JURNAL ILMU KEFARMASIAN INDONESIA, April 2016, hlm. 13-18 Vol. 14, No. 1
ISSN 1693-1831

Activity Tests of Bioactive Material of Salung Leaf (*Psychotria viridiflora* Reinw. Ex. Blume) against *Salmonella thypi* Bacteria *In Vitro* And *In Vivo*

Uji Aktivitas Bahan Bioaktif dari Daun Salung (P*sychotria viridiflora* Reinw. Ex. Blume) terhadap Bakteri *Salmonella thypi* secara *In Vitro* dan *In Vivo*

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Diterima 27 November 2015, Disetujui 7 Februari 2016

Abstract: Activity test of bioactive material of Salung leaf (P*sychotria viridiflora* Reinw. ex. Blume) against *Salmonella thypi in vitro* and *in vivo* has been carried out. Bioactive material was obtained from the maceration and followed by fractionation of liquid-liquid fractionation. Antibacterial activity test performed *in vitro* to determine the value of the minimum inhibitory concentration (MIC) and *in vivo* to determine the ability of bioactive cure diarrhea in in rats (*Rattus norvegicus*) infected by *Salmonella typhi*. Treatment of bioactive material given is 0, 10, 50 and 100 mg kg^{-1} of weight. The results showed that the MIC of salung leaf's bioactive material to *Salmonella typhi* was 250 µg mL^{-1}. Bioactive ingredient at dose of 10 and 50 mg kg^{-1} were able to decrease the number of bacterial colonies to $4.14x10^6$ cfu g-1 and $5.4x10^5$ cfu g^{-1}, less than $5.04x10^6$ cfu g^{-1} as control. Bioactive material in weight of 100 mg kg^{-1} of weight could reduce the population of *Salmonella typhi* to zero after 12 days of treatment. The ability to reduce the amount of bacterial colonies of the bioactive material 100 mg kg^{-1} of weight is equal to the ability of positive control chloramphenicol 10 mg kg^{-1} of weight.

Keywords: antibacterial, bioactive material, *Psychotria viridiflora, Salmonella thypi, Rattus norvegicus.*

Abstrak: Telah dilakukan uji aktivitas bahan bioaktif dari daun Salung (*Psychotria viridiflor*a Reinw. ex. Blume) terhadap bakteri *Salmonella thypi* secara *in vitro* dan *in vivo*. Bahan bioaktif diperoleh dari proses maserasi dan dilanjutkan dengan fraksinasi secara fraksinasi cair-cair (FCC), Pengujian aktivitas antibakteri dilakukan secara *in vitro* untuk menentuan nilai konsentrasi hambat minimum (KHM) dan pengujian secara *in vivo* pada tikus putih (*Rattus norvegicus*) untuk mengetahui kemampuan bioaktif menyembuhkan penyakit diare yang diinfeksi dengan *Salmonella typhi*. Perlakuan bahan bioaktif yang diberikan 0, 10, 50 dan 100 mg/kgbb. Hasil penelitian menunjukkan bahwa KHM bahan bioaktif dari daun salung terhadap bakteri *Salmonella typhi* yaitu 250 µg/ml. Bahan bioaktif pada dosis 10 mg/kgbb dan 50 mg/kg mampu menurunkan jumlah koloni bakteri menjadi $4,14x10^6$ dan $5,4x10^5$ cfu/g, lebih sedikit dibanding kontrol 5,04 x 10^6 cfu/g. Bahan bioaktif 100 mg/kgbb dapat menurunkan populasi bakteri *Salmonella typhi* sampai nol setelah 12 hari pengobatan. Kemampuan bahan bioaktif 100 mg/kgbb sama dengan kontrol positif kloramfenikol 10 mg/kgbb.

