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Active Compounds of Leaf Mint (*Mentha piperita*) Extract and *In Vitro* Antibacterial Inhibitory Effect

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***Abstract*—** *This research aimed to examine active substances and inhibitory effect of leaf mint (Mentha piperita) extract toward the growth of Lactic Acid Bacteria (LAB), Salmonella* sp. *and Escherichia coli. The materials were mint leaf, nutrient agar media, and other supporting laboratory equipments. The methods were laboratory experiment to both determine active compounds and in vitro determination of inhibitory effect. The design experiment was Completely Randomized Design which had 5 treatments, consisting of P0: aquadest, P1 addition of 0.01% antibiotic Zinc Bacitracin, P2 addition of leaf mint extract with concentration of 25%, P3 addition of leaf mint extract with concentration of 50% and P4 addition of leaf mint extract with concentration of 100%. Each treatment was repeated 3 times. The results on flavonoid, phenol and antioxidant of mint leaf water extract were 3.5%, 6.0% and 56%, respectively. On the inhibitory effect, mint leaf extract showed a significant effect (P<0.01) toward LAB, Salmonella* sp, *and Escherichia coli. Conclusion of this research was mint leaf extract contained important active compounds which showed an antibacterial effect against pathogenic bacteria.*

***Keywords*—** *Mint leaf extract, active substances, antibiotic, antibacterial effect.*

I. Introduction

The use of antibiotic growth promoter (AGP) for poultry has been banned more a decade ago in Europe, but it has just been implemented in Indonesia last year. The primarily concern of negative effect of AGP is with regard to the human health. Residue of antibiotic which might cause resistance of bacteria makes more difficult to cure diseases. Replacement of AGP gets a lot of intention from researchers around the world. More specifically, elaboration particularly from different natural herb products is widely attempted. Extract of turmeric [1] and soursop [2] leaves have been reported to show antimicrobial effect. In addition, mint extract has also been reported to have similar effect [3], [4], [5] and [6]. The report of [7] indicated that mint plant of *Mentha piperita* could kill *Bacillus fastidiosus, Staphylococcus aureus, Proteus mirabilis, Proteus vulgaris, Salmonella choleraesuis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Serratia odorifera*. It has been reported that each gram of mint leaf contained flavonoid of 1.84 mg, total phenol of 2.72 mg and antioxidant level of 72.5% [8][9]. Flavonoid is usually directly related with antimicrobial effect. It has an ability to destroy cell membrane of pathogenic bacteria, so that effect toxic of flavonoid might then cause disruption of DNA and protein synthesis leading to death.

The mint plant is a plant in the family of Labiatae, which has a specific smell and taste. It has been identified by [10] that there are 17 species of mint. Mentha local has 2 different verieties based on the flower colour, which are purple and white. Other varieties are for example *Mentha aquatic*, *Mentha piperita*, *Lavender mint*, *Lemon mint spp*, etc. The mint plant can be easily find in Indonesia, is *Mentha piperita*. It has commonly been used for spices, ornament of food, for aromatherapy, and other purposes. Less has been known with regard to the use of mint leaf as feed additive for poultry. The previous research results showed that mint leaf in the form of powder [11] and alcoholic extraction [12] on broiler performances had been reported. Lack of data examined the effect of mint leaf extract obtained by squeezing method. The current experiment aimed to report active substances contained in mint leaf extract and tested in vitro the effect of its product on antibacterial activities against *LAB, Salmonella* sp, and *Escherichia coli.*

II. Materials and Methods

A. Materials

Materials used in this research were mint leaf, Zinc Bacitracin as commonly used antibiotic, isolates of BAL, *Salmonella* sp, and *Escherichia coli*, Nutrient Agar, aquadest, alcohol 70%, yarn, craft paper, medical cotton, tissue, aluminum foil, label paper. The equipments used were cotton cloth, beaker glass, test tube, erlenmeyer, tube flask, micro pipet, Bunsen, incubator, caliper, vortex, blue tip, autoclave, magnetic stirrer, bar, and analytical balance.

B. Extraction of Leaf Mint

The leaf of mint was taken and separated from branch, then the only fresh leaf mint was put in cotton cloth and manually squeezed by hand to get the extract. From 1 kg of fresh leaf mint could be produced 280 ml extract.

