



**UNIVERSITI PUTRA MALAYSIA**

***IN VITRO ANTHELMINTIC ACTIVITY OF NEEM LEAVES  
(AZADIRACHTA INDICA) CHLOROFORM EXTRACT AGAINST THE  
THIRD-STAGE LARVAE OF STRONGYLES FROM SHEEP***

**NURUL HAIRUNNISA BINTI SUHAIMI**

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**NURUL HAIRUNNISA BINTI SUHAIMI**

**A project paper submitted to the**

**Faculty of Veterinary Medicine, Universiti Putra Malaysia**

**In partial fulfillment of the requirement for the**

**DEGREE OF DOCTOR OF VETERINARY MEDICINE**

**Universiti Putra Malaysia**

**Serdang, Selangor Darul Ehsan**

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It is hereby certified that we have read this project paper entitled “*In vitro* anthelmintic activity of neem leaves (*Azadirachta indica*) chloroform extract against the third-stage larvae of strongyles from sheep”, by Nurul Hairunnisa Binti Suhaimi and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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DR SITI ZUBAIDAH RAMANOON

DVM (UPM), MSc (Guelph)

Senior Lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Supervisor)

---

DR WAN MASTURA SHAIK MOHAMED MOSSADEQ

DVM (UPM), MSc (Guelph), PhD (UPM)

Senior Lecturer

Faculty of Veterinary Medicine

Universiti Putra Malaysia

(Co-Supervisor)

## **DEDICATION**

I dedicate this thesis with love and appreciation to:

### **My parents and siblings**

SUHAIMI BIN MOHD MUKHTAR and ZAIDAH BINTI ZAKARIA

NOR FARAHIYAH, NUJAIMI, BAZILAH, NASUHA, IZZAH and ZIA

### **My Friends and FYP mates**

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## LIST OF ABBREVIATIONS

ACE	Neem Leaves ( <i>A. indica</i> ) Chloroform Extract
<i>A. indica</i>	<i>Azadirachta Indica</i>
df	Degree of freedom
DMSO	Dimethyl sulfoxide
EPG	Egg per gram
GI	Gastrointestinal
<i>H. contortus</i>	<i>Haemonchus contortus</i>
IBM SPSS	IBM Statistical Software for Social Sciences
KW	Kruskall-Wallis
L3	Third-stage larvae
p	p-value

## **ABSTRAK**

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

Akhir

### **AKTIVITI ANTELMINTIK *IN VITRO* EKSTRAK KLOROFORM DAUN SEMAMBU (*AZADIRACHTA INDICA*) PADA LARVA STRONGIL**

#### **PERINGKAT KETIGA DARIPADA BEBIRI**

Oleh:

Nurul Hairunnisa Binti Suhaimi

2016

Penyelia: Dr. Siti Zubaidah Ramanoon

Penyelia bersama: Dr. Wan Mastura Shaik Mohamed Mossadeq

Penyakit berparasit menyebabkan kematian dan morbiditi pada bebiri di Malaysia. Semambu (*Azadirachta indica*) telah dibuktikan mempunyai nilai perubatan seperti anti-kulat, anti-bakteria, anti-radang dan antelmintik. Kajian ini dijalankan untuk menentukan kesan antelmintik *in vitro* daun semambu pada larva peringkat ketiga (L3)

strongil dari bebiri. Sampel najis terkumpul daripada 22 bebiri yang mempunyai sejarah gastrousus berparasit dikulturkan untuk menuai L3. Tiga ribu L3 telah dibahagikan kepada lima kumpulan mengandungi enam ceper petri setiap satu, yang mana dalam setiap ceper petri terkandung seratus L3. Tiga kumpulan telah diuji dengan ekstrak kloroform daun semambu *Azadirachta indica* (ACE) berkepekatan 5, 10 dan 15mg/ml, satu kumpulan kawalan positif (levamisol, 10 mg/ml), dan satu negatif (0.01% DMSO + air ternyahion). Mortaliti L3 diperhatikan dalam masa 2, 4, 6, dan 24 jam. Keputusan menunjukkan bahawa peratus mortaliti L3 adalah sebanyak 93% pada kumpulan rawatan ACE 5 mg/ml selepas 24 jam; dan 83% ACE 10 mg/ml, selepas 2 jam. Kumpulan ujian ACE menunjukkan keberkesanan antelmintik ketara pada L3 berbanding dengan kumpulan kawalan negatif ( $KW = 93,55$ ,  $df = 4$ ,  $p <0.05$ ). Kajian lanjut berkaitan dengan kesan ACE berkepekatan lebih rendah terhadap mortaliti L3 strongil pada bebiri dicadangkan.

