Media Peternakan, December 2011, pp. 155-158
EISSN 2087-4634
Accredited by DGHE No: 66b/DIKTI/Kep/2011

Online version:
http://medpet.journal.ipb.ac.id/
DOI: 10.5398/medpet.2011.34.3.155

Antibacterial Activity of Garlic Extract Against some Pathogenic Animal Bacteria

M. Safithri[a], M. Bintang[a], & M. Poeloengan[b]
[a]Department of Biochemistry, Faculty of Mathematic and Natural Science
Bogor Agricultural University, Darmaga Campus, Bogor 16680
[b]Indonesian Research Center for Veterinary Science
Jln. R.E. Martadinata No. 30, Bogor 16114 Indonesia
(*Received 14-12-2009; accepted 22-07-2011*)

ABSTRAK

Aktivitas antibakteri ekstrak bawang putih terhadap beberapa jenis bakteri Gram positif maupun Gram negatif sudah banyak dipelajari. Namun demikian, aktivitas antibakteri ekstrak bawang putih terhadap beberapa bakteri patogen pada ternak di Indonesia, belum banyak diketahui. Tujuan penelitian ini adalah untuk mempelajari potensi ekstrak air dan etanol bawang putih terhadap bakteri *Salmonella typhimurium* penyebab penyakit pada ayam, dan bakteri *Streptococcus agalactie*, *Escherichia coli*, dan *Staphylococcus aureus* penyebab mastitis pada sapi perah. Ekstrak bawang putih yang digunakan untuk menentukan aktivitas antibakteri terhadap *S. typhimurium* adalah filtrat hasil perasan bawang putih segar dengan konsentrasi 5%, 10%, 20%, 30%, dan 40% b/v, sedangkan ekstrak bawang putih yang digunakan untuk menentukan aktivitas antibakteri terhadap *S. agalactie*, *E. coli*, dan *S. aureus* adalah ekstrak air dan ekstrak etanol bawang putih dengan konsentrasi 5%, 10%, 15%, 20%, and 25% b/v. Hasil penelitian menunjukkan bahwa aktivitas antibakteri filtrat bawang putih 30% setara dengan tetrasiklin 10% terhadap *S. typhimurium*. Selanjutnya, untuk aktivitas antibakteri terhadap *S. agalactie*, *E. coli*, dan *S. aureus*, ekstrak air bawang putih menunjukkan hasil yang lebih baik jika dibandingkan dengan ekstrak etanol bawang putih. Ekstrak air bawang putih dengan konsentrasi 20% memiliki aktivitas yang setara dengan 0,01% ampisillin terhadap *S. galactie*, *E. coli*, dan *S. aureus*. Ekstrak bawang putih dapat digunakan untuk menghambat pertumbuhan *S. typhimurium* dan bakteri penyebab mastitis.

Kata kunci: antibakteri, bawang putih, bakteri patogen, mastitis

ABSTRACT

The antimicrobial activity of garlic extract against Gram-positive and Gram-negative bacterial isolates was well studied. However, reports on antibacterial activity of garlic extract against some pathogenic bacteria in animals in Indonesia, are still limited. Therefore, the aim of this study was to evaluate the antibacterial activity of water and ethanol extracts of garlic against *Salmonella typhimurium* in chickens, and *Streptococcus agalactie, Escherichia coli,* and *Staphylococcus aureus* causing mastitis in dairy cows in Indonesia. A filtrate of fresh garlic was used to determine the antibacterial activity against *S. typhimurium* at concentrations of 5%, 10%, 20%, 30%, and 40% w/v, whereas, the antibacterial activity of water and ethanol extracts was determined against *S. agalactie, E. coli,* and *S. aureus* at concentrations of 5%, 10%, 15%, 20%, and 25% w/v. Results showed that antibacterial activity of 30% garlic filtrate was equivalent to 10% tetracycline. Meanwhile, antibacterial activity of garlic aqueous extract on mastitis bacteria was better than that of the garlic ethanol extract. Aqueous extract of garlic at 20% had the same antibacterial activity as 0.01% ampicillin on mastitis bacteria. Filtrates of fresh garlic can be used to inhibit growth of *S. typhimurium* and mastitis bacteria.

