

SCREENING FOR ANTIBACTERIAL PROPERTIES OF THREE MEDICINAL PLANTS AGAINST *SALMONELLA* SP. ISOLATES OBTAINED FROM BROILER CARCASS IN INDONESIA

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ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi potensi antimikroba tiga tanaman obat yang banyak digunakan oleh masyarakat Indonesia, yaitu: sirih (*Piper betel*), bawang putih (*Allium sativum*) dan jinten hitam (*Nigella sativa*) terhadap isolat bakteri *Salmonella* sp. Uji antibakteri dilakukan pada ekstrak air dan ekstrak etanol ketiga jenis tanaman itu. Hasil yang diperoleh menunjukkan bahwa ketiga jenis tanaman obat tersebut memiliki sifat anti bakteri terhadap isolat *Salmonella* sp. Ekstrak air *Allium sativum*, ekstrak etanol *Nigella sativa*, dan *Piper betel*, berturut-turut menghasilkan zona hambat sebesar 17 mm, 15 mm dan 13 mm. Sebagai pembanding, uji sensitivitas antibakteri tiga jenis antibiotika komersial: chloramphenicol, tetracyclin, dan gentamycin terhadap *Salmonella* sp. menghasilkan zona hambat sebesar 19,7-27,3 mm. Jadi, bawang putih dapat digunakan sebagai dekontaminan *Salmonella* sp. untuk memertahankan kualitas karkas ayam broiler dan memperpanjang masa simpannya.

Kata kunci: antimikroba, Salmonella sp., Allium sativum, Piper betel, Nigella sativa

ABSTRACT

Antibacterial properties of three medicinal plants used widely amongst the native Indonesians, i.e., sirih (*Piper betel*), garlic (*Allium sativum*) and jinten hitam (*Nigella sativa*) were screened and evaluated against *Salmonella* sp. isolates. For this purpose, the three plants' powder, aqueous and ethanol extracts were prepared. The tests' results demonstrated the three plants' anti *Salmonella* sp. activities. In this conjunction, the aqueous extract of *Allium sativum*, the ethanol extracts of *Nigella sativa*, and *Piper betel*, consecutively produced 17 mm, 15 mm and 13 mm diameter of bacterial growth inhibition zones. As a comparison, sensitivity tests of three commercial antibiotics, i.e., chloramphenicol, tetracycline, and gentamycin on *Salmonella* sp. isolates produced 19.7-27.3 mm growth inhibition zones. Garlic having antimicrobial potential was comparable to the commercial antibiotics, can be used as a decontaminant against *Salmonella* sp. to maintain the quality of the broiler carcasses and therefore prolonging the carcass shelf-life.

Keywords: antimicrobial, Salmonella sp., Allium sativum, Piper betel, Nigella sativa

INTRODUCTION

The spread of multi-drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages, herbs and spices have evoked interest as sources of natural products for their potential uses as alternative remedies to heal many infectious diseases (Parekh *et al.*, 2005), including gastroenteritis. In relation to this, Amieva (2005) reported that *Salmonella* infection was responsible for 10 to 15% of acute gastroenteritis cases.

Basically, *Salmonella* organisms are gram

negative rods that belong to the family of *enterobacteriaceae*. This group contains the etiologic agents of food-borne salmonellosis as well as the agents that cause typhoid and paratyphoid fever (Jay, 1996). Eggs, poultry, meat, meat products and chocolates are the most common sources of food borne salmonellosis (Amieva, 2005).

Salmonellosis is the most prevalent food borne disease in many countries worldwide (D'Aoust, 1997). Chicken products are widely acknowledged to be a significant reservoir for *Salmonella* and have frequently been incriminated as a source of *Salmonella* contamination (Mulder

1999; Baeumler *et al.*, 2000).

