

Sains Malaysiana 42(2)(2013): 143–147

Antimicrobial Activity of *Nigella sativa* Seed Extract (Aktiviti Antimikrob Ekstrak *Nigella sativa*)

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ABSTRACT

Pathogenic bacterial infections have become a major health problem worldwide. New antimicrobial agents are urgently needed to overcome this problem. In this study, antibacterial activity of Nigella sativa seed extract against some pathogenic bacterial strains (Streptococcus pyogene, Pseudomonas aeruginosa, Klebseilla pneumoniae and Proteus vulgaris) was evaluated. Methanol extract at the concentration of 100 mg/mL had a remarkable sensitivity towards all tested bacteria in this study. Klebseilla pneumonia and Proteus vulgaris showed resistance against aqueous extract at 20 mg/mL. Methanol extract of Nigella sativa exhibited significant antibacterial activity at the concentration of 50 mg/mL ($p \leq 0.01$) against Streptococcus pyogenes with a greater inhibition zone of 19 mm, while a 15 mm zone of inhibition was observed in Pseudomonas aeruginosa, Klebseilla pneumonia and Proteus vulgaris. Kruskal Wallis analysis showed that both aqueous and methanol extract of black seed exhibited a greater inhibition on Gram positive bacteria (Streptococcus pyogenes) compared with Gram negative bacteria (Pseudomonas aeruginosa, Klebseilla pneumoniae and Proteus vulgaris). Our study also showed that species, strains and concentrations of Nigella sativa extract are some of the factors that may influence the sensitivity of the tested bacteria. A significant correlation was observed between zone of inhibition and concentration of extract.

Keywords: Antimicrobial activity; disk diffusion method; methanol extract; Nigella sativa; pathogenic bacteria

ABSTRAK

Jangkitan bakteria patogenik telah menjadi masalah kesihatan utama di seluruh dunia. Agen-agen antimikrob yang baru amat diperlukan untuk mengatasi masalah ini. Dalam penyelidikan ini, aktiviti antibakteria daripada ekstrak biji Nigella sativa telah dikaji terhadap beberapa strain bakteria patogenik (Streptococcus pyogene, Pseudomonas aeruginosa, Klebseilla pneumoniae dan Proteus vulgaris). Ekstrak metanol pada kepekatan 100 mg/mL menunjukkan kepekaan terhadap semua bakteria yang diuji dalam kajian ini. Klebseilla pneumoniae dan Proteus vulgaris menunjukkan rintangan terhadap ekstrak akues pada 20 mg/mL. Ekstrak metanol Nigella sativa menunjukkan aktiviti antibakteria yang ketara pada kepekatan 50 mg/mL ($p \leq 0.01$) terhadap Streptococcus pyogenes dengan zon perencatan yang lebih besar iaitu 19 mm, manakala 15 mm zon perencatan yang sama diperhatikan dalam Pseudomonas aeruginosa, Klebseilla pneumoniae dan Proteus vulgaris. Analisis Kruskal Wallis yang dijalankan menunjukkan kesan perencatan yang lebih besar terhadap bakteria Gram positif (Streptococcus pyogenes) berbanding dengan bakteria Gram negatif (Pseudomonas aeruginosa, Klebseilla pneumoniae dan Proteus vulgaris) pada kedua-dua ekstrak larutan dan metanol jintan hitam. Kajian ini juga menunjukkan, spesies, strain dan kepekatan ekstrak Nigella sativa adalah antara faktor yang mungkin mempengaruhi kepekaan bakteria ujian. Hubungan korelasi yang signifikan dapat diperhatikan antara zon perencatan dan kepekatan yang digunakan bagi kedua-dua ekstrak.

Kata kunci: Aktiviti anti-mikrob; bakteria patogen; cakera kaedah resapan; ekstrak metanol; Nigella sativa

INTRODUCTION

The use of synthetic drugs containing microbes that are biochemically and genetically modified as a treatment of common infectious disease are not reliable due to many controversial issues. Synthetic drugs are not only expensive and inadequate but also often had issues with adulterations and side effects. Customers are more concerned about the pathogenicity and the high mortality rate of the product they used. Therefore, with the advancement of the technology, scientists are challenged to come out with new ideas of alternative

and novel drugs to overcome the usage of microbial resistant drugs.