Key words: antibacterial, garlic, pathogenic animal bacteria, mastitis

* Corresponding author:
Telp/fax: +62 251 8623247, E-mail: safithri@ipb. ac.id

INTRODUCTION

Garlic (*Allium sativum*) can be used as a spice in food and medicine (Ross *et al.*, 2001). Garlic has antibacterial activity against bacterial pathogens including Gram positive and Gram negative bacteria (Sadeghian & Ghazvini 2002; Iwalokun *et al.*, 2004; Shokrzadeh & Ebadi 2006; Eja *et al.*, 2007; Jazani *et al.*, 2007; Durairaj *et al.*, 2009). A bioactive compound in garlic that has antibacterial activity is allicin, which is a volatile compound containing sulfur (Harris *et al.*, 2001; Johnston, 2002). Other bioactive compounds namely, dialildisulphide, and dialiltrisulphide, have antibacterial activity (Avato *et al.*, 2000; Tsao & Yin 2001a; Tsao & Yin 2001b).

The use of several types of antimicrobial agents in the field of medicine, the food industry, feed industry, and crop protection have led to resistance, thereby reducing the effectiveness of these antimicrobial agent. The use of antibiotics has been controversial, because it can lead to residues and resistance in animals so it would be dangerous to the consumer. The study of molecular techniques have shown that the use of antibiotics in animal feed contributed to the occurrence of antimicrobial resistance in humans (McEwen & Fedorka-cray 2002; Swartz 2002), which resulted in high rates of morbidity and mortality and rising health costs (WHO, 2005). Besides antibiotic residues problems, the use of antibiotics also has an impact on bacterial resistance. The results of Salmonella isolates from samples of chicken carcasses obtained from an existing market in Jakarta, showed 14.28% of isolates of *S. enteritidis* resistant to chloramphenicol, while those resistant to tetracycline and amoxicillin were 28.57%. The resistance level of isolates of *S. hadar* was 12.5% to chloramphenicol, 50% to amoxicillin, and 75% to tetracycline, and it was found that one isolate of *S. enteritidis*, and one isolate of *S. hadar*, was resistant to the three antibiotics tested (Noor *et al.* 2006).

Antibacterial activity of aqueous extracts of garlic showed satisfying results on 17 types of pathogenic bacteria in humans such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus* spp. that were resistant to several types of antibiotics. Aqueous extracts of garlic had a minimum inhibitory concentration of 6-11 mg/ml for Gram-positive bacteria, and 7-21 $mg.ml^{-1}$ for Gram negative (Durairaj *et al.*, 2009). In addition, aqueous extracts garlic also had antibacterial activity against bacteria that was found for aquaculture products including *Citrobacter freundii*, *E. coli*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*, with minimum inhibitory concentration ranging from 7.81 to 62.5 $mg.ml^{-1}$ (Seong & Musa 2008). Nevertheless, only little research has been done to determine the antibacterial activity of garlic extract against bacterial pathogens in farm animals in Indonesia. Therefore, this study aims to determine the antibacterial activity of garlic extract on *S. typhimurium* mostly found in poultry in Indonesia, and mastitis bacteria (*Streptococcus agalactie*, *E. coli*, and *S. aureus*) on dairy cattle in Indonesia.

MATERIALS AND METHODS

Materials

Garlic was taken from Pasar Induk Kemang, Bogor. *S. typhimurium*, *S. agalactie*, *E. coli*, and *S. aureus* were taken from collections of BALITVET (Veterinary Research Institute), Cimanggu Bogor. The media used to grow *S. typhimurium* were nutrient broth (NB) and nutrient agar (NA), while for mastitis bacteria used blood agar, Mac Conkey agar, and PYG (peptone, yeast, and glucose) liquid and agar. Materials for extraction were distilled water and 96% v/v ethanol, obtained locally.

Methods

Preparation of garlic extracts for antibacterial *S. typhimurium* activity test. Ten grams of garlic was peeled, sliced into small pieces and mashed in a mortar. The mash was then separated from the filtrate with the residue using filter paper. The filtrate was used for testing antibacterial at concentrations of 5%, 10%, 20%, 30%, and 40% w/v with distilled water as solvent (Kusmiyati & Agustini, 2007).

