

## SHORT COMMUNICATION

### **Antibacterial Activity of Ethanol Extract of *Portulaca oleracea* L. Herb from Various Extraction Methods against *Salmonella typhimurium***

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*Salmonella typhimurium* bacteria could cause gastroenteritis and its growth could be controlled by the active compounds from natural products, such as *Portulaca oleracea* L. herb. *Portulaca oleracea* is proven to have antibacterial activity against *S. typhimurium*. This herb contained flavonoids, alkaloids, terpenoids, and coumarins compounds which had different characteristics towards temperature extraction. This study aims to determine the antibacterial activity of ethanol extract of the *P. oleracea* herb from various extraction methods against *S. typhimurium* bacteria. Extraction of *P. oleracea* herb was carried out with four variations methods which were the cold method (maceration and percolation) and heat method (soxhlet and refluxes) using 96% ethanol solvent. The four types of extract were tested for their antibacterial activity by disk diffusion at concentrations of 30%, 35%, 40%, 45%, and 50% (b/v). The positive control was chloramphenicol 30 µg/disk, while the negative control was DMSO solvent. The results of antibacterial activity test in the form of zone of inhibition were statistically analyzed by Two Way Anova. The results showed that the ethanol extract of the *P. oleracea* herb from various extraction methods had antibacterial activity against *S. typhimurium*. There was a significantly difference in the antibacterial activity of ethanol extract of the *P. oleracea* herb obtained from the reflux method with other methods (maceration, percolation and soxhlet) against *S. typhimurium*.

Key words: antibacterial, ethanol extract of *Portulaca oleracea* L. herb, *Salmonella typhimurium*, various extraction methods

Bakteri *Salmonella typhimurium* merupakan bakteri yang dapat menyebabkan gastroenteritis dan pertumbuhannya dapat dikendalikan oleh senyawa aktif dari bahan alam, salah satunya herba krokot (*Portulaca oleracea* L.). Krokot sudah terbukti memiliki aktivitas antibakteri terhadap *S. typhimurium*. Herba krokot mengandung senyawa flavonoid, alkaloid, terpenoid, dan kumarin yang memiliki karakteristik berbeda terhadap suhu ekstraksi. Penelitian ini bertujuan untuk mengetahui aktivitas antibakteri ekstrak etanol herba krokot dari berbagai metode ekstraksi terhadap bakteri *S. typhimurium*. Ekstraksi herba krokot dilakukan dengan 4 variasi metode yaitu cara dingin (maserasi dan perkolasi) dan cara panas (soklet dan refluks) menggunakan pelarut etanol 96%. Keempat jenis ekstrak tersebut diuji aktivitas antibakterinya secara difusi cakram pada konsentrasi 30%, 35%, 40%, 45%, dan 50% (b/v). Kontrol positifnya adalah kloramfenikol 30 µg/disk, sedangkan kontrol negatifnya pelarut DMSO. Hasil uji aktivitas antibakteri berupa Diameter Daerah Hambat (DDH) yang dianalisis secara statistik *Two Way Anova*. Hasil penelitian menunjukkan adanya aktivitas antibakteri ekstrak etanol herba krokot dari berbagai jenis metode ekstraksi terhadap *S. typhimurium*. Ada perbedaan signifikan aktivitas antibakteri ekstrak yang dihasilkan dari metode refluks dengan metode lainnya (maserasi, perkolasi dan soklet) terhadap *S. typhimurium*.

Kata kunci: antibakteri, ekstrak etanol herba krokot (*Portulaca oleracea* L.), *Salmonella typhimurium*, variasi metode ekstraksi

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A gastroenteritic infection could be caused by pathogenic bacteria namely *Salmonella typhimurium*. *Salmonella* is one of four key global causes of diarrhoeal diseases, which are the most common illnesses resulting from unsafe food. According to the World Health Organization (WHO) in 2018, about 550 million people falling ill each year, including 220

million children under the age of 5 years. Infection was usually treated by using antibiotics and the use of antibiotics could not be separated from its side effects, such as resistance, hypersensitivity, and allergic reactions (Cunha 2001). In order to find alternative treatments for infection, research was conducted to find other sources mainly from natural products. One of these natural products is the purslane (*Portulaca oleracea* L.) plant. Purslane could be used for the treatment of diarrhoea, dysentery, and gastro protective

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(Kumar *et al.* 2022). Phytoconstituents found in purslane plants were flavonoids (apigenin, kaempferol, quercetin, luteolin, myricetin, genistein, and genistin), alkaloids, coumarins, terpenoids, anthraquinone glycoside, cardiac glycoside, sterols, proteins, vitamins, and minerals (Chugh *et al.* 2019).