Since ancient civilization, natural sources especially plants are used as medicinal therapy because they contain several components which are believed to cure various infectious diseases. The biodiversity of plants provides an important source of chemical compounds, which have many therapeutic application such as antiviral, antibacterial, antifungal and anticancer activities (Pereira et al. 2004). *Nigella sativa* is a herbaceous plant which is better known as black seed, a habitat of Southeast Asia and Mediterranean

countries. Indian folks used this plant as a food preservative as well as a protective and curative treatment for numerous disorders (Merfort et al. 1997).

The black seeds contain 36–38% fixed oil, with proteins, alkaloids, saponins and essential oils making up the rest of the composition (Burtis & Bucar 2000). Although black seed extract or oil has been reported to possess antimicrobial activity (Morsi 2000), antioxidant activity (Burtis & Bucar 2000), antitumor activity (Worthen et al. 1998) and a stimulatory effect on the immune system (Salem & Hossain 2000), its full potential as an antimicrobial agent has not been exploited. This current study was conducted to investigate the antibacterial activity of the seed extract of *Nigella sativa* against pathogenic isolates of bacteria. The results of this study may further strengthen the recommendation for the use of ethnomedicine in the treatment and control of microbial infections.

MATERIALS AND METHODS

PREPARATION OF PLANT SAMPLE

Nigella sativa seeds were bought from a local herbal market and the plant was authenticated at the Biology Unit, Faculty of Applied Science, Universiti Teknologi MARA (UiTM), Negeri Sembilan Branch Campus. The seeds were washed with distilled water thrice and dried on a blotting paper in the laboratory at $37 \pm 1^\circ\text{C}$ for 24 h.

PREPARATION OF BACTERIA SAMPLE

The bacteria *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumonia* (*K. pneumonia*), *Proteus vulgaris* (*P. vulgaris*) and *Streptococcus pyogenes* (*S. pyogenes*) were obtained from the Microbiology Unit, Faculty of Applied Science, UiTM Negeri Sembilan Branch Campus. Bacteria were identified based on the colony, morphology, gram staining and biochemical analysis. Reference strains were obtained from the American Type Culture Collection: *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 700721, *P. vulgaris* ATCC 13315 and *S. pyogenes* ATCC 19615. These strains were then cultured on the nutrient agar (OXOID CM 0003, England) and incubated overnight.

PREPARATION OF EXTRACT

The extraction was done using modified Bligh-Dyer method (Bligh & Dyer 1959). *Nigella sativa* seeds (50 g) were homogenized in a Waring blender for 2 min with a mixture of 50 mL chloroform and 50 mL methanol. Another 50 mL of chloroform was added to this mixture and blended for 30 s. Finally, 50 mL of distilled water was added and blended for 30 s. The homogenate was then filtered through a Whatman No. 1 filter paper on Whatman No. 3 filter paper using a Bucher funnel with a slight suction and the residue was compressed to ensure maximum recovery of the filtrate. The combined filtrates were transferred into a decanter. After settling for 5 min to complete the

separation and clarification the bottom layer that contains the chloroform and lipid was mixed with a small amount of anhydrous Na_2SO_4 (1.5–2.5 g). Upon completion of the oil extraction, chloroform was removed from the oil using a rotary evaporator. A range of 1.0 mg/mL and 100 mg/mL stock solution were prepared using distilled water via serial dilution method.

Aqueous extraction was performed following the method of Charhhalchi et al. (2007) where 100 g of black seeds were boiled in 1 L of distilled water for 1 h. Then the solution was filtered using filter paper Whatman No. 1. Finally, the solution was centrifuged at 3000 rpm for 15 min using eppendorf centrifuge 5810R. A range of 1.0 and 100 mg/mL stock solution was prepared using distilled water via serial dilution method. Sterile water and commercial antibiotic were used as positive and negative control.

ANTIBACTERIAL TEST

Antibacterial test was done by preparing bacterial suspension followed by the disc diffusion test. For the preparation of bacterial suspension, one single colony bacteria was picked and inoculated onto 5 mL nutrient broth (MERCK, Germany) and incubated at 37°C overnight. The concentration of the bacteria for disc diffusion assay was standardized to 10^7 cell/mL based on the McFarland standard. An amount of 300 μL from the bacterial suspension which was kept overnight was diluted into 10 mL Mueller Hinton broth (MERCK, Germany). A sterilized cotton bud was dipped into suspension prepared and spread evenly on the surface of the Mueller Hinton agar (OXOID CM0337, England). Next, the commercial antibiotic disc was placed at the middle of the plate to serve as control positive and then five sterilized Whatman filter paper disc (Whatman No. 6) with a diameter of 6 mm were placed around it. One of the paper discs was dipped into sterile distilled water as control negative while the remaining four discs were dipped in four different concentrations of the extract. This method was performed nearby the Bunsen burner to ensure sterility. Incubation was done overnight with a minimum of 8 h to obtain the inhibition result of extracts upon the bacterial strain.