Preparation of garlic extracts for *S. agalactie*, *E.coli*, and *S. aureus* antibacterial activity test. Garlic sliced up to 2 mm in thickness, were dried in an oven at 40-50 °C for 30-36 hours, so that the moisture content was 8%. Garlic was then milled and screened, to produce powder size of 30 mesh and aliquots weighed of 5, 10, 15, 20, and 25 g. The powder was dissolved in distilled water to give a final volume of 100 ml, and mixed using a vortex. The solution was filtered and the filtrate was used for antibacterial activity tests.

Garlic-ethanol-extract was made by using 100 g of garlic powder, with a size of 30 mesh, macerated with 200 ml of 96% ethanol, using a shaker at room temperature for 24 hours. The suspension was concentrated by rotary evaporation at 50 °C, and then distilled to remove volatile fractions. The non-volatile fraction was used for antibacterial activity testing (Kusmiyati & Agustini, 2007).

Antibacterial-activity-tests. One ose bacterial culture was grown in 10 ml of NB, then incubated with shaker at 37 °C for 24 hours. 50 µl of these cultures (10^8 CFU/ml) were inoculated in 20 ml of NA. Wells were made with a diameter of 5.5 mm using a puncher, and was inserted 100 µl of garlic extract into the well. NA were incubated at 37 °C for 24 hours. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. Each experiment was repeated at three times and mean of the diameter of inhibition zones was calculated (Kusmiyati & Agustini, 2007).

Statistical Analysis

All the values of inhibition zone were analyzed using ANOVA and Tukey test. Differences between groups were considered significant at $P<0.05$ levels.

RESULTS AND DISCUSSION

The results of the inhibition zone measurement, using the filtrate of fresh garlic against *S typhimurium*, showed that the higher concentration of the filtrate of fresh garlic, the stronger the antibacterial activity as indicated by the larger zone of inhibition (Table 1). In inhibiting the growth of *S. typhimurium*, 30% filtrate of fresh garlic had antibacterial activity similar to 10% tetracycline (P<0.05). Durairaj *et al.* (2009) reported that the antibacterial activity of 25% filtrate of fresh garlic could inhibit *S. typhimurium* with inhibition zone was 8 mm. Those results did not differ with the results of this study; the 30% filtrate fresh garlic could inhibit *S. typhimurium* (8.75 mm). The antibacterial activity of garlic was lower compared to the garlic water extract. The inhibition zone of 10% filtrate of fresh garlic against *S. typhimurium* was 4.54 mm, while the results of Suharti *et al*. (2005) research showed 10% garlic aqueous extract had 8.00 mm inhibition zones. It showed that filtrate of fresh garlic had less bioactive compounds to inhibit the growth of *S. typhimurium* than that of garlic aqueous extract. It assumed that allicin in filtrate of fresh garlic began to break down quickly to produce diallyl sulphide. It did not have the strong antibacterial properties of allicin (Verma & Verma, 2008).

The results of the inhibition zone measurement of aqueous garlic extract against *S. agalactie*, *S. aureus*, and *E. coli* mastitis bacteria, showed that the higher concentration of garlic water extract, the fewer bacteria could grow, as indicated by the larger inhibition zone (Table 2). The antibacterial activity of 10% garlic water extract against *S. agalactie* was greater (P<0.05) than *S. aureus* and *E. coli*. The antibacterial activity of garlic water extract against *S. agalactie* did not differ with ampicillin 0.01% started at concentration 10% b/v, while against *S. aureus* and *E. coli* started at 20% b/v and 15% b/v, respectively(Table 2).

