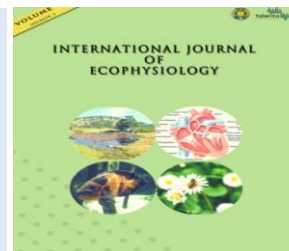




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The Activities of Plant Extracts Heritage of Melayu Culture-Riau Archipelago on Bacteria Causing Diarrhea

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Abstract. Diarrhea is one of the major health problems in Indonesia. Lingga Malay ethnic in Riau archipelago use alternative medicines in a form of a obat pahit potion to treat diarrhea. This research aimed to determine the antibacterial activity of obat pahit extraction against *Escherichia coli* ATCC 11775, *Salmonella* ATCC 14028 and *Shigella flexneri* ATCC 12022. Antibacterial activity can be seen with the formation of inhibitory zone using diffusion method. The extract concentration used were 100%, 75%, 50% and 25%. Based on the antibacterial activity test, the largest inhibitory zone against *E. coli* was found at 100% *Cnestis palala* extract of 8.29 mm and the smallest inhibitory zone was found at 25% traditional medicine practioner (TMP) 2 SP4 of 5.73 mm. The more over, the largest inhibitory zone to *S.* was found at 100% *Bauhinia semibifida* of 8.81 mm; and the smallest inhibitory zone was found at 50% TMP 2 SP4 of 6.30 mm. Antibacterial activity with the largest inhibitory zone against *S. flexneri* at 75% *C. palala* of 6.21 mm and the smallest inhibitory zone was found at 50% TMP 2 SP4 of 5.62 mm.

Keywords: *Bauhinia semibifida*, *Escherichia coli*, Lingga Malay ethnic, *Salmonella typhi*, *Shigella flexneri*.

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1. Introduction

Diarrhea is one of the main health problems in developing countries including Indonesia. The disease can last for days and cause dehydration. Severe dehydration lead to weakness, shock and even death, especially in infants and children (Ganong, 1999). Diarrhea occurs due to several factors such as sanitation, nutrition, hygiene, malabsorption, chemical poisoning, allergies, viruses, parasites and bacterial infections (Suharyono, 2008). Bacteria that cause diarrhea are: *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perferingens*, *Escherichia coli*,

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Campylobacter jejuni, *Klebsiella pneumoniae*, *Salmonella*, *Shigella flexneri* and *Vibrio cholerae* (Meliawati, 2009).

In an effort to deal with infection by microorganisms, it requires optimal working power of medicines with minor side effects. Current use of antibiotics is very high, but infection is still one of the global problems due to bacterial resistance to antibiotics. Thus, it is necessary to develop traditional medicines to support the improvement of public health (Ardiansyah, 2002). The World Conservation Monitoring Center reports that the territory of Indonesia is an area with various types of medicinal plants that have been utilized to reach 2,518 species (Galingging and Bhermana, 2010). The knowledge of local people in the use of medicinal plants is different, namely in a simple and complex manner. In simple terms, the community uses one type of plant while in a complex way the community utilizes various medicinal plants or mixed ingredients called herbs to cure various disease complaints (Hanadari, 2014). This herb is made based on ancestral recipes, customs, beliefs, habits and traditional knowledge and experience (Dewoto, 2007). This traditional treatment can still be found in various regions in Indonesia, one of which is known as the Bitter Medicinal Herb / Medicinal Rebus, in Lingga District, Lingga Regency, Riau Islands. Bitter medicine made by Traditional Medicine Practitioners (POT).