STATISTICAL ANALYSIS

Kruskal Wallis test was performed to test for the differences in sizes of inhibitory zones formed by oil against different bacteria. Mann-Whitney U test was performed to compare antimicrobial effects of *Nigella sativa* between methanol and aqueous extract. Bivariate correlation analysis using Pearson's test was done to find relationships between concentration of oil and inhibition zones. All tests were done using SPSS software 13.0 version.

RESULTS AND DISCUSSION

This study reports the antimicrobial activity of 8 concentrations of *Nigella sativa* against *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*

and *Proteus vulgaris*. The results of the antimicrobial activity of the investigated extract are shown in Tables 1 and 2. In this study, both methanol and aqueous extracts showed no inhibition against all the bacteria tested at lower concentrations (<50 mg/mL). Generally, the methanol extract of *Nigella sativa* exhibited higher antibacterial effect compared with aqueous extracts. The extraction of the biologically active compounds from the plant material depends on the type of solvents used in the extraction procedure. According to Parekh et al. (2006), methanol, ethanol and water are the most commonly used solvents for determining the antimicrobial activity in plants. The diameter of inhibition zone in methanol extract are higher (Mdn=11.5) than aqueous extract (Mdn=11.0). On the contrary, statistical analysis using Mann-Whitney analysis showed that there are no significant differences between methanol extract and aqueous extract used, $U=282.0$, $z=-0.142$ with $p\text{-value}\geq 0.01$. This is because different sources of the extracts, agro-climate factor, handling of experiment and phytochemical ingredients in the extract also contribute to the differences of results obtained (Erdman et al. 2007).

In this study, positive result was only observed in methanol extracts of *Nigella sativa* at 20 mg/mL against *Streptococcus pyogenes* measured at 10 mm; ($p\leq 0.01$) while all other bacteria were resistant in aqueous extract. At concentration of 100 mg/mL, the highest antibacterial activity of 19 mm was recorded in *Streptococcus pyogenes* and similar activity was recorded in *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* with an inhibition zone measuring around 15 mm (Table 3).

Aqueous extract of *Nigella sativa* had a remarkable sensitivity towards *Pseudomonas aeruginosa* and *Streptococcus pyogenes* with inhibition zones of 20 mm and 15 mm at concentration of 100 mg/mL, respectively. At concentration of 50 mg/mL, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae* exhibited modest inhibition at 10, 12, 12 and 11 mm, respectively. There was no difference in inhibition zone showed by *Klebsiella pneumoniae* when the concentration was increased from 50 mg/mL to 100 mg/mL (Table 4).

On the basis of the above results, it showed that methanol extract of *N. sativa* exhibited a greater inhibition compared with aqueous extract. Parekh et al. (2006) reported that most of the antimicrobial active compounds were soluble in polar solvent such as methanol instead of water. This result is comparable to the study by de Souza et al. (2004) using methanol extract of *L. sibiricus* that showed effective antibacterial activity on *Bacillus subtilis*. Bajwa and Shafique (2008) showed that methanol fraction of *A. rabiei* exhibited more promising results in suppressing the fungal growth rather than aqueous extract. This was also reported by Zafar et al. (2002), where chloroform extract of *Melia azedarach* leaves was active against *Fusarium chlamidosporum* while water extract of the leaves did not show any positive results.

