



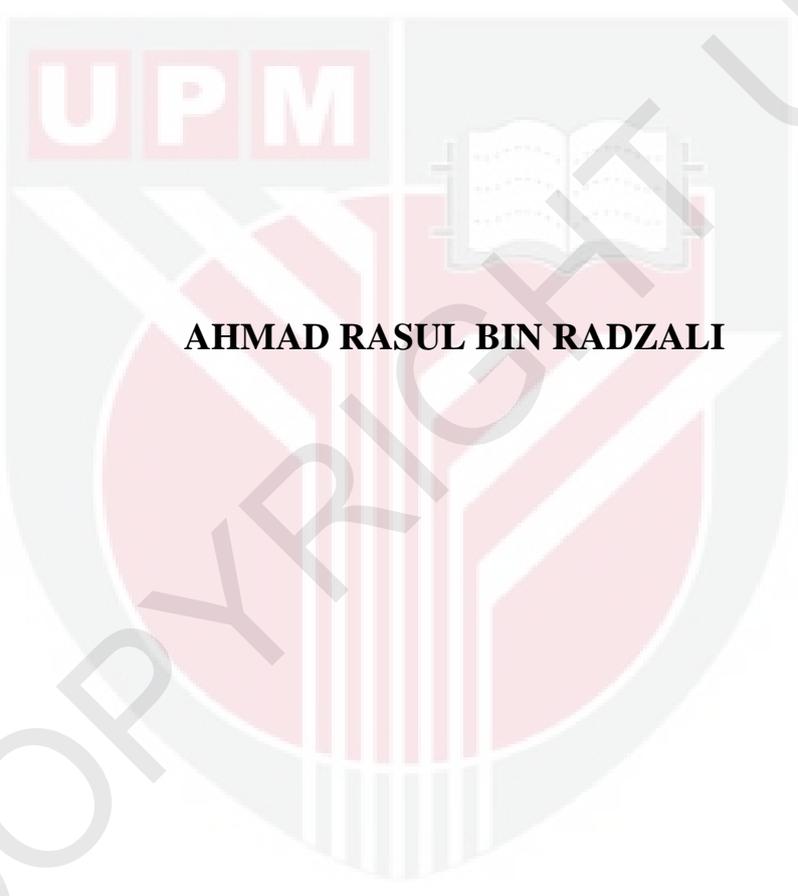
**UNIVERSITI PUTRA MALAYSIA**

***ANTIMICROBIAL ACTIVITY OF NIGELLA SATIVA  
(BLACK SEED) OIL AGAINST LEPTOSPIRA SPECIES***

**AHMAD RASUL BIN RADZALI**

**FPV 2015 27**

**ANTIMICROBIAL ACTIVITY OF *NIGELLA SATIVA*  
(BLACK SEED) OIL AGAINST *LEPTOSPIRA* SPECIES**

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**FACULTY OF VETERINARY MEDICINE,  
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SERDANG, SELANGOR.**

**2015**

**ANTIMICROBIAL ACTIVITY OF *NIGELLA SATIVA* (BLACK SEED) OIL  
AGAINST *LEPTOSPIRA* SPECIES**

AHMAD RASUL BIN RADZALI

A project paper submitted to the  
Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfilment of the requirement for the  
DEGREE OF DOCTOR OF VETERINARY MEDICINE

Universiti Putra Malaysia

Serdang, Selangor Darul Ehsan.