Kata kunci: antibakteri, bahan bioaktif, salung, *Salmonella thypi, Rattus norvegicus.*

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INTRODUCTION

DIARRHEA remains a global health problem, both in developed countries and developing countries. The severity of the problem is evident from the high morbidity and mortality due to diarrhea. WHO estimates that 4 billion cases of diarrhea occur in the world in 2004 and 2.2 million of them died, mostly children under the age of 5 years[1].

Diarrhea can be caused by infection or non-infection. Most diarrhea occur is infectious diarrhea. Infectious diarrhea can be caused by bacteria, viruses, and parasites. Diarrhea infection of *Shigella flexneri* contributes 60% of diarrhea cases in developing countries. *Shigella sonnei* causes 77% of diarrhea cases in developed countries and 15% in developing countries. *Shigella dysenteriae* is usually the cause of dysentery epidemic, especially in populations that are restricted such as a place of evacuation. *Salmonella typhi*, one of species of the genus *Salmonella* cause acute gastrointestinal with diarrhea as most prominent symptom, accompanied by nausea and vomiting. *Salmonella typhi* also can cause typhoid fever[2].

From 37 species of medicinal plant in South Sumatera that had been examined, the antibacterial activity of 33 species were confirmed, and the most active from each district were obtained from Muara Enim, namely kayu kuman (*Rhinacanthus nasutus* (L.) Kurz), Musi Rawas namely daun salung plant (*Psychotria viridiflora* Reinw. ex Blume), Musi Banyuasin namely temengo plant (*Melochia umbellata* [Houtt.] Stapf), Ogan Ilir namely tahi babi (*Adenostemma lavenia* Linn.) and Lahat namely kilinyu plant (*Eupathorium Odoratum* L). These plants have high potential to be developed as herbal medicine to cure the skin infection and as sources of new antibacterial compound[3].

Traditionally, salung leaf (*Psychotria viridiflora* Reinw. Ex. Blume) has been used to treat diarrhea and skin infections. This plant has high potential as source of bioactive materials to treat diarrhea and as source of antibacterial compounds. Ethyl acetate fraction of salung leaf have strong antibacterial activity against bacteria. Ethyl acetate fraction isolated is EA1 isolate antibacterial compounds. Minimum Inhibitory Concentration (MIC) of EA1 isolate against *Escherichia coli* ATTC 25922 and *Staphylococcus aureus* ATTC 25923 was 62.5 μg mL^{-1}. EA1 isolate is included in antibacterial compounds that have a very strong activity[4]. Further test of EA1 isolate activity against *Salmonella typhi* and *Shigella disentriae* had been carried out, MIC values of EA1 isolate against *Salmonella typhi* and *Shigella dysentriae* was 62.5 μg mL^{-1}[5].

In order to determine the potential of bioactive materials (ethyl acetate fraction) salung leaf to treat diarrhea caused by the bacterium *Salmonella typhi*, research of "activity test of leaf Salung bioactive materials (P*sychotria viridiflora* Reinw. Ex. Blume) against *Salmonella typhi* bacteria *in vitro* and *in vivo*" needs to be carried out.

MATERIALS AND METHOD

MATERIALS. Materials used in this study includes salung leaf (*Psychotria viridiflora* Reinw. Ex. Blume), *Salmonella typhi* from Biofarma Bandung, *Rattus novergicus* from ITB Bandung, paper disc 6 mm, filter paper, nutrient agar (NA), medium nutrient broth (NB), *n*-hexane, ethyl acetate, methanol, dimethyl sulfoxide solvent and silica gel GF_{254}.

Instruments. The equipment used includes autoclave, hot plate, incubator, thin layer chromatography, column chromatography, laminar air flow cabinet, magnetic stirrer, electric heating, water bath, the capillary pipette, serological pipette, rotary evaporator, Soxhlet and Spectronic 20- D.

METHOD. Extraction. Dried Salung leaf were crushed using a blender to obtain a simplicia powder. A total of 1000 g of simplicia powder extracted by maceration with methanol. Extraction is done for ± 2 days, then filtered, with 2 repetition. Liquid methanol extract was evaporated with a rotary evaporator to obtain a thick extract. Thick extract is dried with a water bath.