Several studies showed that flower of rosella (*Hibiscus sabdariffa* L.) has inhibitory effect on *E. coli* (Ryaniarti and Susilo, 2015). Sawitti *et al* (2013) study bitter leaf extract against *E. coli*, Dewanti & Teguh (2011) study the antimicrobial activity of *Foliasyzygium polyanthum* Wight bay leaf infusions on *E. coli* in vitro, Bakhriansyah *et al* activity test of sago root infusion (*Metroxylon sagu*) against *S. typhi*. Information about the activity of bitter medicinal herbs and their composition in inhibiting the growth of diarrhea-causing bacteria is unknown. Therefore, it is necessary to conduct a study to test the antibacterial activity of bitter medicinal herbs extract of Lingga Malay culture, single plant *Bauhinia semibifida* and *Cnetis palala* in inhibiting the growth of diarrhea-causing bacteria (*E. coli* ATCC 11775, *S. typhi* ATCC 14028 and *S. flexneri* ATCC 12022).

2. Materials and Methods

This research was carried out by making five extracts, namely three bitter medicinal herbs and two single herbs ((Kangkang Katup (*Bauhinia semibifida*) and Seven Lapis Root (*Cnetis palala*)). Samples were obtained from Kalan Village, SP4 Village and Linau Village in Lingga District, Regency Lingga, Riau Islands and test bacteria were obtained from the UPT of the Health and Environment Laboratory of the Riau Province Health office. The five samples were made with a decoction extract with concentrations of 100%, 75%, 50% and 25%, as a negative control of 100 mL aquadest and positive control of chloramphenicol 30 µg. The test bacteria suspension was prepared as much as 10⁸ CFU/ml. Test was carried out on MHA medium. Repeated treatment was 3 replications and antibacterial activity of the extract was observed to

inhibit the growth of diarrhea-causing bacteria through the inhibitory zone formed during incubation for 24 hours.

Plant Extracts Preparation

Bitter medicine and single herbs (simplicia) as much as 100 g in 1 L distilled water (infundation) in a container (cauldron) heated on a bath \pm 15 minutes counted when the water began to boil, carried out for 3 consecutive days, after the first boiling (day 1), the sample is left in the container, to be boiled again on the second and third days (Nurhalimah *et al.* 2015) The results of the decoction were filtered using filter paper, 200 ml of extract was used and considered as 100% concentration, dilution was carried out to obtain concentration variations of 100%, 75%, 50% and 25% (Rahmawati and Siti 2014).

Nutrient Agar (NA) Medium

This media NA is used to breed bacteria, making it by weighing 20 g of NA dissolved in 1 L of distilled water using a stirring rod and heated on a hot plate. Sterilized using an autoclave with a pressure of 15 psi at 121°C for 15 minutes (Aswarita 2013).

Mueller Hinton Agar (MHA) Medium

MHA media was used for testing antibacterial activity, making it with MHA weighing 38 g dissolved in 1 L distilled water using a stirring rod and heated on a hot plate. Sterilized using an autoclave with a pressure of 15 psi at 121°C for 15 minutes.

Preparation of Test Bacterial Suspension

The test bacteria were inoculated as much as one ounce into 100 ml MHB from the inclined NA media and incubated 18-24 hours in a shaker incubator (150 rpm, 37°C). The number of bacterial colonies grown in petri dishes was calculated using the total plate count (TPC) method to obtain 10⁸ CFU/ml colonies (Sutton, 2011)

Antibacterial Activity Test (Disc Diffusion Method)

Test bacteria (10⁸ CFU / ml) of 1 ml were inoculated into petri dishes, then poured 15 ml of MHA (pour plate) and allowed to solidify (Oktavia *et al.* 2013). Each extract was dripped on 100 μ L sterile disc paper, allowed to stand for \pm 15 minutes (Atikah 2013). After condensing media positioned on the surface of the media: herb extracts and medicinal plants with various concentrations (100%, 75%, 50% and 25%), sterile distilled water as much as 100 μ L (negative control) and chloramphenicol 30 μ g (positive control). After being incubated for 24 hours, antibacterial activity was observed by measuring the diameter of the inhibitory zone (mm) using a calipse.