Karlina *et al.* (2013) reported screening phytochemical from the purslane herb ethanol extract obtained by maceration method contained tannins, saponins and flavonoids. The purslane leaves ethanol extract from the soxhlet extraction contained secondary metabolites such as alkaloids, saponins, tannins, flavonoids, diterpenes, and proteins (Wasnik and Tumane 2014). Different extraction methods of purslane plant could affect the chemical content in the extracts. Both hot and cold extraction methods could affect the amount of chemical content extracted, because the heat in the extraction process could extract more active compounds but also affects the stability of these compounds. Some compounds in natural products are likely less stable to heat.

Sudaryati and Nusandari (2017) reported that antibacterial activity against *Salmonella typhimurium* from the ethanol extract of macerated purslane herb occurred at concentration range of 80-100%. According to Londonkar and Nayaka (2011), the purslane herb ethanol extract from the soxhlet process contained many flavonoid compounds that had antibacterial activity against *Salmonella typhimurium*. Another research by Nayaka *et al.* (2014) reported that apigenin compound that was isolated from purslane had antibacterial activity against *Salmonella typhimurium*.

The research about the antibacterial activity of the purslane herb ethanol extract with several extraction methods against *Salmonella typhimurium* had never been reported. This study was conducted to provide information about the best extraction methods in obtaining the purslane herb ethanol extract with the greatest antibacterial activity against *Salmonella typhimurium*. This study could also determine any significantly differences in the antibacterial activity of the purslane herb ethanol extract against *Salmonella typhimurium* based on the extraction method variations.

Purslane herb were obtained from Putat Nganten village, Grobogan regency, Central Java, Indonesia. The plant was harvested from cultivated fields with characteristics dark green leaves, yellow flowers and reddish stem. The plant parts used are the roots, stems, leaves and flowers. The purslane herbs were sorted,

then washed with water to remove impurities. Fresh herbs were dried at temperature 55 °C and powdered.

**The Extraction Process.** The maceration process was carried out by weighed dried purslane herb powder for 600 grams. The solvent used was 75 parts (4500 mL) of 96% ethanol and immersed for 3 days, protected from the sunlight, at room temperature with several stirring. The mixture was filtered to obtain macerate (1). The waste was soaked again with 25 parts of solvent (1500 mL) for 2 days. Then mixture was stirred and filtered again to obtain macerate (2). Macerate (1) and (2) were mixed and stored in a closed container and cool place, protected from the sunlight. The filtrate was stored for 24 hours. The filtrate was then filtered and concentrated by using a rotary vacuum evaporator at 55 °C until thick extract was obtained (Indonesian Ministry of Health 2006).

The percolation method was perform by measuring 900 mL 96% ethanol and then poured into 600 grams of purslane herb powder. The damp powder was stored into a percolator along with 1000 mL solvent, and then left for 2 hours. The percolates were collected together with the addition of the solvent continuously. Percolation was carried out until a clear solution obtained from the percolator. Percolates were concentrated by using a rotary vacuum evaporator at 55 °C until thick extract was obtained (Indonesian Ministry of Health 2000).

The reflux method was perform by weighed 600 grams of dried purslane herb powder and then stored into a round bottom flask, then 530 mL of 96% ethanol solvent was added until the dried powder was immersed in it. The reflux equipment was assembled, and the sample was extracted at 78 °C for 2 hours. The extracted filtrate was filtered with gauze and filter paper, and then stored into an erlenmeyer. This process was repeated for three times. The filtrate obtained was evaporated by using a rotary vacuum evaporator at 55 °C until a concentrated extract was obtained (List and Schmidt 2000).

