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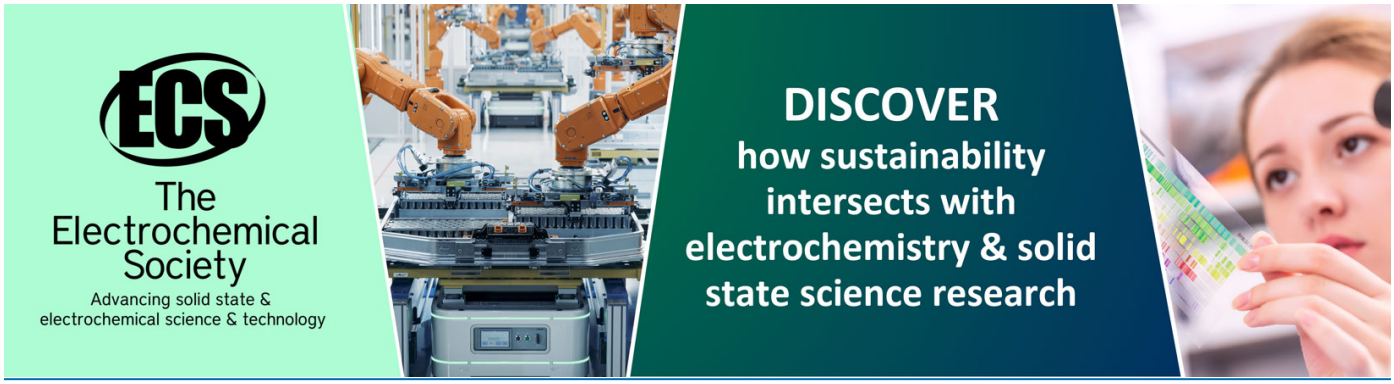

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Activities inhibition methanol extract Laban Leaf (*Vitex pinnata*) on growth of bacteria *S. mutans* Atcc 31987

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Abstract. People in Aceh, especially in the area of *Ie Seu'um* using the surrounding plants to treat diseases. One of the medical plants is laban (*Vitex pinnata*). Laban leaves are used to treat fever, hypertension, and toothache. Various bacteria found in the oral cavity, but only a few bacteria cause dental caries, including *S. mutans*. Based on the laban plant ethnobotany information, an evaluation of antibacterial activity on bacterial bioindicator of *S. mutans* and phytochemical screening had been performed. Laban plants contain flavonoids, saponins, and tannins. This study aims to determine the inhibition activity of laban leaf methanol extract (*Vitex pinnata*) on the growth of *S. mutans* bacteria in vitro. The antimicrobacterial activity was tested by using the Kirby-Bauer method with the extract concentration of 30%, 40%, 50% and 60%. The results showed that there was an inhibition activity with the significance of 0.000 < 0.05, therefore the hypothesis in this research can be accepted that Laban leaf methanol extract (*Vitex pinnata*) has inhibition activity, the higher the concentration the higher inhibition power.

1. Introduction

The use of traditional medicines to treat diseases is quite attractive to most of Indonesian people, because of its mild side effects [1]. People in Aceh, especially in the area of *Ie Seu'um* use the surrounding plants to treat diseases. One of the plants used as alternative medicine is laban plants (*Vitex pinnata*), known under various names, Aceh (*bak mane*) [2], Indonesia (*laban*), *Millettia pinnata* (L.) *Panigrahi*, *Pongamia glabra Vent*, *Derris indica*, *keranja* (Hindi, Bengali, Sanskrit), *ki pahang laut*, *sea-nut*, and *pongam* oil tree (Malay) [3].

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Laban plants contain the components of flavonoids, saponins, and tannins. Laban leaves (*Vitex pinnata*) can be used for the treatment of diseases such as fever and hypertension [4]. The Punjabi (Pakistan) community uses laban (*Vitex pinnata*) plants to treat oral diseases [3]. Phytochemical test results of laban leaves shows the content of alkaloids, flavonoids, saponins, sterpenoids, tannins,[2]. Therefore, laban plant (*Vitex pinnata*) is potential as an antibacterials [5].