Fractionation. Fractionation was conducted using liquid-liquid fraction method. The extract was added with methanol and water (with ratio of 3:7). After that, *n*-hexane of 250 mL was added up to 4 times (4 x 250 mL). Fractions of methanol and *n*-hexane were separated by a separator funnel in order to obtain the fraction of *n*-hexane. Fraction of methanol-water is added with 250 mL of ethyl acetate up to 4 times (4x250 mL), then separated by a separator funnel to obtained fraction of ethyl acetate. *N*-hexane fraction, ethyl acetate and methanol-water fractions were evaporated with a rotary evaporator and water bath. From the fractionation, obtained three fractions, namely fraction of *n*-hexane, ethyl acetate, and methanol in the form of pasta. Ethyl acetate fraction is the bioactive material.

Determination of Minimum Inhibitory Concentration (MIC). MIC determination was done by agar diffusion method using a paper disc with diameter of 6 mm. The procedure was as follows: Bioactive materials (ethyl acetate fraction) was prepared at a concentration of 4000, 2000, 1000, 500, 250, and 125 μg mL^{-1} using DMSO (dimethyl

sulfoxide). Test bacteria was inoculated into Nutrient Broth media about 1-2 ose needle, then incubated for 24 hours at 37 °C. The bacterial suspension was shaken by a vortex. The transmittance on Spectronic 20-D was measured with wavelength of 580 nm. Transmittance is set at 25% by the addition of bacterial or liquid medium. The bacterial suspension of 0.1 mL was added to petri dish, then added by nutrient agar medium of 10 mL. Paper discs were inserted and solution of 10 µL bioactive materials dropped into medium containing bacteria. After incubated for 24 hours at a temperature of 37 °C, diameter of inhibitory measured using vernier calipers.

Activity Test of Bioactive Materials *In vivo*. Test of the activity of bioactive materials *in vivo* conducted on mice with treatment as follows: test bacteria (as control), bacteria + fraction of ethyl acetate 10 mg kg^{-1} of weight, bacteria + fraction ethyl acetate 50 mg kg^{-1} of weight, bacteria + fraction of ethyl acetate 100 mg kg^{-1} of weight and bacteria + antibiotics. The mice infected by *Salmonella typhi* bacteria on day 0, bioactive material given orally for 12 days. During the experiment, mice were given food and drink. Observation to the condition and symptoms of abnormality was carried out every day. The feces of mice were taken every 2 days. Feces were weighed 1 g then dilution performed 5 times, ranging from 10^{-1} to 10^{-5}. After that, the last 3 dilution series were taken, sown in SSA medium, incubated at room temperature for 24 hours. Then, the number of *Salmonella typhi* bacteria found in feces was calculated. The comparison antibiotics were also given daily after incubation period of 8 days, the comparison antibiotic of chloramphenicol used was 10 mg kg^{-1} (modification[6]).

RESULTS AND DISCUSSION

Extraction and Fractionation. The extraction of 1000 g of salung leaf (*Psychotria viridiflora*) simplicia with methanol was performed by maceration methods. The result obtained as much as 415 g. Table 1 show the result of fractination from methanol extracts. The fractionation results of 415 g salung leaf extract obtained *n*-hexane fraction of 30 g (7.25%), ethyl acetate fraction of 135 g (32.50%) and 250 of methanol-water fraction (60.25%). Solvents used in the fractionation process have different abilities in attracting compounds contained in extracts. Ethyl acetate fraction (bioactive materials) activity was tested in vitro and *in vivo* against *Salmonella typhi* bacteria.

Minimum Inhibitory Concentration (MIC) of Ethyl Acetate Fraction. The antibacterial activity test

Table 1. Results of fractination of the methanol extract from salung leaf using different solvents.