The development of antimicrobial resistance among pathogenic bacteria has emerged as a major public health concern. This has led to an intensive discussion concerning the prudent use of antimicrobial agents, especially in veterinary medicine, nutrition and agriculture (Caprioli *et al.*, 2000). The utilization of antimicrobial substances has played an important role in animal husbandry, since they are used in prophylaxis, treatment and growth promotion.

Overall, the largest quantities of antimicrobials are used as regular supplements for prophylaxis or growth promotion in the feed of animal herds and poultry flocks. These results in the exposure of a large number of farm animals, irrespective of their health, to frequently sub therapeutic concentrations of antimicrobials (Dupont and Steele, 1987; Franco *et al.*, 1990). Furthermore, antibiotics given to animals and their closely related compounds used in human therapy have been exerting selective pressure on their target bacteria for decades (Witte, 1998), and therefore, can generate a reservoir of antimicrobial resistant bacteria (Endtz *et al.*, 1991, Smith *et al.*, 2002).

The presence of antimicrobial-resistant bacteria in animal origin human food stuffs threaten the efficacy of human drugs if the antimicrobial-resistant bacteria or the antimicrobial-resistance genes become incorporated into the human bacterial populations (Smith *et al.*, 2002). The use of agricultural antibiotic increases the frequency of antibiotic resistant zoonotic pathogens such as *Salmonella* sp. (Smith *et al.*, 2002). Most antimicrobial-resistant *Salmonella* sp. infections are acquired from eating contaminated foods from animal (Angulo *et al.*, 2000).

The husbandry practices used in the poultry industry and the widespread use of medicated feeds in broiler and layer operations made poultry a major reservoir of antimicrobial-resistant *Salmonella* sp. (D'Aoust *et al.*, 1992). Resistance in *Salmonella* sp. limits the therapeutic options available to veterinarians and physicians in the treatment of certain human cases of salmonellosis (Witte, 1998). Saad *et al.* (2007) reported that *Salmonella* sp. was found about 17.53% in the broiler chicken carcasses sold in local markets and 7.25% in the chicken carcasses released from slaughterhouse.

In this conjunction, this study assessed the potentials of antimicrobial activities of three

medicinal plants, i.e.: Sirih (*Piper betle*), Garlic (*Allium sativum*) and Jinten Hitam (*Nigella sativa*) that are widely used amongst native Indonesians to cure various health disturbances. Powder, aqueous and ethanol extracts of the plant materials were prepared and evaluated against isolates of *Salmonella* sp.

Firstly, *Allium sativum* or garlic, a member of the *Liliaceae* family, is one of the cultivated medicinal plants in Indonesia. It is commonly used in many cultures as a seasoning. According to the US Food and Drug Administration survey, garlic is the second most utilized supplement amongst the Echinaceae (Timbo *et al.*, 2006). The genus *Allium* includes garlic, scallions, onions, and leek. They contain sulfur compounds which are medicinally active. The majority of reported medicinal effects of garlic appear to come from the sulfur containing compounds, high trace mineral content, and enzymes. The types of sulfur found in whole garlic mostly are S-alkylcysteine sulfoxide and the γ -glutamyl-S-alkylcysteine. The most abundant sulfur compound in garlic is alicin (S-alkylcysteine sulfoxide) (Bongiorno *et al.*, 2008).

Secondly is *Nigella sativa* Linn. (Black cumin), is an herbaceous plant that has been used for centuries to treat various ailments, including infectious disease. The seeds are commonly used in recipes in Asian countries (Ali and Blunden, 2003; Randawa and Al-Ghamdi, 2002). Thirdly is *Piper betel* Linn. It is one of the important medicinal plants in different regions. It belongs to the family of *Piperaceae*. *Piper betel* leaves extract is reported to contain a large number of bioactive molecules like polyphenol, alkaloids, steroid, saponin, and tannin. The genus *Piper* (*Piperaceae*) has been distributed in tropical and subtropical regions of the world (Shalini *et al.*, 2012). Besides, *Piper betel* is cultivated in India, Srilanka, Malaysia, Indonesia, Philippines (Parmer *et al.*, 1997).