Various bacteria are found in the oral cavity, but only a few bacteria cause dental caries, one of which is *S. mutans*. These bacteria can be easily mount the tooth surfaces [6]. *S. mutans* is a bacteria that can grow well in an acidic atmosphere and can produce acid as a result of carbohydrate fermentation. The acid produced by this bacterium can cause tooth demineralization [7]. Therefore, the activity of inhibition of methanol leaf extract of Laban leaf (*Vitex pinnata*) in *Ie Seu'um* to *S. mutans* bacteria is needed, and Laban leaf methanol extract (*Vitex pinnata*) can be used as a raw material of anti-bacterial drugs that can prevent dental caries. The area of *Ie Seu'um* is one of the Manifestations of the existing geothermal system in Aceh Besar. This area is located in the geothermal outflow zone of Mount Agam, Aceh Besar, located 20 km from the top of the mountain and 35 km from the city of Banda Aceh [8]. Associated with the inhibitory activity of methanolic extract of laban leaves (*Vitex pinnata*) against *S. mutans* bacteria in vitro until now has never been published. In Particular laban plant (*Vitex pinnata*) from the area of *Ie Seu'um* Aceh Besar.

2. Materials and Methods

2.1. Sampling of Plants

Laban leaf (*Vitex pinnata*) collections were collected on February 3rd, 2018, from *Ie Seu'um* Mesjid Raya, Aceh Besar District, Aceh Province with purposive sampling method. Sampling was done at four locations with following coordiantes: sample point 1 at coordinates (5°32'48.9" N 95°32'55.1" E), sample point 2 (5°32'51.4" N 95°32'54.3" E), sample point 3 (5°32'53.7" N 95°32'54.6" E), and the sample point 4 (5°32'45.7" N 95°32'57.8" E). Plant identification was done in Biology Department Faculty of Mathematics and Natural Sciences of Syiah Kuala University with number 558 / UN11.1.28.1 / DT / 2018. Coordinate *Ie Seu'um* and Laban (*Vitex pinnata*) can be seen in Figure 1 and Figure 2 respectively.

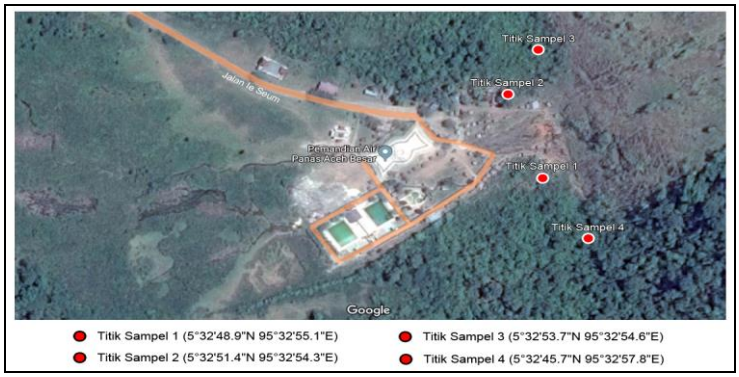

Figure 1. Sampling coordinates in *Ie Seu'um* area, Aceh Besar.

Figure 2. Laban (*Vitex pinnata*) in *Ie Seu'um* Area

2.2. Instruments and Materials

Instruments used included an analytical balance sheet, hot plate, media bottle, ose needle, shaker, tweezers, spiritus burner, autoclave, incubator (ecocell), colony counter, and sliding range. Materials used were Laban leaf (*Vitex pinnata*), *S. mutans* ATCC 31987 from Faculty of Veterinary Medicine Unsyiah, Nutrient agar (NA), Nutrient Broth (NB), Muller Hinton Agar (MHA), Disk Blank, Alcohol 70% , Aquades, Plastic wrap, and Aluminum foil, sulfoxide) [9].

2.3. Procedure

2.3.1. Maceration.

1 kg of dried leaves sample were mashed [10]. The obtained simplicia was then weighed [11], macerated with methanol solvent for 3 × 24 hours, evaporated with a rotary evaporator to obtain the methanol extract [12]. The extract was divided into several concentrations, 30%, 40%, 50% and 60%. Sterile aquadest was used as the negative control, while the positive control was *Chlorhexidine* 0.2%.