The soxhlet method was carried out by weighed 600 grams of dried purslane herb powder then wrapped in filter paper and tied at both ends, after that it was inserted into the soxhlet extractor (Zhang *et al.* 2018). The ethanol was poured into a round bottom flask until it filled 2/3 parts of the flask. The extraction was carried out at 78 °C. The soxhlet was assembled with a condenser and extraction was carried out until the liquid in the siphon tube was colorless (clear). The filtrate obtained was evaporated by using a rotary vacuum evaporator at 55 °C until a concentrated extract was obtained.

**Antibacterial Activity Test.** Pure culture of *S. typhimurium* was obtained from the laboratory of microbiology, University of Muhammadiyah Semarang. Two point five (2.5) mL *Salmonella typhimurium* suspension was added into 22.5 mL of warm media Nutrient Agar and then poured into a sterile petri dish, and let stand for the media to become solid (test media). Ten (10)  $\mu$ L extract from various extraction methods with 30%, 35%, 40%, 45%, and 50% (b/v) concentration to organic solvent (dimethylsulfoxide/DMSO) respectively, were dropped onto the surface of the paperdisk in empty sterile petri dish and were left for 10 minutes. This procedure was also carried out for 10  $\mu$ L of DMSO solvent as the negative control. Paperdisk that already contained the test solution and DMSO is affixed to the surface of the test media. The paperdisk contain 30  $\mu$ g of chloramphenicol (positive control) was also attached to the test media. The petri dishes were then incubated for 24 hours at 37 °C. The test results were observed by looking at the zone of inhibition formed around the disk after the incubation period and then measured by using a caliper. The test was repeated for 3 times (Banjara *et al.* 2012). The data were analyzed statistically with 95% level of confidence.

The zone of inhibition resulted from maceration extract was 9.36-10.16 mm; percolation was 9.23-10.21 mm; reflux was 8.54-9.91 mm; and soxhlet was 9.52-10.20 mm (Table I.). The two way Anova test showed sig <0.05 in the zone of inhibition of all extracts from the four extraction methods, and the concentration series from each extraction method also showed significant differences ( $p < 0.05$ ). The zone of inhibition produced by chloramphenicol was very large (27.11-28.98 mm) compared to the maceration and reflux extract (8.54-10.16 mm), therefore the antibacterial activity was significantly different. Meanwhile, the difference with DMSO solvent is due to the fact that the solvent did not provide any inhibition, while the extract had a zone of inhibition in a certain range.

Result from Tukey's test for post-hoc analysis of the zone of inhibition in the percolated extract concentration series showed a significant difference between the 30-35% and 45-50% concentration. These results showed that the zone of inhibition from the 45-50% concentration was relatively greater compared to zone of inhibition from the lower concentrations (30-35%), and this was statistically different ( $p < 0.05$ ). Result from Tukey's test for post-hoc analysis of the zone of inhibition in the concentration series from

soxhlet extract showed a significant difference between low concentrations (30%) and high concentrations (50%). Meanwhile the other concentration series (35%, 40%, 45%) did not give a significant difference ( $p > 0.05$ ).

Extraction by maceration, percolation, reflux and soxhlet method resulted in 47.7, 66.3, 61.4, and 62.8 grams viscous extract respectively. The extract organoleptic from four extraction methods showed the same characteristics, which were blackish brown color, thick consistency and distinctive odor of purslane. The antibacterial activity test of purslane herb ethanol extract (PHEE) against *S. typhimurium* was shown by the presence of a radical inhibition zone in all test concentrations. The display of PHEE antibacterial test results from each extraction method could be seen in Figure 1. The radical inhibition zone was characterized by the presence of a clear area around the disk paper with no bacterial growth. If a radical inhibition zone was formed, an antibacterial agent could be considered to have bactericidal properties (Mycek 2001).

The statistic test result amongst the extraction methods showed that there were differences in the zone of inhibition produced by extract reflux method with the extracts of other methods including maceration, percolation and soxhlet. The extraction method of reflux produced the lowest zone of inhibition among the three other extraction methods, which meant that the reflux method caused the lowest PHEE antibacterial activity. Reflux method extraction was an extraction method that used heat by boiling the dried powder with a solvent. It was possible that the heat used in the process caused some of the active compounds to break down and affect its antibacterial activity against *S. typhimurium*. Li *et al.* (2017) reported the isolation of compounds from purslane herb, it had the oleraciamide A and oleraciamide B alkaloids. These compounds had 74.5-75.5 °C as its melting point which was lower than the boiling point of ethanol 96% (78 °C), so there is a possibility that these compounds was damaged due to the heat.