The objectives of this study were to screen and to evaluate the antibacterial properties of three medicinal plants used widely amongst the native Indonesian, i.e., Sirih (*Piper betel*), Garlic (*Allium sativum*) and Jinten Hitam (*Nigella sativa*) against *Salmonella* sp. Isolates.

MATERIALS AND METHODS

Extracting the Antimicrobial Plants Ingredients

The dried plant materials (*Piper betle* leaves,

Nigela sativa seeds and *Allium sativum bulbs*) were finely ground into powder. Except for the *Allium sativum* powder, ethanol was then added to the two other plants' powder. The mixtures of *Piper betle* and the *Nigela sativa* powder and alcohol were shaken for one hour to homogenize the solution. The agitation was required to accelerate the dilution of the two plants' active substances in the ethanol solvent.

Those mixtures were kept for 24 hours. Each of the crude plant extract was filtered by paper filter. Then each of the obtained ethanol solution contained the plant active substances was poured into a florentine tube. The tube was then placed in a rotary evaporator to evaporate the ethanol solvent at 40°C at 140-160 rpm and at 15-20 lbs of pressure.

Sterile aquadest was added to each obtained extract to make four concentrations of the plant extract, i.e. 50, 25, 12.5 and 6.25 percents. Then, 50 micro liters of each concentration was dropped at a sterile paper disc. Each disc was then laid on a Mueller Hinton agar medium that previously had been inoculated with the bacterial isolate and then was incubated for 24 hours at 37°C.

The bacterial growth inhibition zones were observed and measured. The size or the diameter of the growth inhibition zones indicated the effectiveness of the plant substances (the aqueous extract of *Allium sativum*, and the ethanol extracts of *Piper betle*, and *Nigella sativa*) in controlling the bacterial infection.

Culture Collection

Isolates of *Salmonella* sp. were obtained from broiler carcass samples collected from some local markets in Bogor, West Java, Indonesia. These specimens were then used for bacterial verification. The bacterial specimens were brought to the Indonesia Research Center for Veterinary Science (IRCVS) laboratory in Bogor. They were cultivated in blood agar media plates. The inoculated blood agar plates were incubated for 24 hours at 17°C. The bacterial isolates grown in the nutrient agar plates were identified by employing the Cowan and Steel methods (2003).

Antibacterial Effect of the Medicinal Plant

Susceptibility of *Salmonella* sp. was determined by agar disc diffusion method using Mueller Hinton agar. Blank discs (Oxoid) filled with each concentration of the plant solutions were laid at the agar media containing 0.1 ml of *Salmonella* sp. 10⁶ cfu/ml equal to 0.5 McFarland

Standard. After 24 hours of incubation at 37°C, the bacterial inhibition zones were measured and recorded.

Antibiotics Susceptibility Test

Susceptibility to different antibiotics was tested using the Mueller Hinton agar media. In this case, various commercial antibiotics discs i.e.: Chloramphenicol 30 µg, Tetracycline 30 µg, and Gentamycin 10 µg were employed. The antibacterial activity was carried out by employing 0.1 ml solution containing 10⁶ cfu/ml *Salmonella* sp., that was inoculated onto the agar media by spreading them over the media's surface. The antibiotics discs were laid at the cultured media (Bauer *et al.*, 1966). The susceptibility of the *Salmonella* sp. isolates to these antibiotics was recorded after 24 hour incubation at 37°C.

Experimental Design and Data Analysis

There were two main factors observed in this *in vitro* study. The first factor was the type of medicinal plants. This factor had three levels i.e.: the *Piper betle*, the *Allium sativum*, and the *Nigella sativa*. The second factor was the concentration of each medicinal plant substance. This factor had four levels, i.e.: 50, 25, 12.5 and 6.25 percents. In this conjunction, as a comparison, commercial antibiotics were also tested against *Salmonella* sp. isolates. In this case, antibiotic discs successively containing 30 µg Chloramphenicol, 30 µg Tetracycline and 10 µg Gentamycin were employed in the tests. The observed dependent variable of this investigation was the diameter of the bacterial growth inhibition zones produced by the medical plant extracts and the antibiotics.