JANUARY 2015

It is hereby certified that we have read this project paper entitled “Antimicrobial Activity of *Nigella Sativa* (Black Seed) Oil against *Leptospira* Species” by Ahmad Rasul bin Radzali, and in our opinion it is satisfactory in term of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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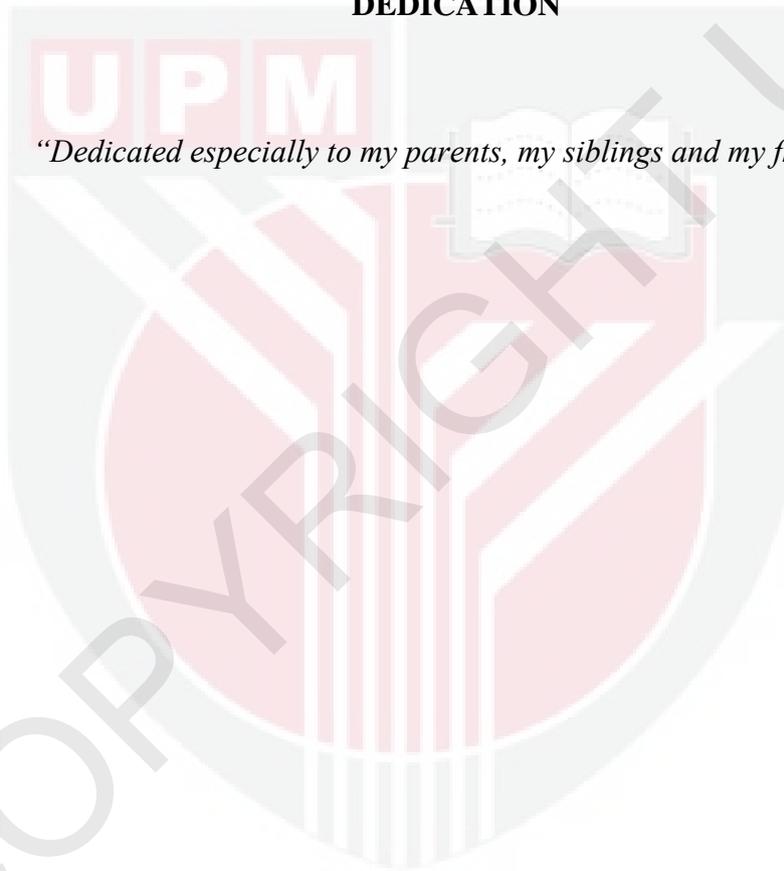
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**DEDICATION**

*“Dedicated especially to my parents, my siblings and my friends”*



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## ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

### **AKTIVITI ANTI-MIKROB MINYAK *NIGELLA SATIVA* (HABBATUS SAUDA) TERHADAP SPESIS *LEPTOSPIRA***

Disediakan oleh

Ahmad Rasul bin Radzali

2014

Penyelia: Prof. Madya Dr. Siti Khairani binti Bejo

Penyelia Bersama: Prof. Dato' Dr. Abdul Rani bin Bahaman

Aktiviti antimikrob minyak *Nigella sativa* telah dikaji terhadap lima serovar *Leptospira interrogans* iaitu *L. pomona* , *L. hardjobovis* , *L. australis* , *L. canicola* , dan *L. icterohaemorrhagiae*. Kepekatan perencatan minima (MIC) telah ditentukan dengan menggunakan kaedah *broth microdilution* dengan memerhatikan perencatan lengkap motilitas *Leptospira* melalui mikroskop *dark-field* pada pelbagai tempoh inkubasi (1 jam , 1 hari, 3 hari, dan 7 hari). Minyak *N. sativa* telah dilarutkan dalam dimetil sulfoksida (DMSO) sebelum dilarutkan dalam medium cecair Ellinghausen , McCullough , Johnson dan Harris (EMJH) dengan kepekatan akhir dari 0.1 hingga

50mg/ml. Semua serovar *Leptospira* adalah sensitif kepada minyak *N. sativa* pada setiap tempoh inkubasi dengan nilai-nilai MIC yang berbeza-beza dari 0.39 hingga 6.25 mg/ml. *Leptospira pomona* adalah lebih sensitif kepada minyak *N. sativa* berbanding dengan serovar-serovar lain, dengan nilai MIC yang paling rendah dalam setiap tempoh inkubasi (1 jam = 1.5 6mg/ml , 1 hari = 0.78 mg/ml , 3 hari = 0.78 mg/ml , 7 hari = 0.39 mg/ml), manakala *L. australis* adalah yang paling kurang sensitif terhadap minyak *N. sativa* dengan nilai MIC 6.25 mg/ml pada tempoh inkubasi 7 hari. Penicilin G dan DMSO telah dipilih sebagai kawalan positif dan negatif untuk eksperimen ini. Terdapat perbezaan yang signifikan antara serovar-serovar *Leptospira* yang dirawat dengan minyak *N. sativa* pada MIC yang diperolehi dalam setiap tempoh inkubasi; 1 jam ( $p = 0.014$ ), 1 hari ( $p = 0.016$ ), 3 hari ( $p = 0.026$ ) dan 7 hari ( $p = 0.010$ ). Walau bagaimanapun, tidak terdapat perbezaan yang signifikan di antara tempoh inkubasi yang berbeza-beza dengan nilai-nilai MIC yang diperolehi ( $p = 0.332$ ).

