

Antibacterial Effect of *Nigella sativa* L. Seed from Indonesia

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ABSTRACT

Nigella sativa L. was allegedly to has antibacterial effect. The aim of this research is to investigate antibacterial effect of ethanolic extracts of 96% *Nigella sativa* L. from Indonesia toward *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus epidermidis*. The Antibacterial activity of of *Nigella sativa* L toward *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus epidermidis* using the well method. The 96% ethanolic extract of 12,5; 25;50 and 100% (mg/ml) inhibit growth of *Staphylococcus aureus* (with inhibition zone 11.06; 29.58; 28.22; 30.84 mm respectively) and *Streptococcus epidermidis* (20.90; 31.90; 29.93; 33.07 mm respectively). Ethyl acetate fraction of 96% ethanolic extract of *Nigella sativa* concentration of 6,25; 12,5; 25;50 and 100% (mg/ml) inhibit growth of *Staphylococcus aureus* (12.91; 15.06; 19.19; 37.48; 46.18 mm respectively) and *Streptococcus epidermidis* (19.07; 19.21; 20.22; 21.62; 40.00 mm respectively). Ethanolic 96% extract and ethyl acetate fraction of ethanolic extract of *Nigella sativa* have antibacterial effect toward *Staphylococcus aureus* and *Streptococcus epidermidis* *in vitro*.

Key words: *Nigella sativa* L., Antibacterial, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus epidermidis*.

INTRODUCTION

Black cumin (*Nigella sativa* L.) has been widely used as a natural remedy to cure several diseases. The seeds are also used as a spice in cooking. *Nigella sativa* is a well-known medicinal plant because its seeds are used for various ailments. In traditional medicine, Black cumin (*Nigella sativa* L.) is used to treat gastrointestinal pain and can act as diuretic.^{1,2} Research by Muhtadi *et al* showed that *N. sativa* has hypouricemic effect.³

Previous research stated that ethanol extract from Black cumin can inhibit the growth of bacteria *Methicillin Resistant Staphylococcus aureus* (MRSA) and *Escherichia coli*.⁴⁻⁶ Another research by Putra *et al*. showed that *Nigella sativa* oil had a significant inhibitory effect on the growth of *Staphylococcus aureus*.⁷ Research by Emeka *et al*. showed there is antimicrobial activity of *Nigella sativa* L. seed oil against multi-drug resistant *Staphylococcus aureus* isolated from diabetic wounds.⁸

This study was performed to determine antibacterial effect of *N. sativa* L. from Indonesia toward *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus epidermidis*.

MATERIALS AND METHOD

This study is an experimental study *in vitro* test (pre-clinical research). Research design is a post-test with control group design.

Materials test plants: *N. Sativa* L was found Gede Market Solo, Central of Java, Indonesia.

Bacterial test: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus epidermidis*.

Procedure

Extract preparation

Amount 500 g of dry *N. sativa* seed was washed

by water and then was blended to be powder. The powder was macerated using 96% ethanol for 3 days. The filtrate was poured into the Becker glass. The residue was re- macerated using 96% ethanol for 3 days for 3 days. The filtrate was added to the first filtrate and then the filtrate was fractionated using ethyl acetate.

Bacteria Inoculation

The bacteria were inoculated on nutrient agar, then incubated at 37°C for 24 hours. Some Bacteria were taken from bacterial isolates and then put in test tube containing sterile physiological NaCl, shaken until homogeneous. Then compare with 0.5 Mc Farland suspension. Bacteria were taken with a sterile cotton swab, placed on the edge test tube, and smeared on agar Mueller Hinton.

Antibacterial testing

The method of antibacterial affect referred to Rahman *at al* method [9]. The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6-8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the extracts (at concentration of 100;50;25;12,5 and 6,25% (mg/ml) respectively); positive control (K+) (chloramphenicol 10%) and negative control (K-) DMSO (Dimethyl sulfoxide) were dripped into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

RESULTS AND DISCUSSIONS

Results of studying the effects of ethanolic extract and ethyl acetate fraction on growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus epidermidis* are presented in 1 and table 2.