No.	Solvent	Weight (g)	Weight (%)
1.	*n*-hexane	30	7.25
2.	Etylacetat	135	32.50
3.	Methanol-water	250	60.25
	Total	414	100

Table 2. Minimum inhibitory concentration (MIC) of ethyl acetate fraction of salung leaf extract against *Salmonella typhi.*

No.	Concentration (µg mL^{-1})	Average of inhibition diameter (mm) *Salmonella typhi*
1.	4000	12.25 ± 0.50
2.	2000	10.25 ± 0.50
3.	1000	8.75 ± 0.50
4.	500	8.25 ± 0.50
5.	250	7.00 ± 0.82
6.	125	0

Figure 1. Minimum Inhibitory Concentration(MIC) of salung leaf bioactive materials against *Salmonella typhi.*

to the fraction of *n*-hexane, ethyl acetate and methanol-water showed ethyl acetate fraction have the strongest activity to the *Escherichia coli* and *Staphylococcus aureus* [4]. In this study, the determination of MIC of ethyl acetate fraction to the bacteria *Salmonella typhi* can be seen in Table 2.

Table 2 and figure 1 shows the diameter of inhibition zone formed around the paper disc which is indication of the strength of antibacteria activity from ethyl acetate fraction. The result showed that the lower concentration, the smaller diameter of the inhibition zone was formed. The largest diameter of inhibition zone of ethyl acetate fraction is 12.25 mm against *Salmonella typhi* at a concentration 4000 µg mL^{-1}, while the smallest diameter of inhibition zone is 7.00 mm against *Salmonella typhi* at a concentration of 250 µg mL^{-1}. Based on diameter of inhibition zone, the fraction of ethyl acetate can be classified into a strong antimicrobial. This is in accordance with the opinion of[7], bactericidal ability in inhibiting the growth of bacteria by regional obstacles have a certain range. Inhibition zone of 20 mm or more is classified very strong, inhibition zone of 10-20 mm

is classified strong, inhibition zone of 5-10 mm is classified moderate, inhibition zone of 5 mm or less is classified weak.

In Table 2, the smallest concentration of materials bioactive which capable to inhibit the growth of *Salmonella typhi* is 250 µg mL-1, this concentration is namely the MIC value of materials bioactive. The bioactive materials is classified into strong antibacterial activity because the bioactive material has MIC between 100-500 µg mL^{-1}. This is in accordance with the opinion of[8] that based on the value of the MIC, the antibacterial compounds can be divided into four: active compounds that have a MIC of less than 100 µgmL^{-1} were classified as a compound which has a very strong antibacterial activity. This compound is very good to be used as drug compounds. Active compounds which have MIC values between 100-500 µg mL^{-1} were classified as compounds that have antibacterial activity strong enough. Active compounds which have MIC values between 500-1000 µg mL^{-1} were classified as compounds that have weak antibacterial activity and compounds active compounds which have more than 1000 MIC µg mL^{-1} were classified as compounds having antibacterial activity.

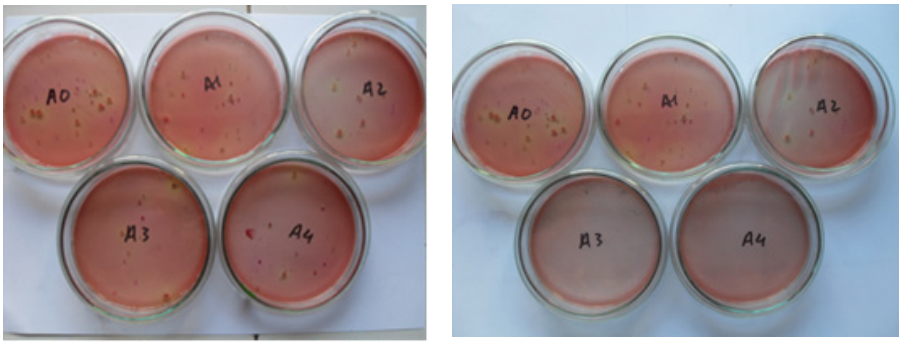
Bioactive materials such as ethyl acetate fraction found in this study has a MIC value almost the same as antibacterial fractions derived from other plants such as the antibacterial activities of ethyl acetate fraction of methanol extract from *Kalanchoe pinnata* Pers. leaf both to Gram positive bacteria and Gram negative bacteria. MIC value of the fraction to Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) is 192 mg mL^{-1} and Gram negative bacteria which are *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* is 192 mg mL^{-1}, but the MIC value to *Salmonella typhi* is 128 mg mL^{-1}. TLC identification show that the fraction contains flavonoid[9].