Kata-kata kunci: *Leptospira*, *Nigella sativa*, Kepekatan perencat minima (MIC)

## ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Project.

### ANTIMICROBIAL ACTIVITY OF *NIGELLA SATIVA* (BLACK SEED) OIL AGAINST *LEPTOSPIRA* SPECIES

By

Ahmad Rasul bin Radzali

2014

Supervisor: Assoc. Prof. Dr. Siti Khairani binti Bejo

Co-supervisor: Prof. Dato' Dr. Abdul Rani bin Bahaman

The antimicrobial activity of *Nigella sativa* oil was studied against five serovars of *Leptospira interrogans* which are *L. pomona*, *L. hardjobovis*, *L. australis*, *L. canicola*, and *L. icterohaemorrhagiae*. The minimum inhibitory concentration (MIC) was determined using broth microdilution method by observing complete motility inhibition of the *Leptospira* through dark-field microscopy at various incubation periods (1 hour, 1 day, 3 days, and 7 days). The *N. sativa* oil was dissolved in dimethyl sulfoxide (DMSO) prior to further dilution in Ellinghausen, McCullough, Johnson and Harris (EMJH) liquid medium with final concentration ranged from 0.1 to 50mg/ml. All *Leptospira* serovars were sensitive to *N. sativa* oil dilution at every incubation period with MIC values varying from 0.52 to 5.21mg/ml. *Leptospira*

*pomona* was more sensitive to *N. sativa* oil compared to the other strains, with the lowest MIC value obtained in every incubation period (1 hour = 1.56mg/ml, 1 day = 1.56mg/ml, 3 days = 0.78mg/ml, 7 days = 0.52mg/ml), while *L. australis* was the least sensitive towards *N. sativa* oil which was 5.21mg/ml at 7-day incubation period. Penicilin G and DMSO were chosen as positive and negative controls for the experiment respectively. There were significant differences among the *Leptospira* serovars treated with *N. sativa* oil on MIC values at 1-hour ( $p = 0.014$ ), 1-day ( $p = 0.016$ ), 3-day ( $p = 0.026$ ) and 7-day ( $p = 0.010$ ) incubation periods. However, there was no significant difference among different incubation periods on the MIC values of *N. sativa* oil ( $p = 0.332$ ).

Keywords: *Leptospira*, *Nigella sativa*, Minimal inhibitory concentration (MIC)

## 1.0 INTRODUCTION

Leptospirosis is a zoonotic disease caused by any of the pathogenic members of the genus *Leptospira*. The disease occurs worldwide and is more common in tropical and subtropical areas with high rainfall (WHO, 2003). In Malaysia, the first diagnosed case of leptospirosis in human was in 1926 by Fletcher (Lim *et al.*, 2011). The leptospirosis cases in human have increased significantly starting from 263 cases in year 2004 to 1418 cases in 2009, and continued to rise with 1876 cases in 2010, followed by 2268 cases in 2011 and 3665 cases in 2012 (Suhailah *et al.*, 2014).

There are nearly 300 serovars of *Leptospira* distributed worldwide among both pathogenic and non-pathogenic species (Adler *et al.*, 2010). Most of them have their primary reservoirs in wild and domestic animal or livestock. In humans, leptospirosis may be presented with broad range of clinical manifestations from mild influenza-like illness to severe infection with multiple-organ failure (WHO, 2003). In livestock animals, leptospirosis could lead to abortion, pre-mature birth, infertility, low milk production and death that would cause great loss to the livestock industry (JPV, 2011).

Various antibiotics have been suggested to be used in treating leptospirosis. In severe cases of leptospirosis, high doses of intravenous penicillin is recommended, while less severe cases can be treated with oral antibiotics like amoxicillin, ampicillin, doxycycline or erythromycin (WHO, 2003). Nevertheless, the

development of antibiotic resistance should be concerned especially with the use of the same antibiotics over a period of time, as well as improper antibiotic administration. Thus, there is a need to find alternatives to the antibiotic therapy, and herbal medicine may be one of them.

*Nigella sativa*, commonly known as black seed or Habbatussauda, is herbaceous plant that is well recognised for its medicinal properties in treating various diseases in humans. Its seed extract as well as its essential oil has been reported to have significant antimicrobial properties to variety of pathogenic bacteria including some bacterial strains that have multidrug resistance (Salman *et al.*, 2007). However, there is lack of information on the antimicrobial activity that *N. sativa* seed on *Leptospira* species. Hence, the objectives of this study were

1. to investigate the antimicrobial activity of *N. sativa* oil against *Leptospira* species.
2. to determine the Minimum Inhibitory Concentration (MIC) of *N. sativa* oil on *Leptospira* species.

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