The other three extraction methods which were the maceration, percolation and soxhlet, did not present any difference in zone of inhibition statistically ( $p > 0.05$ ). This meant that there was no difference in PHEE antibacterial activity against *S. typhimurium*. From the three extracts produced, it meant that the antibacterial potential is likely same among these extracts. The soxhlet extraction method used heat in the process, but the dried herb powder was not boiled along with the solvent, so the process did not involve direct

Table 1 Antibacterial activity test results of the purslane herb ethanol extract with various extraction method against *Salmonella typhimurium*

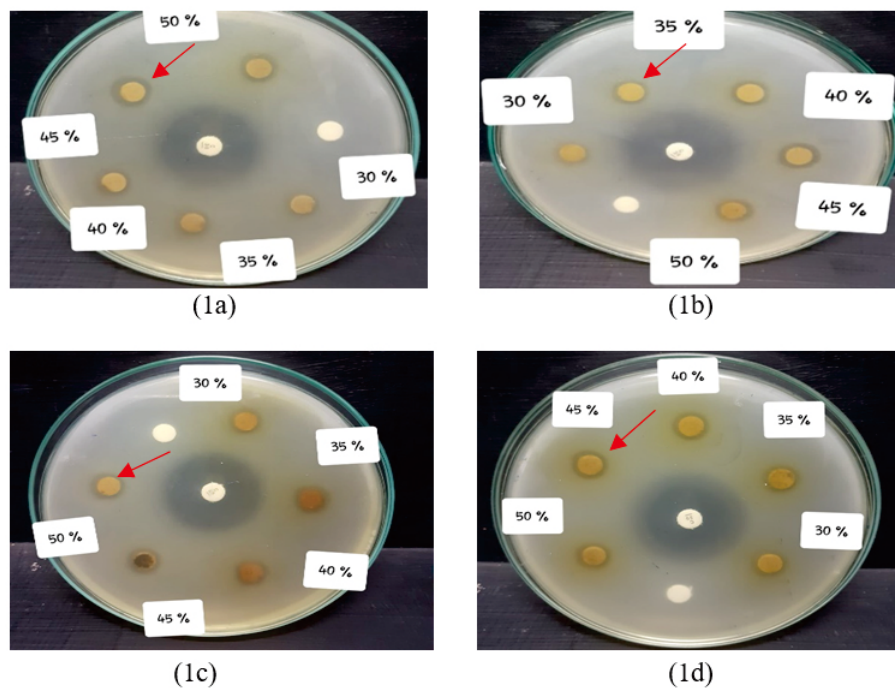
Extraction Method	Treatment	Zone of inhibition (mm)			Average ± SD
		1 <sup>st</sup> attempt	2 <sup>nd</sup> attempt	3 <sup>rd</sup> attempt	
Maceration	PHEE 3000 µg/disk	9.96	9.09	9.03	9.36±0.52 <sup>b</sup>
	PHEE 3500 µg/disk	9.97	9.71	9.33	9.67±0.32 <sup>b</sup>
	PHEE 4000 µg/disk	9.98	9.73	9.38	9.69±0.30 <sup>b</sup>
	PHEE 4500 µg/disk	10.00	9.91	9.39	9.76±0.32 <sup>b</sup>
	PHEE 5000 µg/disk	10.55	10.30	9.65	10.16±0.46 <sup>b</sup>
	Chloramphenicol 30 µg/disk	29.03	29.03	28.99	28.98±0.02
	DMSO solvent	-	-	-	-
Percolation	PHEE 3000 µg/disk	9.16	9.03	9.51	9.23±0.24 <sup>b</sup>
	PHEE 3500 µg/disk	9.44	9.39	9.54	9.45±0.07 <sup>b</sup>
	PHEE 4000 µg/disk	9.62	9.40	9.70	9.57±0.15 <sup>b</sup>
	PHEE 4500 µg/disk	10.15	9.85	10.51	10.17±0.33 <sup>b</sup>
	PHEE 5000 µg/disk	10.18	9.90	10.55	10.21±0.32 <sup>b</sup>
	Chloramphenicol 30 µg/disk	29.50	28.99	29.69	29.39±0.36
	DMSO solvent	-	-	-	-
Reflux <sup>a</sup>	PHEE 3000 µg/disk	8.55	8.26	8.82	8.54±0.28 <sup>b</sup>
	PHEE 3500 µg/disk	9.38	9.23	9.04	9.21±0.17 <sup>b</sup>
	PHEE 4000 µg/disk	9.54	9.29	9.12	9.31±0.21 <sup>b</sup>
	PHEE 4500 µg/disk	9.55	9.44	9.40	9.46±0.07 <sup>b</sup>
	PHEE 5000 µg/disk	9.57	10.04	10.12	9.91±0.29 <sup>b</sup>
	Chloramphenicol 30 µg/disk	29.14	26.10	26.10	27.11±1.75
	DMSO solvent	-	-	-	-
Soxhlet	PHEE 3000 µg/disk	9.31	9.55	9.72	9.52±0.20 <sup>b</sup>
	PHEE 3500 µg/disk	9.51	10.13	9.86	9,83±0,31 <sup>b</sup>
	PHEE 4000 µg/disk	9.67	10.16	9.93	9,92±0,24 <sup>b</sup>
	PHEE 4500 µg/disk	9.70	10.39	9.94	10,01±0,35 <sup>b</sup>
	PHEE 5000 µg/disk	10.25	10.40	9.97	10,20±0,21 <sup>b</sup>
	Chloramphenicol 30 µg/disk	29.20	29.10	28.91	29,07±0,14
	DMSO solvent <sup>l</sup>	-	-	-	-