Kata kunci: daun semambu, *Azadirachta indica*, ekstrak kloroform, antelmintik, strongil, larva, bebiri

## **ABSTRACT**

An abstract of the project paper presented to the Faculty of Veterinary Medicine, UPM  
in partial requirement of the course VPD 4999- Final Year Project

### ***IN VITRO ANTHELMINTIC ACTIVITY OF THE NEEM LEAVES (AZADIRACHTA INDICA) CHLOROFORM EXTRACT AGAINST THE THIRD-STAGE LARVAE OF STRONGYLES FROM SHEEP***

**By**

**Nurul Hairunnisa Binti Suhaimi**

**2016**

**Supervisor: Dr. Siti Zubaidah Ramanoon**

**Co-supervisor: Dr. Wan Mastura Shaik Mohamed Mossadeq**

Parasitic disease causes mortality and morbidity of sheep in Malaysia. Neem plant (*Azadirachta indica*) has been shown to possess medicinal values such as anti-fungal, antibacterial, anti-inflammatory and anthelmintic activity. This study was conducted to determine the *in vitro* anthelmintic effect of neem leaves on the third-stage larvae (L3) of strongyles from sheep. Pooled faecal samples from 22 sheep with the history of gastrointestinal parasitism were cultured to harvest the L3. Three thousands L3 were

divided into five groups of six petri dishes each containing one hundred L3 per dish. Three groups were tested with the neem leaves (*Azadirachta indica*) chloroform extract (ACE) at 5, 10 and 15mg/ml concentrations, one positive control group (levamisole, 10 mg/ml) and one negative (0.01% DMSO + deionized water). The L3 mortality was observed for 2, 4, 6, and 24 hours. Results showed that the percentage L3 mortality were 93% (ACE 5 mg/ml of 24 hours post-treatment) and 83% (ACE 10 mg/ml 2 hours post-treatment). All ACE treated groups showed significant anthelmintic efficacy against L3 compared to the negative control group (KW=93.55, df=4, p<0.05). Further studies related to the effect of using lower ACE concentrations on L3 mortality of strongyles from sheep are suggested.

Keywords: neem, *Azadirachta indica*, chloroform extract, anthelmintic strongyles, larvae, sheep

## **Chapter 1**

### **INTRODUCTION**

#### **1.1 Background**

Helminthiasis is considered the major cause of mortality and morbidity in goats and sheep in Malaysia (Sani *et al.*, 2004) and elsewhere throughout the humid tropical/subtropical countries of the world (Perry *et al.*, 2002). Neem (*Azadirachta indica*, *A. indica*), a tree in the mahogany family Meliaceae, is native to India, Pakistan and Burma, growing in tropical and semi-tropical regions (Neem Foundation, 2001). Some parts of neem have been used in traditional medicine to treat diseases such as gastrointestinal (GI) nematodes infection (Arunachal *et al.*, 2002). Literature on the usage of neem leaves as anthelmintics for sheep especially in reducing the worm load of *Haemonchus contortus* (*H. contortus*) is very limited. Though Costa *et al.* (2006) reported that there was no significant anthelmintic activity observed after feeding sheep with neem leaves. Furthermore, studies on anthelmintic properties of neem through feeding the leaves to sheep are limited. Therefore, there is a need to study the anthelmintic effect of neem leaves in sheep.

## **1.2 Justification**

Drug resistance from prolong use at high levels and increasing frequency and inappropriate doses of chemical anthelmintics, to control gastrointestinal parasitism in sheep is considered an important problem in small ruminant husbandry. Additionally, anthelmintic drugs are costly and may cause drug residues in food products that will cause public health concern for food safety. Neem leaves as an alternative anthelmintic treatment for GI parasitic infections in sheep need to be investigated.

## **1.3 Study Objectives**

- a) to determine the *in vitro* anthelmintic efficacy of ACE on the L3 of strongyles from sheep
- b) to determine whether or not ACE has the same anthelmintic efficacy as levamisole

## **1.4 Study Hypothesis**

- i. ACE at different concentrations show anthelmintic activity against L3 of strongyles from sheep.
- ii. ACE has same efficacy as levamisole

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