C. Procedure of Testing Active Substances

C.1. Flavonoid measurement

Total flavonoid was tested according to [13] with modification as follow: 10 ml of acetyl-acetate fraction of leaf mint extract was put in the tube flask, added with 1 ml of $AlCI_3$ solution (2 g dissolved in 100 ml of acetate glacial-methanol). Then, another addition wad done up to 25 ml indicator in tube flask. Similar steps were also done for blank, but without addition of sample. The solution was then allowed to stand for 30 minutes before measurement with spectrophotometry at 425 nm wave length.

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C.2. Total Phenol Measurement

Total phenol was measured according to Pourmorad method [14]

C.3. Antioxidant measurement

Antioxidant activity was measured according to Molyneux method [15]

D. Microbiological Analysis

D.1. Sterilization of equipments

All equipments including petri disk, test tube, tube flask used were wrapped by using craft paper, roped and sterilized by using autoclave. The respective temperature, pressure and time were adjusted to 121°C, 1.5 atmosphere and 20 minutes.

D.2. Preparation of Nutrient Agar

Nutrient Agar was dissolved with aquadest at the ratio of 50 g Nutrient Agar : 1000 ml aquadest. The solution was then stirred and homogenized by using magnetic stirrer. Next, it was then being sterilized with condition similar to those of equipments except only taking 15 minutes. The sterilized solution was then distributed to each petri disk about a quarter of the volume or 15 ml.

D.3. Handling bacterial isolates

Each bacterial isolate was treated similarly, of which it was diluted with aquadest at the ratio of 1:10 and put into an Erlenmeyer of 250 ml. Then, 1 ml of isolate solution was taken, put into the petri disk and shaken by forming like 8 number till homogeny. The media was then dig by using blue tip having diameter of 6 mm to put sample. Then, 100 µl of sample according to the treatment was poured to the hole, and incubated 35-37°C for 24 hour.

E. Diameter of Inhibition Zone Measurement

Measurement was using caliper, it was done in 2 directions: horizontal and vertical on clear zone circle. The diameter of inhibition zone was measured by different between clear zone and original hole diameter.

Experimental Design

The experiment had 5 treatments consisted of:
P0: addition of 0.01% antibiotic Zinc Bacitracin
P1: addition of leaf mint extract with concentration of 25%
P2 addition of leaf mint extract with concentration of 50% and
P3 addition of leaf mint extract with concentration of 100%.

Each treatment was repeated 3 times, so there were 12 units of experiment.

F. Variables Observed

The variables observed in this study were:
a) Total flavonoid of ileal content and characteristics included intestinal length and weight,
b) Total phenol content,
c) Antioxidant level
d) Antibacterial activity against *Escherichia coli*, *Salmonella* sp. and *Lactic Acid Bacteria* (LAB)

G. Statistical Analysis

All data obtained in this research were analyzed with one way analysis of variance (ANOVA) and continued with Duncan's Multiple Range Test (DMRT) if there were significant differences.

III. Results and Discussion

A. Active substances of mint leaf extract. The results on analysis of active substances of mint leaf is showed in Table I:

TABLE I. Active compounds of Mint Leaf Extract

Compound Name	Concentration
Flavonoid	(1.32±0.02)%
Polyphenol	(5.76±0.01) % b/b
Antioxidant	(0.77) mg/ml

The results indicated that mint leaf contained flavonoid, polyphenol and antioxidant, but the concentration of each substance was less than previous report [8][9]. It was reported that each gram of mint leaf contained flavonoid of 1.84 mg, total phenol of 2.72 mg and antioxidant level of 72.5% when mint leaf was extracted by using alcohol or tested in the powder form. The different should be attributable to different method of extraction or preparation.

B. Effect of Leaf Mint Extract Concentration on Diameter of Inhibition Zone

One of indicator of antimicrobial activity of any herb or substance might be indicated by measuring diameter of inhibition zone. Each microbe will response differently toward an antimicrobial agent. It is expected that a good microbial agent will suppress microbial growth indicated by having larger diameter of inhibition zone, but it would not affect diameter zone of LAB. Effect of leaf mint extract concentration on diameter of inhibition zone of different microbial responses showed in Table II and pictures showed in the figure 1.