Bioactive material (ethyl acetate fraction) activity *in vivo*. Ethyl acetate fraction activity *in vivo* was indicated from the amount of colonies of *S. typhi* bacteria that grow on the medium SSA by the feces of mice in treatment. Feces of mice weighed 1 g was dissolved in 10 mL of sterile distilled water, dilution up to 5 times. The diluted feces of 1 mL was inserted into a petri dish, added with SSA medium, and then incubated for 24 hours. The amount of bacterial colonies growing calculated after incubation. The result number of colonies can be seen in Table 3, Figure 2 and 3.

Table 3. Amount of *Salmonella typhi* bacterial cell in the mice feces in 12 days of treatment by ethyl acetate fraction of salung leaf and chloramphenicol.

Treatment (mg kg^{-1} of weight)	Bacterial cell (x 10^5) cfu g^{-1} feses Days						
	0	2	4	6	8	10	12
A1 Control	0	0.52	10.40	52.60	84.20	112.80	50.40
A2 10	0	0.54	10.20	48.20	66.20	92.40	41.40
A3 50	0	0.50	9.80	34.60	51.40	54.20	5.40
A4 100	0	0.54	9.60	21.20	42.20	24.60	0.00
A5 chloramphenicol 10	0	0.54	9.60	19.80	38.40	21.40	0.00

Note:
A1= control (no treatment), A2=10, A3= 50, A4=100 mg kg^{-1} of weight (treatment with salung leaf) and A5= 10 mg kg^{-1} of weight (treatment with chloramphenicol).



(a) **(b)**

Figure 2. *Salmonella typhi* bacterial breed at 6 (a) and 12 days (b) in mice feces with the treatment by ethyl actetate fraction of salung leaf extract. A1= control (no treatment) , A2=10, A3= 50, A4=100 mg kg^{-1} of weight (treatment with salung leaf) and A5= 10 mg kg^{-1} of weight (treatment with chloramphenicol).

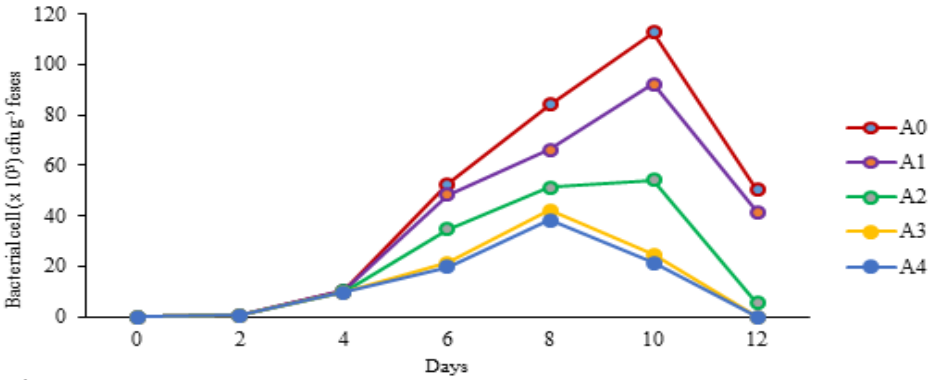


Figure 3. Amount of *Salmonella typhi* bacterial cell in the mice feces in 12 days of treatment by ethyl acetate fraction of salung leaf and chloramphenicol. A1= control, A2=10, A3=, A4=100 mg kg^{-1} of weight and A5= chloramphenicol 10 mg kg^{-1} of weight.