Data analysis

Antibacterial activity of bitter medicinal herb extract, *B. semibifida* and *C. palala* on the growth of *E. coli* ATCC 11775, *S. typhi* ATCC 14028 and *S. flexneri* ATCC 12022 with data (inhibition zone diameter) obtained were analyzed descriptively, displayed in the form of tables and picture. Antibacterial activity of extracts in the form of inhibitory zones formed in inhibiting the growth of test bacteria was categorized according to Susanto *et al.* (2012) in Rachmawaty (2016), the diameter of the inhibitory zone was <5 mm, the activity was categorized as weak, the diameter of the inhibition zone was 6-10 mm categorized as moderate, the diameter of the inhibition zone was 11-20 mm categorized as strong and the diameter of the inhibitory zone > 21 mm was categorized as very strong.

3. Result and Discussion

In this study to obtain extracts, the infundation method is used, namely the method of extraction of heat using water solvents. In Table 1 it can be seen the average inhibition zone shown by five bitter medicinal herb extracts on three test bacteria that cause diarrhea (*E. coli* ATCC 11775, *S. typhi* ATCC 14028 and *S. flexneri* ATCC 12022). The largest inhibitory zone for all herb extracts and test bacteria was shown from *B. semibifida* at 100% concentration of 8.81 mm against *S. typhi* ATCC 14028 and the smallest inhibition zone of 5.62 mm from POT 2 SP4 ingredients at a concentration of 25% to *S. flexneri* ATCC 12022. Poeloengan & Andriani (2013), stated that the inhibition zone diameter was influenced by the absorption capacity of the extract as an antibacterial compound into the agar medium and the sensitivity of the bacteria to the extract. Extracts can inhibit bacterial growth due to the content of secondary metabolites which can act as antibacterial agents because they are able to inhibit bacterial growth Nurhalimah *et al.* (2015).

Table 1 shows the largest inhibition zone against *E. coli* ATCC 11775 was shown by *C. palala* extract at a concentration of 100% which is 8.29 mm and the smallest inhibition zone of POT 2 SP4 at a concentration of 25% which is 5.73 mm. Antibacterial activity in the form of inhibitory zones formed in inhibiting the growth of *E. coli* ATCC 11775 was categorized as moderate and weak based on Susanto *et al.* (2012) in Rachmawaty (2016). Research by Roslizawaty *et al.* (2013), from ant nest extract (*Myrmecodia* sp.) Showing inhibition zone of 6.67 mm. Sawitti *et al.*⁹, using the sambiloto leaf extract obtained a inhibition zone of 10.063 mm at a concentration of 100%. The types and composition of plants in these three bitter medicinal herbs can affect the results of antibacterial activity. This is in line with Fitriani *et al.* (2016), the existence of synergism or antagonism between compounds so that each other increases or decreases the effect in activity.

Table 1. The average of inhibition zone plants extract on bacteria causing diarrhea

Plant extract	Concentration (%)	Inhibition zone (mm)		
		<i>Escherichia coli</i> ATCC 11775	<i>Salmonella typhi</i> ATCC 14028	<i>Shigella flexneri</i> ATCC 12022
POT 1 KALAN	100	7,28 ± 0,51	6,53 ± 0,20	-
	75	5,76 ± 0,37	6,58 ± 0,40	-
	50	6,13 ± 0,53	6,43 ± 0,39	5,66 ± 0,23
	25	5,90 ± 0,28	6,42 ± 0,35	5,75 ± 0,55
POT 2 SP 4	100	6,57 ± 0,28	7,44 ± 0,09	5,76 ± 0,09
	75	6,83 ± 0,31	7,06 ± 0,32	5,83 ± 0,00
	50	6,18 ± 0,60	6,30 ± 0,13	5,62 ± 0,36
	25	5,73 ± 0,06	6,31 ± 0,26	5,63 ± 0,14
POT 3 LINAU	100	6,79 ± 0,09	6,88 ± 0,13	5,86 ± 0,44
	75	6,09 ± 0,06	6,50 ± 0,21	5,99 ± 0,22
	50	5,78 ± 0,07	6,27 ± 0,27	-
	25	-	6,11 ± 0,12	-
<i>Bauhinia semibifida</i>	100	7,83 ± 0,97	8,81 ± 1,94	5,80 ± 0,31
	75	6,97 ± 0,46	8,41 ± 0,64	6,00 ± 0,79
	50	7,18 ± 0,00	7,55 ± 0,61	5,78 ± 0,00
	25	6,82 ± 0,28	6,18 ± 0,14	5,91 ± 0,40
<i>Cnestis palala</i>	100	8,29 ± 1,33	7,30 ± 0,44	6,11 ± 0,48
	75	6,22 ± 0,17	7,24 ± 0,25	6,21 ± 0,33
	50	6,42 ± 0,43	6,59 ± 0,34	5,68 ± 0,33
	25	6,57 ± 0,24	6,34 ± 0,18	5,64 ± 0,09
Positive control	chloramphenicol	17,03 ± 0,00	24,76 ± 0,00	18,7 ± 0,00
Kontrol negatif	distilled water	-	-	-

