Afduha Nurus Syamsi et al/Animal Production. 21(1):38-42, 2019
Accredited by Kemenristek Dikti No 32a/E/KPT/2017. ISSN 1411-2027

Inhibition Activity of Garlic (Allium sativum) Skin Extract on Mastitis Causing Microorganisms

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Abstract. The study was aimed to identify the effectiveness of the inhibitory activity of garlic skin extract (GSE) with different concentrations on the growth of *Staphylococcus aureus, Streptococcus mutants, Escherichia coli*, and *Candida albicans*. The study used the Kirby-Bauer method in a completely randomized (CRD) design with five treatments (positive control, negative control, 5% GSE, 10% GSE, and 15% GSE) and three replicates. The extract was obtained through evaporation of garlic skin macerated with aquadest solvent. The data were subjected to ANOVA, continued with an Honestly Significant Difference (HSD) test. The results showed that GSE concentration (minimum 5-10%) was highly significant to inhibit the growth of mastitis-causing microorganisms.

Keywords: mastitis, microorganisms, dairy, garlic skin extract, inhibition activity

Abstrak. Penelitian ini bertujuan untuk mengidentifikasi efektivitas daya hambat ekstrak kulit bawang putih (EKBP) dengan konsentrasi berbeda terhadap pertumbuhan *Staphylococcus aureus*, *Streptococcus mutants*, *Escherichia coli* dan *Candida albicans*. Penelitian menggunakan metode Kirby-Bauer dengan rancangan acak lengkap yang terdiri atas 5 perlakuan (kontrol positif, kontrol negatif, EKBP 5%, EKBP 10%, dan EKBP 15%), masing-masing di ulang sebanyak 3 kali. Ekstrak didapatkan melalui evaporasi kulit bawang putih yang dimaserasi dengan pelarut aquadest. Data penelitian dianalisis menggunakan ANOVA dan dilanjutkan dengan uji beda nyata jujur. Hasil menunjukkan bahwa EKBP berpengaruh sangat nyata terhadap daya hambat pertumbuhan mikroorganisme penyebab mastitis. Berdasarkan hasil penelitian dapat diambil simpulan bahwa EKBP memiliki kemampuan penghambatan terhadap mikroorganisme penyebab mastitis dengan konsentrasi minimum 5-10%.

Kata kunci : mastitis, mikroorganisme, ternak perah, ekstrak kulit bawang putih, aktivitas daya hambat

Introduction

Mastitis is a common disease in mammals including dairy livestock industry, which is very detrimental since it decreases the quantity and quality of milk production. Surjowardojo et al. (2009) stated that the negative impact of mastitis is the reduction of milk production up to 4.4-8.3 L/day or equal to 28.4-53.5% of healthy cattle's milk production. Firmansyah et al. (2013) explained that mastitis could reduce milk quality, as marked with reduction of protein, fat and lactose contents. Also, mastitis may result in livestock death.

In general, mastitis is caused by various microorganisms, such as Streptococcus agalactiae, S. disgalactiae, S. uberis, S. zooepidermicus, Staphylococcus aureus, S. epidermidis, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Klebsiela, Mycoplasma spp., Candida spp., Geotrichum spp. and Nocardia spp. (Riyanto et al., 2016; Hameed et al., 2006). Rady and Sayed (2009) reported that the main agents of subclinical cause of mastitis include Staphylococcus aureus (52.5%), Streptococcus agalactiae (31.25%), and Escherichia coli (16.25%). This is supported by Sani et al. (2011) that the major bacterial cause of mastitis includes Staphylococcus and Streptococcus. On the contrary, yeast group causes more clinical mastitis. According to Hastiono (1984), yeast species that often attack Indonesian dairy cattle includes Geotrichum spp., Rhodoturulla spp., Candida spp. and Saccharomyces spp. Ahmad (2011) stated that the highest prevalence of yeasts responsible for mastitis involved Saccharomyces spp. (37.5%) and Candida spp. (33.7%); however, Candida spp. contributes more to mycotic mastitis

A common preventive measure for mastitis is *teat dipping* procedure; the teat is dipped into germicidal fluid such as iodine, chlorine, dodecyl benzene sulfonic acid (DDBSA), glycerol monolaurate, and nisinwhich may reduce mastitis occurrence up to 50-95% (Nickerson, 2001). The germicidal fluid used in Indonesia is commercial iodine solution which is known effective to prevent mastitis occurrence on dairy livestock, but it will leave residue in the milk. According to Boodie and Nickerson (1989), repeated usage of 1% iodine in teat dipping solution will leave iodine residue in the milk for about 80-100 µg/L. Iodine residue is hazardous to human health if continuously consumed. Therefore, a safe and cheap natural antimicrobial alternative with capability equal to or better than iodine is required.