Amount of *S. typhi* bacteria cell in the feces before treatment is 0.0. After two days of treatment, the amount of *S. typhi* bacteria cell is at the ranges from $5.2x10^4$ to $5.3x10^4$. The amount of bacterial cells in control increase with the highest amount on the tenth day after the infection is 1.128×10^7. At a dose of 10 mg kg^{-1} of weight, amount of bacterial cells on day 12 was 4.14×10^6, less than the control which is 5.04×10^6. The amount of bacterial cells in a dose of 50 mg kg-1 of weight on day 12 was 5.4×10^5, much less than control. The amount of bacterial cells in a dose of 100 mg kg^{-1} of weight after 12 days is zero. The value is equal to the amount of bacterial cells in the control treatment chloramphenicol 10 mg kg-1 of weight. The higher dose of the bioactive material given, the higher antibacterial activity and the less amount of colonies of bacterial found in feces of mice. This is caused by the increasing amount of antibacterial compounds contained in the bioactive substance. According to[6] that the activity of a fraction is related to the content of the active compound in the fraction. The more active compound content so the higher the activity of the fraction.

The treatment of bioactive materials from *Psychotria viridiflora* leaf can be inhibit to the growth of *Salmonella typhi* bacteria in vivo at mice treatment. It is caused in the bioactive material contained antibacterial compounds of EA1 isolate. The EA1 isolate in vitro was active to the several bacterial such as *Escherichia coli* 25922 and *Staphylococcus aureus* ATTC 25923 with MIC value of 62.5 ug mL^{-1} [4]. Furthermore, EA1 isolates in vitro was also active to the bacterial of *Salmonella typhi* and *Shigella disentriae* with MIC values of 62.5 ug mL^{-1}[5]. The bioactive material at a dose of 100 mg kg^{-1} of weight can cure diarrhea that caused by *Salmonella typhi* in mice after 12 days of treatment. The capacity of the bioactive ingredient 100 mg kg^{-1} of weight is almost the same as the positive control, chloramphenicol 10 mg kg^{-1} of weight.

The capacity of the bioactive materials of the leaf salung better than beluntas leaf extract (*Pluchea indica* L). Leaf extract of beluntas need a dose of 600 mg kg^{-1} of weight while the bioactive materials of salung was 100 mg kg^{-1} of weight [10]. Obtained that the activities of leaf extract beluntas in curing diarrhea in mice that induced by *Salmonella typhi* leaf extracts. The result obtained is beluntas have antidiarrheal effect at doses of 150 and 300 mg kg^{-1} of weight and at a dose of 600 mg kg^{-1} of weight give effect comparable with loperamide.

CONCLUSIONS

The conclutions of the research show that the Minimum Inhibitory Concentration (MIC) of bioactive materials (ethyl acetate fraction) of *Psychotria viridiflora* leaf to the *Salmonella typhi* is 250 µg mL^{-1}. The bioactive materials is classified as the antibacterial materials that have a strong activity. The bioactive materials of *Psychotria viridiflora* leaf with 10 to 50 mg kg^{-1} of weight can decrease the amount of Salmonella typhi bacteria cells *in vivo* to the mice more than control. The bioactive materials at a dose of 100 mg kg^{-1} of weight can cure diarrhea that caused by *Salmonella typhi* after 12 days of treatment. The capability of the bioactive materials of 100 mg kg^{-1} of weight is equal to the positive control chloramphenicol 10 mg kg^{-1} of weight.

ACKNOWLEDGMENT

We would like to thank the Sriwijaya University and DIKTI for financial support “Hibah Bersaing 2015”.

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