2.3.2. S. mutans bacterial culture.

Cultures of *S. mutans* was grown in NA agar medium, subsequently was incubated at 37° C for 2x24 hours in an incubator. One ose needle cultured *S. mutans* in NA medium was taken and inserted into a liquid tube of NB. The tube was inserted into the anaerobic jar, incubated at 37° C for 24 hours in the incubator. The turbidity was compared with one of McFarland 0.5. If the turbidity of *S. mutans* in liquid medium NB equal to the turbidity of McFarland 0.5 then the number of *S. mutans* is estimated to be 1.5 x 108 CFU/ml.The next was performing gram staining procedure. Each disc was dipped into a petri dish containing Laban leaf methanol extract (*Vitex pinnata*) with concentrations of 30%, 40%, 50% and 60%. A sterile cotton bud was dipped into a *S. mutans* culture in suspension of NB medium, then was smeared evenly on the surface of MHA media, the disc was placed on the surface of MHA media.The disk paper was previously dipped into *Chlorhexidine* 0.2% before being placed on the MHA media surface. Then it was incubated at 37^{o} C for 24 hours [13]. The procedure was conducted with three repetitions, then the inhibition zone was measured [14].

3. Result and Discussion

The results of the inhibitory activity of Laban leaf methanol extract (*Vitex pinnata*) can be seen in Table 1.

Table 1. Analysis and statistic of confidence level of the inhibition activity of Laban leaf methanol extract (*Vitex pinnata*) against *S. mutans* ATCC 31987

Experimental treatment	Average (mm)	Confidence Interval 95%		Standard Deviation*
		Lower Limit	Upper Limit	
30%	6.567	6.423	6.71	0.06
40%	6.867	6.487	7.246	0.15
50%	7.2	6.952	7.448	0.1
60%	10.433	9.916	10.95	0.21
Chlorhexidine 0.2 % Control (+)	19.6	15.696	23.504	1.57
Aquades Control (-)	6	6	6	0

* Three repetitions

Table 1. shows the number of treatment measures for each three times repetition, with different mean values with concentrations of 30%, 40%, 50% and 60%. This shows an increase in inhibition activity of laban leaf methanol extract (*Vitex pinnata*) against *S. mutans* bacteria. All the experimental treatment showed relative low standard deviations.

Table 2. ANOVA table to see the effect of laban concentration on inhibition power of *S. mutans*.

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square	F	P-value
Treatment	407.824	5	81.565	191.917	0.000
Error	5.100	12	0.425		
Total	412.924	17			

Table 2 is the result of ANOVA test showing that there is an inhibition activity with significance of 0.000 < 0.05, therefore the hypothesis in this research is acceptable in which the extract of laban leaf methanol (*Vitex pinnata*) has inhibitory activity against *S. mutans* bacteria.

Table 3. Duncan's advanced test

Treatment	Subset for alpha = 0.05
30%	6.567
40%	6.867
50%	7.200
60%	10.433
Chlorhexidine 0.2% Control (+)	19.600
Aquades Control (-)	6.000

Table 3. exhibit the mean values of treatments at concentrations of 30%, 40%, 50% and 60% were not significantly different. However the mean values of treatments 30%, 40%, 50% and 60% in comparison to positive control and negative control treatments were significantly different. Meanwhile the mean value of positive control treatment was significantly different from the negative control treatment.

The test results showed an increase in inhibitory activity of laban leaf methanol extract (Vitex pinnata) against S.mutans bacteria. Each experimental treatment has a different standard deviation value. Positive methanol extract contained alkaloids, steroids, terpenoids, saponins, flavonoids and phenolics [2]. In line with S. Thenmozhi's research, laban plants are potential antibacterial [5]. The result of ANOVA test confirmed that there was an inhibitory activity to S.mutans bacteria with significance of 0.000 <0.05. This is due to the greater concentration of laban extract, the more inhibitory zone to S. mutans. This difference was caused by several factors, including the amount of inoculum, incubation time, extract concentration, and antibacterial type. The larger the inoculum the smaller the inhibit zone is formed. The inhibitory zone formed in Chlorhexidine 0.2% (the positive control) was greater than the inhibitory zone of laban leaf methanol extract. Meanwhile, the sterile aquadest (the negative control) did not form a zone of inhibition. The antibacterial activity of laban leaf methanol extract was in accordance with the results of the study that extracts with 60% concentration had a larger inhibitory zone compared with concentrations of 30%, 40% and 50%.

4. Conclusion

Based on the results of a test study of inhibitory activity laban leaves methanol extract (*Vitex pinnata*) against *S. mutans* bacteria in vitro, it can be concluded that laban leaves methanol extract (*Vitex pinnata*) has an activity in inhibiting the growth of bacteria *S. mutans* in vitro. Based on the results of in this study, it can be concluded that the higher the concentration, the higher the inhibition power.

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