A material which potentially serves to be a natural antimicrobial function which is not much studied is garlic (*Allium sativum*) skin. Garlic skin is a waste of agricultural industry which is not yet maximally utilized and wasted away. Indonesia Ministry of Agriculture (2016) reported that Indonesian garlic production in 2015 reached 20,294 tons. According to Stefania et al. (2015), the morphology of garlic consists of 88% tuber and 12% tuber skin. This shows that there is potentially 2,435.28 tons of garlic skin waste to be utilized. A phytochemical screening test showed that garlic skin contained bioactive compounds, i.e. alkaloid, quinone, flavonoid, saponin, and polyphenol (Wijayanti and Rosyid, 2015). Ifesan et al. (2014) reported that garlic skin ethanol extract contained 355.50 µg/ml polyphenol and 33.27 µg/ml flavonoid, including allicin group. Naganawa *et al*. (1996) stated that allicin could extensively inhibit microbial growth including bacteria (Gram positive and negative), protozoa, yeast and fungi.

Therefore, it is important to investigate garlic skin extract on the growth of the main microorganism, particularly those causing mastitis in vitro. The information regarding garlic skin potential in terms of availability and bioactive content is the basis that its ability as an antimicrobial agent for mastitis in dairy cattle needs to be assessed. The research is conducted to examine the effect of garlic skin extract on the growth of the main mastitis-causing microorganism, and to identify the effective concentration of garlic skin extract to inhibit the growth the main mastitis-causing microorganism.

Materials and Methods

The materials used are garlic (*Allium sativum*) skin, cultures of *Staphylococcus aureus, Streptococcus mutants, Escherichia coli* and *Candida albicans*, Nutrient Agar (NA) media, Manitol Salt Agar (MSA), Potato Dextrose Agar (PDA), Nutrient Broth (NB), Potato Dextrose Broth (PDB), aquadest, alcohol 70%, physiological solution NaCl 0.85%, aluminum foil, cotton, Whatmann paper no.41, and *paper disc* with diameter 6 mm. The experiment device included autoclave, incubator, rotary evaporator, Petri dish, Erlenmeyer flask, graduated cylinder, analytical balance, vortex, micropipette, sample bottle and Laminar Air Flow (LAF).

The garlic skin (simplicia) was washed under flowing water, finely sliced and oven-dried at maximum temperature of 60°C. The simplicia then mashed in a blender, sieved with a mesh sieve 12, and weighed 2x50 gr for extraction process using a rinse method. Each simplicia portion was soaked in aquadest solvent with a ratio 1:10 (1 gr simplicial:10 ml solvent) for about 3x24 hours, and then filtrated using Whatman paper 41. Afterwards, the solution was solidified using a rotary vacuum evaporator at 50°C (Shams et al., 2003).

The *Staphylococcus aureus, Streptococcus mutants,* and *Escherichia coli* cultures were recultured on NA medium, and *Candida albicans* culture on PDA medium. The inhibitory power of garlic skin extract was tested using the broth culture of each isolate. *Staphylococcus*

aureus, Streptococcus mutants, and *Escherichia coli* were grown on the NB medium and the *Candida albicans* culture on PDB medium.

The inhibitory activity of garlic skin extract was tested using the Kirby-Bauer method (Hudzicki, 2016) as follows:

1. The tested microorganism was inoculated using a pour plate method on the MSA medium for *S. aureus, Str. mutants,* NA medium for *E. coli*, and PDA medium for *C. albicans*.
2. 20 µL garlic skin extract was dripped on the paper disc and put on the test microbial culture.
3. The *S. aureus, Str. mutants,* and *E. coli* cultures were incubated for 1-2x24 hours, and *C. albicans* culture for 3-5x24 hours.
4. The diameter of inhibitory zone was observed and measured using the following formula:

$$\frac{D1+D2+D3+...+Dn}{n}=.....cm$$

The experiment used a completely randomized design (CRD) with five treatments and three replications each. The data were subjected to ANOVA and continued with an Honestly Significant Difference (HSD) test. The examined treatments were as follows:

P1: Positive control (without treatment)
P2: Negative control (iodine 10%)
P3: Garlic skin extract 5%
P4: Garlic skin extract 10%
P5: Garlic skin extract 15%

Results and Discussions

Analysis of variance result showed that the garlic skin extract was highly significant (P <0.01) in inhibiting the growth of mastitis-causing microorganisms. Also, aqueous garlic skin extracts effectively inhibited the growth of *S. aureus*, *Str. mutants* and *C. albicans*, but less effective on *E. coli* (Table 1). Lupoae et al. (2013) stated that the bioactive content of garlic plant is the water-soluble organosulphur compound, which includes thiosulfinate as the potential antibiotic. Hughes and Lawson (1991) stated that the antimicrobial activity of garlic plant would deplete if one content of thiosulfinate compounds (allicin) is eliminated. Similarly, when allicin is reduced to diallyl-disulfide, the antibacterial activity would greatly suffer.

The aqueous garlic skin extract in this research was tested using the Kirby-Bauer method and showed an inhibitory activity against the tested microbes. Using a different test method, Rambet et al. (2017) reported that 25% squeezed garlic concentration effectively inhibited the growth of *C. albicans*. Meanwhile, Shams et al. (2003) claimed that 10% aqueous garlic extract exhibited an antifungal activity. Comparatively, this study reported that 5% garlic skin extract was effective to inhibit the growth of *C. albicans* compared to positive or negative controls.

C. albicans' growth inhibitory activity by the bioactive compounds in garlic skin is to inhibit lipid synthesis. Adetumbi et al. (1986) stated that nucleic acid and protein synthesis inhibition by aqueous garlic extract was in line with the growth of *C. albicans*, but lipid synthesis was absent. It indicated that lipid synthesis blockade was garlic's main activity against pathogenic microbes.

Candida albicans is a unicellular fungus with different characteristics from bacteria such as *S. aureus, Str. mutants* and *E. coli. E. coli* is a Gram negative bacteria with a peptidoglycan layer on the cell wall and an outer membrane (lipid layer) which prevents the external antibacterial compound's activity. When a Gram negative bacterium is exposed to aqueous garlic extract, the lipid layer on the outer membrane will dissolve into a hole to inhibit cell membrane permeability and cause cell death (Zaika, 1988).

Allicin blocked lipid synthesis and suppressed RNA synthesis rate to inhibit the growth of *S. aureus* and *Str. mutants* (Gram

Table 1. The inhibition zone of aqueous garlic skin extract against microbes

Treatment	Inhibition zone (mm)			
	S. aureus	*Str. mutans*	*E. coli*	*C. albicans*
positive control	34.67 ± 0.33[a]	35.33 ± 25.33[a]	20.60 ± 3.13[a]	7.40 ± 0.73[c]
negative control	6.00 ± 0.00[d]	6.00 ± 0.00[c]	6.00 ± 0.00[c]	6.00 ± 0.00[c]
5% extract	8.33 ± 0.00[cd]	13.17 ± 0.00[b]	6.00 ± 0.00[c]	23.33 ± 0.00[b]
10% extract	15.33 ± 0.00[bc]	14.83 ± 0.00[b]	10.67 ± 0.00[b]	28.33 ± 0.33[a]
15% extract	19.25 ± 0.00[b]	15.67 ± 0.00[b]	12.67 ± 0.00[b]	27.00 ± 0.33[ab]

[abc] Values bearing equal superscript within column are not significantly different (P>0.05).

positive bacteria). Deresse (2010) stated that lipid on Gram positive bacteria's cell wall help allicin penetrate into the membrane and inhibit RNA synthesis rate.

Based on Kirby-Bauer test, this study reported that the minimum concentration of aqueous garlic skin extract to inhibit microbial growth was from 5 to 10%. Table 1 shows that the inhibition zone increases at a concentration of 5% to 10%, but not significantly different at higher concentrations (15%). It was in line with Nejad et al. (2014) that the minimum concentration of aqueous garlic extracts to inhibit bacterial growth is 7.5%.

Conclusions

Garlic skin extracted using aquadest could inhibit the growth of mastitis-causing microorganism. The minimum concentration of aqueous garlic skin extract to effectively inhibit the growth of microorganism causing mastitis is ranged from 5-10%.

Acknowledgement

We would like to thank the Research and Community Service Institution, Jenderal Soedirman University for funding this research through BLU's 2018 *Riset Dosen Pemula* grant scheme.

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