration. The fermentation product using 70% ethanol is able to inhibit the growth of *S. Aureus* approaching the positive control with 6,25% concentration.

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