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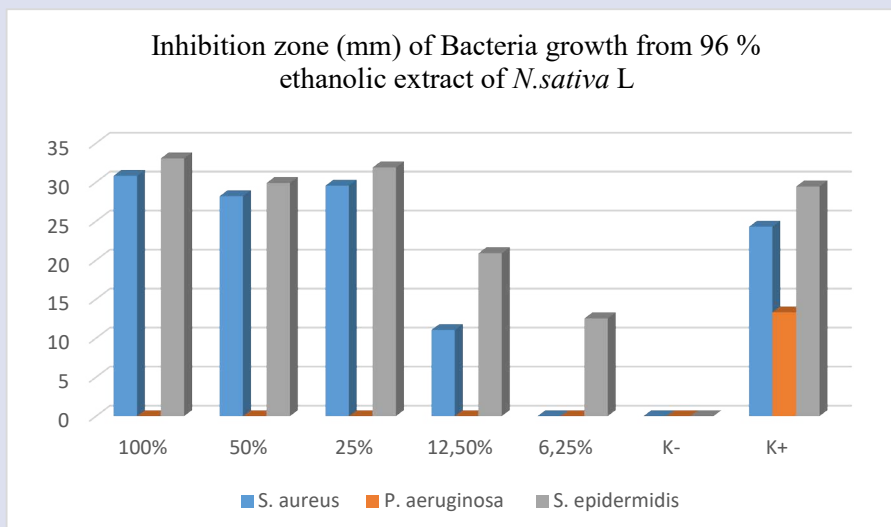


Figure 1: Inhibition zone (mm) of bacteria growth from 96 % ethanolic extract of *N. sativa* L.

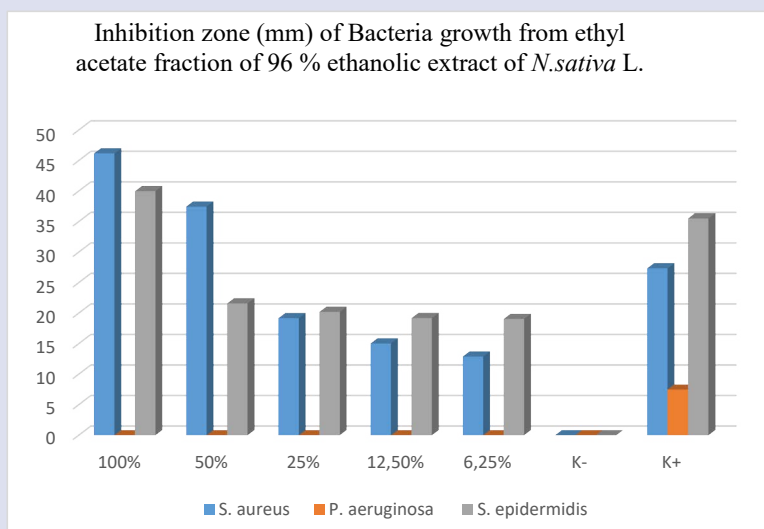


Figure 2: Inhibition zone (mm) of bacteria growth from ethyl acetate fraction of 96 % ethanolic extract of *N. sativa* L.