TABLE II. Diameter of inhibition zone after exposure toward *Escherichia coli*, *Salmonella* sp. and LAB

Treatment	Diameter of Inhibition Zone (mm)		
	Escherichia coli	*Salmonella* sp.	LAB
P0	6.80 ± 1.35^{a}	3.07 ± 1.62^{a}	6.80 ± 1.35^{a}
P1	15.20 ± 3.21^{b}	14.45 ± 2.34^{b}	16.33 ± 2.88^{b}
P2	18.08 ± 0.98^{b}	13.68 ± 5.69^{b}	21.20 ± 3.98^{b}
P3	17.87 ± 1.15^{b}	22.42 ± 7.18^{b}	25.95 ± 4.83^{b}

Notes: The different superscripts in the same row showed highly significant differences (P<0.01), and ns indicated no significant effect

Effect of leaf mint extract on diameter of inhibition zone showed that statistically significant increases were noted to all microbes (P<0.01). This indicated that the growth of pathogenic microbes (*Escherichia coli* and *Salmonella* sp) as well as non-pathogenic LAB could be inhibited by leaf mint extract regardless the concentration used. The suppression of LAB might be negatively affect the growth performance of poultry, however, the inhibitory effect of mint leaf extract is as expected. The previous reported finding [16] showed similar result when flavonoid containing stem or leaf of mint or [17] for skin root of awar-awar plant. Based on membrane structure which contains peptidoglycan, it was reported by [18] that membrane cell of pathogenic bacteria is thinner than non-pathogen one.

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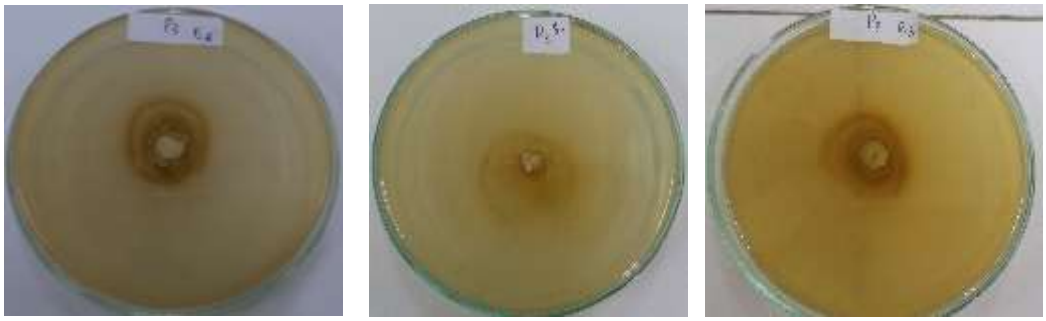

Escherichia coli *Salmonella* sp. LAB

This makes flavonoid could easily penetrate the cell wall to disrupt metabolic process resulted a death cell. The ability of flavonoid to inhibit or even kill bacteria has also been reported [19]. On the other hand, non-pathogenic bacteria which grouped as LAB have a thicker cell wall. It is expected to more resistant toward flavonoid exposure, in fact, flavonoid might positively increase clear zone diameter indicated it might inhibit or kill LAB. Previous report [20] said that the addition of date juice inhibit or kill LAB.

IV. Conclusion

Based on research results, it could be concluded that leaf mint extract contains active substances of flavonoid and total phenol and shows antioxidant activity. In addition, the use of leaf mint extract positively inhibit pathogenic bacteria (*Escherichia coli* and *Salmonella* sp) and also non-pathogenic LAB.

Reference

[1] Widodo, E. 2015. Selected Plant Phytobiotics Feed Additive Based On Antimicrobial Property And Application in Meat-Type Duck. Proceeding of International seminar improving tropical animal production for food securty, Universitas Halu Oleo, Kendari, Southeast Sulawesi, Indonesia, 3-5 November, 2015. P. 80-86.

[2] Sari. Y. D., S. N. Djannah, dan L. H. Nurani. 2010. Uji Aktivitas Antibakteri Infusa Daun Sirsak (*Annona muricata* L.) Secara in Vitro terhadap *Staphylococcus aureus* ATCC 25923 dan *Escherichia coli* ATCC 35218 Serta Profil Kromatografi Lapis Tipisnya. *J. Kes Mas*, 4(3): 144-239.

[3] Mehri, M., V. Sabaghi, F. Bagherzadeh-Kasmani. 2015. Mentha piperita (peppermint) in growing Japanese quailsdiet: Performance, carcass attributes, morphology andmicrobial populations of intestine. J. Animal Feed Science and Technology, 207: 104–111.