The result of inhibition zone measurement of garlic ethanol extract on *S. agalactie*, and *E. coli* mastitis bacteria, showed that the higher concentration of garlic ethanol extract, the more bacteria were inhibited to grow, as indicated by the larger inhibition zone. There was no inhibition zone of garlic ethanol extract on *S. aureus* (Table 3) at all concentrations tested. It had shown that 5%-25% garlic ethanol extract did not inhibit grow of *S. aureus*. The antibacterial activity of 10% garlic ethanol extract against *S. agalactie* was greater than for *E. coli*, but there was no difference in 15%-25%. In addition, 10%-25% garlic ethanol extract had antibacterial activity substantially lower than ampicillin 0.01% (Table 3). Thus, the use of ethanol to extract antibacterial bioactive compounds in garlic is not effective. This was consistent with the results of research by Sadeghian & Ghazvini (2002) which showed that the 17% w/v garlic methanol extract was able to inhibit 2% the Shigella bacteria. This indicates that bioactive compounds which acts as antibacterial compounds has a high polarity (Cuttler & Wilson, 2004).

Table 1. Diameter of inhibition zone of fresh garlic filtrate against *S. typhimurium*

Garlic concentration (% b/v)	Inhibition zone (mm)
0	0.00±0.00^{a}
5	3.75±0.63^{a}
10	4.54±0.32^{a}
20	7.54±0.19^{ab}
30	8.75±0.13^{bc}
40	10.08±1.70^{bc}
10% Tetracycline	11.69±0.44^{c}

Note: Means in the same column with different superscript differ significantly (P<0.05).

Table 2. Diameter of inhibition zone of garlic water extract against mastitis bacteria

Garlic concentration (% b/v)	Inhibition zone (mm)		
	S. agalactie	*S.aureus*	*E. coli*
0	0.00±0.00^{i}	0.00±0.00^{i}	0.00±0.00^{i}
5	9.17±0.23^{fg}	7.30±0.26^{h}	7.73±0.71^{gh}
10	12.50±0.15^{de}	9.83±0.51^{f}	9.80±1.65^{f}
15	14.17±0.06^{abcd}	12.13±0.15^{e}	12.97±0.65^{cde}
20	14.58±0.44^{abc}	14.00±0.46^{abcd}	14.70±0.46^{abc}
25	15.63±0.06^{a}	14.53±0.06^{abc}	14.83±0.64^{ab}
Ampicillin 0.01%	14.33±0.15^{abcd}	14.87±1.23^{ab}	13.72±0.85^{bcde}

Note: Means in the same column and row with different superscript differ significantly (P<0.05).

Table 3. Diameter of inhibition zone of garlic ethanol extract against mastitis bacteria

Concentration (% b/v)	Inhibition zone (mm)		
	S. agalactie	*S.aureus*	*E. coli*
5	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{d}
10	1.80±0.09^{c}	0.00±0.00^{d}	1.50±0.20^{cd}
15	2.73±0.21^{bc}	0.00±0.00^{d}	1.97±0.25^{c}
20	2.70±0.30^{bc}	0.00±0.00^{d}	2.58±0.03^{bc}
25	3.67±1.85^{b}	0.00±0.00^{d}	3.03±0.06^{bc}
Ampicillin 0.01% ^{e}	14.33±0.15^{a}	14.33±0.15^{a}	13.72±0.85^{a}

Note: Means in the same column and row with different superscript differ significantly (P<0.05).

CONCLUSION

Filtrates of fresh garlic can be used to inhibit growth of *S. typhimurium* and mastitis bacteria. Thus use of fresh garlic filtrates may reduce the use of antibiotics in the poultry and cattle industries.

ACKNOWLEDGMENT

This research was funded through Basic Research project 2004, The Institute of Research and Community Empowerment (LPPM) IPB.

REFERENCES

Avato, P., E. Tursil, C. Vitali, V. Miccolis, & V. Candido. 2000. Allylsulfide constituents of garlic volatile oil as antimicrobial agents**.** Phytomedicine. 7**:**239-243.

Cutler R. & P. Wilson. 2004. Antibacterial activity of a new, stable, aqueous extract of allicin against methicillin-resistant Staphylococcus aureus. British J. Biomed. Sci. 61:1-4.

Durairaj, S., S. Srinivasan, & P. Lakshmanaperumalsamy. 2009. *In vitro* antibacterial activity and stability of garlic extract at different pH and temperature. Elec. J. Biol. 5: 5-10.

Eja, M. E., B. E. Asikong, C. Abriba, G. E. Arikpo, E. E Anwan, & K. H. Enyi-Idoh. 2007. A comparative assessment of the antimicrobial effects of garlic (*Allium Sativum*) and antibiotics on diarrheagenic organisms. Southeast Asian J Trop Med Public Health. 38: 343-348.