Note: Inhibition zone <5 mm (weak level), 6-10 mm (medium), 11-20 mm (strong), >21 mm (very strong).

The antibacterial activity test against *S. ATCC 14028* with indications of inhibition zone formation resulted from *B. Semibifida* extract at a concentration of 100% which is 8.81 mm which is the largest inhibition zone and the smallest inhibitory zone is shown from the POT 3 Linau herb extract at a concentration of 25% namely 6.11 mm. Antibacterial activity in the form of inhibitory zones formed in inhibiting the growth of *S. typhi* ATCC 14028 is included in the moderate category based on Susanto et al. (2012) in Rachmawaty (2016). In Table 1 can be seen for the concentration of 100% of the four extracts (POT 2 SP4, POT 3 Linau, *B. semibifida* and *C. palala* shows the largest inhibition zone compared to other concentrations. Thus it is known that the antibacterial activity is directly proportional to the concentration of extract. with the

statement of Rahmawati and Siti (2014), that the higher the concentration, the greater the antibacterial activity. On the herb extract of POT 1 Kalan, the largest inhibition zone was obtained from the concentration of 75% (6.58 mm) on the other hand at a concentration of 100% inhibitory zone, namely 6. Based on the antibacterial activity, it was found that extract activity was not directly proportional to the level of concentration. It was thought that the extract did not work stably in inhibiting the growth of *S. typhi*, indicated by the unequal activity between levels of concentration that can be seen from the inhibition zone formed.

Rahmawati's research (2015) using pacing rhizome water (*Costus spiralis*) on *S. typhi* ATCC 14028 resulted in inhibition zones of 8.55 mm at a concentration of 100 mg / ml by disc diffusion method. Virgianti (2016) in his study obtained inhibitory zone against *S. typhi* ATCC 14028 by 15.8 mm at a concentration of 100% from the leaves of Ashibata water (*Angelica keiskei*) which is known to contain alkaloids, tannins and phenolics. Puspita's (2013) study showed antibacterial activity in the form of a low inhibitory zone against *S. typhi* with a range of 1-5 mm inhibition zone diameter from black mangrove decoction (*Rhizospora mucronata*).

The inhibitory test of *S. flexneri* ATCC 12022 was the largest inhibitory zone of *C. palala* extract at a concentration of 75% which was 6.21 mm and the smallest zone was 5.62 mm from POT 2 SP4 at a concentration of 50%. At a concentration of 75% of POT 2 SP4, POT 3 Linau, *B. semibifida* and *C. Palala* obtained the largest inhibition zone. This shows the optimum concentration of four extracts in inhibiting the growth of *S. flexneri* ATCC 12022 is at a concentration of 75%. At a concentration of 100% found activity was lower than 75%, this was due to the extract in an unstable condition so that the activity was not directly proportional to the concentration of the extract. According to Elifah & Esty (2010), the diffusion speed of antibacterial compounds, types of antibacterial compounds and concentration can provide a wide range of inhibitory zones at certain times. The extract concentration has not reached optimal concentration to produce antibacterial activity or has not been effective for removing antibacterial compounds. The decoction extract (infundation method) in this study has not been effective in inhibiting the test bacteria because it has not been able to attract plant metabolites properly. This is reinforced by Alam & Waluyo (2006) using the boiling method of active substances can be taken only 5%. According to Bakhriansyah *et al.* (2011), extraction using water solvents can bind polar active substances such as flavonoid compounds. Rheza's research (2015), the antibacterial activity test of the infusion of bacang mango leaves (*Mangifera foetida* L.) with a concentration of 10% -100% showed no activity against *S. flexneri* growth.