The analysis of variance was used to analyze data regarding the diameter of the bacterial growth inhibition zones. Additionally, the Duncan's Multiple Range Test procedure was also employed to determine the differences amongst the means of the bacterial growth inhibition zone diameters (Walpole, 1968).

RESULTS AND DISCUSSION

Result of analysis of variance presented in Table 1 pointed out that the main effect of type of medicinal plant tested in this study on the bacterial growth inhibition zones was significant. Subsequently, Table 1 revealed that *Allium*

Table 1. Main Effect of Type of Medicinal Plant on Bacterial Growth Inhibition Zones

Form of Plant Substance	Diameter of growth Inhibition Zone (mm)
<i>Nigella sativa extract</i>	10.83 ^b
Aqueous mixture of <i>Allium sativum powder</i>	13.15 ^a
<i>Piper betle extract</i>	9.25 ^c

Different superscript indicates significant different at P<0.05

Table 2. Main Effect of Concentration of Medicinal Plant Substance on Bacterial Growth Inhibition Zones

Concentration of the Plant Substance (%)	Diameter of Bacterial Growth Inhibition Zone (mm)
50	15.11 ^a
25	12.44 ^b
12.5	9.67 ^c
6.25	7.11 ^d

Different superscript indicates significant different at P<0.05

sativum, one of the three medicinal plants tested, produced the greatest effect on the bacterial growth inhibition zones, followed by *Nigella sativa* and *Piper betle*.

Further analysis in Table 2 displayed the main effect of the second factor, i.e.: the concentration of the medicinal plant substance on the bacterial growth inhibition zone. This effect was also significant. Then, Table 2 disclosed also that overall, the 50% concentrations of the three medicinal plant substance tested, produced the largest effect on the bacterial growth inhibition zones, followed by the 25%, the 12.5%, and the 6.25% concentrations.

In this conjunction, garlic that has a broad spectrum of antimicrobial activity could destroy many species of bacteria, virus, parasites, prozoa, and fungi. Additionally, Singh and Shukla (1984) pointed out that garlic is active against strains of bacteria that are highly resistant to antibiotics.

Further observation specifies that garlic is also active against the following bacteria: *Shigella dysenteriae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus* spp., *Salmonella* spp., and *Proteus mirabilis*.

Eventually, the crude extracts and the essential oil of the *Nigella sativa* seeds possess antibacterial activity against several bacteria (Ali *et al.*, 2001; Halwani *et al.*, 1999). The oil shows pronounced dose dependent antibacterial activity which is more against Gram positive than Gram negative bacteria. Among the Gram negative bacteria that is sensitive to the test is *Pseudomonas aeruginosa* (Salman *et al.*, 2008). In contrast to the findings of earlier studies, it turns out that *Salmonella* sp. – the pathogen bacteria tested in this study is sensitive to *Nigella sativa*.

Additionally, *Piper betel* leaves are also reported to contain volatile oil, such as betel phenol and chavicol, tannin, sugar, and vitamin C. Moreover, Betel's leaves possess some other activities like antidiabetic, antiulcer, antitumor (Majumdar *et al.*, 2003) and antimicrobial (Majumdar *et al.*, 2002; Shalini *et al.*, 2012). Thus, the findings of these earlier researches are in accordance with the findings of this study, i.e., disclosing the antimicrobial activities of the three commonly used medicinal plants amongst the native Indonesian against the pathogenic bacteria, *Salmonella* sp.