[4] Talpur, A. D. 2014. Mentha piperita (Peppermint) as feed additive enhanced growth performance, survival, immune response and disease resistance of Asian seabass, Lates calcarifer (Bloch) against Vibrio harveyi infection. J. Aquaculture, 420(421): 71–78.

[5] Emamia, N. K., A. Samiea, H.R. Rahmania, C.A. Ruiz-Feria. 2012. The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. J. Animal Feed Science and Technology, 175: 57– 64.

[6] Durrani, F.R., Abidullah, N. Chand, Z. Durrani and S. Akhtar. 2008. Hematological, Biochemical, Immunomodulatory and Growth Promoting Effect of Feed Added Wild Mint (Mentha Longifolia) in Broiler Chicks. J. Agric, 24(4): 661-664.

[7] Al-Sum, Basheer A. Al-Arfaj, Abdullah A. 2013. Antimicrobial activity of the aqueous extract of mint plant. *Science*, 2(3): 110-113.

[8] Ameri, S. A., F. Samadi, B. Dastar and S. Zerehdaran. 2016. Effect of Peppermint (*Mentha piperita*) Powder on Immune Response of Broiler Chickens in Heat Stress. *Iranian Journal of Applied Animal Science*, 6(2): 435-445.

[9] Khodadust, M. R., F. Samadi, F. Ganji, Y. J. Ahangari and G. H. Asadi. 2015. Effects of peppermint (Mentha piperita L.) Alcoholic extract on carbon tetrachloride-induced hepatotoxicity in broiler chickens under heat stress condition. Poultry Science Journal 3(1): 1-16.

[10] Erum, S., M. Naeemullah and S. Masood. 2012. "*Phenotypic Variation Among Mentha* Spp". *Pakistan J. Agric. Res*., 25(1): 55-62.

[11] Ocak, N., Erener, G., Burak Ak, F., Sungu, M., Altop, A., and Ozmen, A. (2008). Performance of broilers fed diets supplemented with dry peppermint (Mentha piperita L.) or thyme (Thymus vulgaris L.) leaves as growth promoter source. *Czech Journal of Animal Science*, *53*(4), 169.

[12] Nanekarani, S., Goodarzi, M., Heidari, M., and Landy, N. (2012). Efficiency of ethanolic extract of peppermint (Mentha piperita) as an antibiotic growth promoter substitution on performance, and carcass characteristics in broiler chickens. *Asian Pacific Journal of Tropical Biomedicine*, *2*(3), S1611-S1614.

[13] Chang, C. C., Yang, M. H., Wen, H. M., and Chern, J. C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*, *10*(3). 178-182.

[14] Pourmorad, F., Hosseinimehr, S. J., and Shahabimajd, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African journal of biotechnology*, *5*(11). 1142-1145.

[15] Molyneux, P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*, *26*(2), 211-219.

[16] Saeed, S and P. Tariq. 2005. Antibacterial Activities of Mentha Piperita, Pisum Sativum and Momordica Charantia. Pak. J. Bot., 37(4): 997-1001.

[17] Sukadana, I. M. 2010. Aktivitas Antibakteri Senyawa Flavonoid Dari Kulit Akar Awar-Awar (*Ficus septica Burm F*). *Jurnal Kimia*, 4(1): 63-70.

[18] Razak, A., A. Djamal, dan G. Revilla. 2013. Uji Daya Hambat Air Perasan Buah Jeruk Nipis (Citrus aurantifolia s.) terhadap Pertumbuhan Bakteri *Staphylococcus Aureus* Secara In Vitro. *Jurnal Kesehatan Andalas*, 2(1): 5-8.

[19] Lestariningsih, O. Sjofjan dan E. Sudjarwo. 2015. Pengaruh Tepung Tanaman Meniran (*Phyllanthus niruri Linn)* Sebagai Pakan Tambahan terhadap Mikroflora Usus Halus Ayam Pedaging. *J. Agripet*, 15(2): 85-91.

[20] Ngajowa, M., J. Abidjulua danV. S. Kamua. 2013. Pengaruh Antibakteri Perasan Kulit Batang Matoa (*Pometia pinnata*) terhadap Bakteri *Staphylococcus aureus* secara In vitro. *Jurnal Mipa Unsrat Online*, 2(2):128-132.