PHEE : Purslane Herb Ethanol Extract

(-) : no inhibition zone was formed

(<sup>a</sup>) significantly different (p<0.05) from other extraction methods

(<sup>b</sup>) significantly different (p<0.05) against the positive control (chloramphenicol) and negative control (DMSO solvent) group



→ : radical zone

Fig 1 Antibacterial activity test results view of the purslane herb ethanol extract from the extraction methods with (1a) maceration, (1b) percolation, (1c) reflux and (1d) soxhlet against *Salmonella typhimurium*.



heating. This made the extract produced during the soxhlet process, still had antibacterial activity equivalent to cold extraction.

The zone of inhibition profiles resulted from maceration and reflux extracts were likely the same. Among all series of extract concentrations (30%, 35%, 40%, 45%, and 50%) there was no significant difference in the zone of inhibition ( $p > 0.05$ ), but all series of extract concentrations had significantly different of zone of inhibition compared to chloramphenicol as positive control group. This was due to the broad spectrum with high inhibitory effect from chloramphenicol to inhibit and kill bacteria.

High concentration extracts (45-50%) had greater chemical content than low concentration extracts (30-35%). Therefore, there was an increased inhibitory effect on the percolated extract against the growth of *S. typhimurium*. The antibacterial activity of the soxhlet extract at 30% concentration was the lowest compared to other concentration series. The heating process of dried purslane powder apparently very influential on its chemical content, especially compounds that were unstable to heat. The 30% concentration in the soxhlet extract also resulted in the smallest zone of inhibition compared to other concentrations (35-50%).

Based on the research results, with maceration, percolation and soxhlet extraction methods, extracts with the same antibacterial activity could be obtained. These three methods produced extracts with no significant difference in antibacterial activity ( $p > 0.05$ ). However, for the practical purposes of research, the percolation method had the highest yield value (11.05%) compared to maceration and soxhlet (7.95-10.47%). This meant that the percolation method could produce more extracts than the other two methods, and by using percolation extraction for the purslane herb, it would produce extracts that had antibacterial activity against *S. typhimurium* with more obtained extracts amount. From this research, it could be concluded that the purslane herb ethanol extract produced from various extraction methods (maceration, percolation, soxhlet, and reflux) had antibacterial activity against *S. typhimurium*. The antibacterial activity of the purslane herb ethanol extract produced by the reflux method was significantly different from other methods (maceration, percolation and soxhlet) against *S. typhimurium*.

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