Table 3 disclosed the combined effects of the type of medicinal plants (*Allium sativum*, *Piper betle*, and *Nigella sativa*) and the concentration of the medicinal plant substance on *Salmonella* sp. growth inhibition zones. These interaction effects were also significant. Further, Table 3 pointed out that *Allium sativum* produced the largest growth inhibition zones, followed by *Nigella sativa* and *Piper betle* at all concentrations. These bacterial growth inhibition zones differed significantly. In this conjunction, Table 3 remarkably demonstrated that of the three medicinal plants tested in this study, *Allium sativum* at 50% concentration produced the largest bacterial growth inhibition zones.

Additional antibacterial tests were accomplished with commercial antibiotics including chloramphenicol, tetracycline, and gentamicyn. The results are presented in Table 4. All three antibiotics employed were susceptible to bacteria *Salmonella* sp., isolated from broiler chicken carcasses. Susceptibility of *Salmonella*

Table 3. Combined Effect of Type of Medicinal Plant and Concentration of the Medicinal Plant Substance on the Bacterial Growth Inhibition Zones

Combined Effect of Plant Substance Concentration and Types of Medicinal Plant (%)	Medicinal Plant	Diameter of Growth Inhibition Zones (mm)
50 %	<i>Nigella sativa</i>	16.3 ^b
	<i>Allium sativum</i>	17.0 ^a
	<i>Piper betle</i>	13.0 ^{c,d}
25 %	<i>Nigella sativa</i>	12.0 ^d
	<i>Allium sativum</i>	14.3 ^{bc}
	<i>Piper betle</i>	11.3 ^e
12.5 %	<i>Nigella sativa</i>	9.0 ^{f,g}
	<i>Allium sativum</i>	12.0 ^d
	<i>Piper betle</i>	8.0 ^g
6.25 %	<i>Nigella sativa</i>	7.0 ^h
	<i>Allium sativum</i>	9.3 ^f
	<i>Piper betle</i>	5.3 ⁱ

Different superscript indicated significant different at P<0.05

Table 4. Growth Inhibition Zones Antibiotics against *Salmonella* sp.

Antibiotics	Mean Diameter of Growth Inhibition Zone (mm)	Susceptible/Intermediate/Resistance *
Chloramphenicol 30 µg	27.3 ^c	Susceptible
Tetracyclin 30 µg	22.3 ^a	Susceptible
Gentamycin 10 µg	19.7 ^b	Susceptible

Different superscript indicates significant different at P<0.05; * NCCLS (2002)

sp. to antibiotics chloramphenicol, tetracycline and gentamycin were characterized by the size of the bacterial growth inhibition zones of 27.3, 22.3, and 19.7 mm respectively.

As a matter of fact, garlic has been used for thousands of years as food and medicine in many cultures (Braun and Cohen, 2005). Garlic is well known for its anti-infective properties against a wide range of microorganisms (Iwalokun *et al.*, 2004). Garlic can be eaten raw as well as cooked. However, it is more often cooked to enhance flavor as well as to add nutritional benefits. One study mentioned that garlic could be used to

extend the shelf-life of livestock origin food products.

On the other hand, the observed *Salmonella* sp.'s growth inhibition zone elicited by chloramphenicol, tetracycline, and gentamycin indicated that isolate's susceptibilities (Table 4). Yet, the present study demonstrated the largest potency of *Allium sativum*'s antimicrobial effect against *Salmonella* sp. isolate. This pointed out that garlic had more effective susceptibility potency than *Nigella sativa* and *Piper betel* did. Yabaya *et al.* (2010) confirmed this finding. Their research disclosed that fresh garlic aqueous

extract had higher sensitivity against *Salmonella typhi* isolates.

CONCLUSION

The three widely used medicinal plants amongst native Indonesian evaluated in this study, i.e.: *Piper betle*, *Allium sativum*, and *Nigella sativa* had bactericidal effects on *Salmonella* sp. The higher the concentration of the medicinal plant substances, the larger the diameter of the bacterial growth inhibition zones produced. Of the three plants tested, *Allium sativum* persistently produced the most effect on *Salmonella* sp., the gram negative pathogenic bacteria at all concentrations.

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