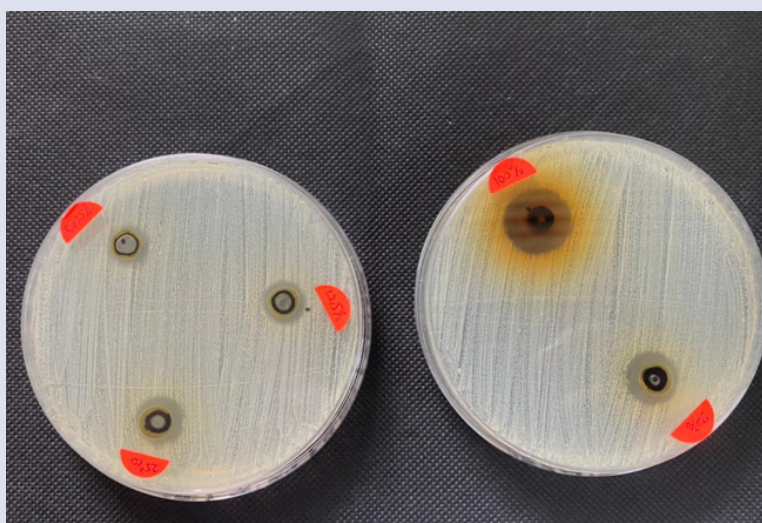


Figure 3: Inhibition zone of bacteria growth in various concentration.

Table 1: Antibacterial effect of 96 % ethanoic extract of *N. sativa* L. toward *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus epidermidis*.

Concentration	Inhibition zone (mm)		
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>
100 %	30.84	00	33.07
50 %	28.22	00	29.93
25 %	29.58	00	31.90
12.5 %	11.06	00	20.90
6.25 %	00	00	12.55
(-)	00	00	00
(+)	24.31	13.32	29.47

Table 2: Antibacterial effect of ethyl acetate fraction of ethanoic 96% extract of *Nigella sativa* toward *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus epidermidis*.

Concentration	Inhibition zone (mm)		
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>
100 %	46.18	00	40.00
50 %	37.48	00	21.62
25 %	19.19	00	20.22
12.5 %	15.06	00	19.21
6.25 %	12.91	00	19.07
(-)	00	00	00
(+)	27.36	7.49	35.54

From the table 1 and 2 above, we can see that 96% ethanolic extract and ethyl acetate fraction of 96% ethanolic extract of *Nigella sativa* L. seed can inhibit the growth of *Staphylococcus aureus* and *Streptococcus epidermidis* with various of diameter of inhibition zone.

This research in line with previous research. Research by Kamel Chaieb *et al*, showed that thymoquinone has antibacterial effect toward coccus Gram positive bacteria (*Staphylococcus aureus* ATCC 25923 dan *Staphylococcus epidermidis* CIP 106510).¹⁰ Research by Derbal and Niar showed that *Nigella sativa* L. seed extracts (ethanol) At concentration of 0.3 g/mL, against *Enterococcus faecalis*, *Escherichia coli*, *Enterobacter sp.*, *Staphylococcus aureus* and *Staphylococcus intermedius*.¹¹ Ethyl acetate of *N sativa* L seed contains, alkaloid, steroid and terpenoid, meanwhile flavonoid, phenol, tannon, cardiac glycoside and saponin was not found in phytochemical screening.¹² Research by Asniyah, 2009 stated that *N sativa* can inhibit the growth of *E.coli in vitro*.¹³ The active compound that inhibit of *Staphylococcus aureus* and *Streptococcus epidermidis* is not known by this research. Several studies reported that thymoquinone in *N sativa* seeds is a pharmacologically active component of this plant.¹⁴⁻¹⁷ Apart from thymoquinone, it is suspected that ditimoquinone, timohydroquinone, thymol, and tannins presumably a compound which functions as an antimicrobial. The mechanism of thymoquinone as an antibacterial is suspected that this chemical compound can form a complex that irreversibly with nucleophilic amino acids bacterial proteins, thereby causing inactivation protein.¹⁸ While the tannins work by forming hydrophobic bonds with proteins that cause inactivation of adhesins, enzymes, and cell wall transport proteins that interfere with bacterial growth.¹⁹

The further research is needed to determine active compound that act antibacterial effect.

CONCLUSION

Ethanolic 96% extract and ethyl acetate fraction of ethanolic 96% extract of *Nigella sativa* have antibacterial effect toward *Staphylococcus aureus* and *Streptococcus epidermidis in vitro*.

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