By referring to Tables 1 and 2, the extracts were found to be more effective on Gram positive than Gram negative bacteria, which is in conformity with a number of earlier studies where compounds derived from plants often show considerable activity against Gram positive

TABLE 1. Inhibitory properties of methanol extract of *Nigella sativa* on pathogenic bacteria

Test bacteria	Black seed concentration (mg/mL)					
	1	5	10	20	50	100
<i>Klebsiella pneumoniae</i>	-	-	-	-	+	++
<i>Proteus vulgaris</i>	-	-	-	-	+	++
<i>Pseudomonas aeruginosa</i>	-	-	-	-	+	++
<i>Streptococcus pyogenes</i>	-	-	-	+	++	++

- : No inhibition zone
 + : Inhibition zone \leq 15 mm
 ++ : Inhibition zone \geq 15 mm

TABLE 2. Inhibitory properties of aqueous extract of *Nigella sativa* on pathogenic bacteria

Test bacteria	Black seed concentration (mg/mL)					
	1	5	10	20	50	100
<i>Klebsiella pneumoniae</i>	-	-	-	-	+	+
<i>Proteus vulgaris</i>	-	-	-	-	+	+
<i>Pseudomonas aeruginosa</i>	-	-	-	+	+	++
<i>Streptococcus pyogenes</i>	-	-	-	+	+	++

- : No inhibition zone
 + : Inhibition zone \leq 15 mm
 ++ : Inhibition zone \geq 15 mm

TABLE 3. Correlation between concentration of *Nigella sativa* extract and inhibition zones against different types of bacteria

Test bacteria	Concentration / disc (5 µL/disc) Diameter of inhibition zones (mm)						Methanol	Spearman's Correlation
	Concentration (mg/mL)							
	1	5	10	20	50	100		
<i>Klebseilla pneumonia</i>	Nil	Nil	Nil	0	11.0 ± 1.8	15.0 ± 4.5	Nil	0.845
<i>Pseudomonas aeruginosa</i>	Nil	Nil	Nil	0	12.0 ± 2.7	15.0 ± 4.0	Nil	0.845
<i>Streptococcus pyogenes</i>	Nil	Nil	Nil	10.0 ± 0.0	16.0 ± 9.9	19.3 ± 1.9	Nil	0.880
<i>Proteus vulgaris</i>	Nil	Nil	Nil	0	10.7 ± 1.0	15.0 ± 4.0	Nil	0.845
Kruskal Wallis df				1	3	3		
P				0.012	0.012	0.341		

TABLE 4. Correlation between concentration of *Nigella sativa* extract and inhibition zones against DIFFERENT types of bacteria

Test Bacteria	Concentration / disc (5 µL/disc) Diameter of inhibition zones (mm)						Methanol	Spearman's Correlation
	Concentration (mg/mL)							
	1	5	10	20	50	100		
<i>Klebseilla pneumonia</i>	Nil	Nil	Nil	0	10.0 ± 0.5	10.0 ± 0.5	Nil	0.778
<i>Pseudomonas aeruginosa</i>	Nil	Nil	Nil	11.0 ± 1.4	12.0 ± 1.5	15.0 ± 0.0	Nil	0.941
<i>Streptococcus pyogenes</i>	Nil	Nil	Nil	11.0 ± 0.5	12.0 ± 1.0	20.0 ± 0.9	Nil	0.941
<i>Proteus vulgaris</i>	Nil	Nil	Nil	0	11.0 ± 0.5	12.0 ± 1.0	Nil	0.845
Kruskal Wallis df				3	3	3		
P				0.021	0.205	0.016		

**Correlation between log dose and size of zones is significant it 0.01 (2-tailed)

(Mean diameter of inhibition zones in mm around 6 mm disc impregnated with *Nigella sativa* extract)

bacteria but not against Gram negative species (Nagi et al. 2008). Gram negative bacteria have effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphiphatic compounds and multidrug resistance pumps that extrude toxins across this barrier (Nagi et al. 2008). It is possible that the apparent ineffectiveness of the plant antimicrobial activity is largely due to this permeability barrier.

Results of the study indicate that black seed extract showed a dose of dependent inhibition against concentration. Statistical analysis using Spearman's Rho, indicates that there is a significant correlation between zone of inhibition and concentration's used. Both extracts showed that all the bacteria tested ($n=4$) showed strong and positive correlation value. Positive correlation value indicates that increasing the concentration will increase the diameter of inhibition zone formed by the bacteria. This finding is in agreement with results reported by Hannan et al. (2008) using the same genus of plant tested.

CONCLUSION

It may be concluded from this study that *Nigella sativa* seed extract exhibits some degree of antibacterial activity towards

Pseudomonas aeruginosa and *Streptococcus pyogenes*. Thus, it shows that *Nigella sativa* has a great potential as an effective antimicrobial agent for medicinal purposes.

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Received: 27 July 2011
Accepted: 9 October 2012