Fitria, Y., R. H. Nugroho, H. B. Sosiawan, Noviarti, & Nurhayati. 2004. Hasil monitoring dan surveilanse cemaran mikroba dan residu antibiotika di kota Padang, Pekanbaru dan Jambi tahun 2004. Bull. Infromasi Kesehatan Hewan. 6: 10–15.

Harris, J. C., S. L. Cottrell, S. Plummer, & D. Lloyd. 2001. Antimicrobial properties of *Allium Sativum* (garlic). Appl. Microbiol. Biotechnol. 57**:** 282-286.

Iwalokun, B. A., A. Ogunledun, D. O. Ogbolu, S. B. Bamiro, & J. Jimi-Omojola. 2004. *In vitro* antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and *Candida* species from Nigeria. J. Med. Food. 7: 327-334.

Jazani, N. H., S. Shahabi, A. A. Ali, S. Zarrin, & N. A. Daie. 2007. *In vitro* antibacterial activity of garlic against isolates of Acinetobacter sp. J. Biol. Sci. 7:819-822.

Johnston, N. 2002. Garlic: A natural antibiotic. MDD. 5**:**12-12.

Kusmiyati & N. W. S. Agustini. 2007. Uji aktivitas senyawa antibakteri dari mikroalga *Porphyridium coventum*. Biodiversitas 8: 48-53.

McEwen, S. A. & P. J. Fedorka-Cray. 2002. Antimicrobial use and resistance in animals. Clin. Infect. Dis. 34:93–106.

Noor, S. M., M. Poeloengan, & Andriani. 2006. Kepekaan isolat *Salmonella enteritidis* dan *Salmonella hadar* yang diisolasi dari daging ayam terhadap antibiotika. *Seminar Nasional Teknologi Peternakan dan Veteriner 2006*. http: peternakan.litbang.deptan.go.id/publikasi/semnas/pro06-113.pdf. *[11 December 2009].*

Ross, Z. M., E. A. OÕGara, D. J. Hill, H. V. Sleightholme, & D. J. Maslin. 2001. Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparison with garlic sulfides and garlic powder. Appl. Environ. Microbiol. 67:475-480.

Sadeghian, A. & K. Ghazvini. 2002. Antimicrobial activity of garlic extract against Shigella. IJMS. 27:142-144.

Seong Wei, L. & N. Musa. 2008. Inhibition of *Edwardsiella tarda* and other fish pathogens by *Allium sativum* L. (Alliaceae) extract. American-Eurasian J. Agric. & Environ. Sci. 3: 692-696.

Shokrzadeh, M. & A. G. Ebadi. 2006. Antibacterial effect of garlic (*Allium sativum* L) on *Staphylococcus aureus*. Pak. J. Biol. Sci. 9: 1577-1579.

Suharti, S., M. Bintang, & K. G. Wiryawan. 2005. Kajian antibakteri temulawak, jahe, dan bawang putih terhadap *Salmonella typhimurium* serta pengaruh bawang putih terhadap performans dan respon imun ayam pedaging. Med. Pet. 28: 52-62.

Swartz, M. N. 2002. Human diseases caused by foodborne pathogens of animal origin. Clin. Infect. Dis. 34: 111 – 122.

Tsao, S. M. & M. C. Yin. 2001a. *In vitro* activity of garlic oil and four diallyl sulfides against antibiotic-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. J. Antimicrob Chemother. 47**:** 665-670.

Tsao, S. M. & M. C. Yin. 2001b. *In vitro* antimicrobial activity of four diallyl sulfides occurring in Garlic and chinese leek oils. J. Med. Microbiol. 50**:** 646-649.

Verma S. K., V. Jain, & D. Verma. 2008. Garlic the spice of life: composition, cooking, chemistry, and preparations. J. Herb. Med. Toxicol 2:21-28.

World Health Organization. 2005. *Containing antimicrobial resistance.*WHO Policy Prespectives on Medicines No. 10, Geneva. http://www.who.int/management/anmicrobialresistance.pdf