Test bacteria in this study are Gram negative bacteria, according to Jawetz *et al.* (2005), these bacteria have three-layered cell walls, namely lipoproteins, phospholipid outer membranes and lipopolysaccharides with lipid content in the cell wall ranging from 11-22%. Phospholipid outer membranes can cause antibacterial chemical components difficult to penetrate the cell wall of Gram negative bacteria. Different things were found in Gram positive bacteria, where the cell

wall of this bacterium was single-layered with a lipid content of 1-4%, making it more likely to inhibit the growth of Gram-positive bacteria compared to Gram-negative bacteria.

The antibacterial activity of each extract is different because of the variation in the number and metabolite of plants in bitter medicine. Hazimi's research (2017), compounds contained in bitter medicinal herbs include: *C. palala* (flavonoids alkaloids, terpenoids, saponins and tannins), cloves (alkaloids, flavonoids, terpenoids, saponins, tannins and steroids), keteng skin (alkaloids, flavonoids, terpenoids, saponins and tannins), seba leaves (alkaloids, terpenoids, saponins and tannins), edible roots (flavonoids, terpenoids, and tannins), key chains (alkaloids, flavonoids, terpenoids, saponins and tannins), piths of the earth (alkaloids, saponins and tannins), root of layer laughter (alkaloids, flavonoids, terpenoids, saponins and tannins) and *B. semibifida* (flavonoids, terpenoids, saponins and tannins).

Pepeljnjak *et al.* (2005) stated that the mechanism of action of flavonoids is to form complexes with extracellular proteins, inactivate enzymes and damage cell membranes. In general, these compounds were able to inhibit the growth of Gram positive and Gram negative bacteria (Cowan 1999). Terpenoids as antibacterials can lyse bacterial cell walls. This compound reacts with porin (transmembrane protein) on the outer wall of bacterial cells, forming a strong polymeric bond which causes damage to the protein. Damage to the porin which is the entrance and exit of the compound will reduce the permeability of the bacterial cell wall so that bacterial cells lack nutrients, as a result bacterial growth is inhibited or dead (Cowan 1999). Alkaloids are able to influence the formation of peptidoglycan in bacterial cells, so that bacterial cells cannot form perfectly and experience death.

Negative control and positive control were used as a comparison in determining antibacterial activity from extracts of bitter and single herbs. Negative control was used by distilled water to see the effect of solvent on the extraction stage on the resulting inhibition zone. Positive control uses chloramphenicol, a broad-spectrum antibiotic. According to Pelczar & Chan (2008) chloramphenicol is relatively non-toxic when used in mammals therapeutically.

4. Conclusion

Based on the research conducted, the following conclusions were obtained:

1. The biggest inhibitory zone against *E. coli* ATCC 11775 was obtained from *C. palala* extract which was 8.29 mm at a concentration of 100% and the smallest inhibition zone of 5.73 mm from POT 2 SP4 at a concentration 25%.
2. The biggest inhibitory zone against *S. typhi* ATCC 14028 was obtained from *B. semibifida* extract which was 8.81 mm at a concentration of 100% and the smallest inhibition zone of 6.11 mm from POT 3 Linau at a concentration of 25%.

3. The biggest inhibitory zone against *S. flexneri* ATCC 12022 was obtained from *C. palala* extract which was 6.21 mm at a concentration of 75% and the smallest inhibition zone of 5.62 mm at a concentration of 50% of POT 2 SP4

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