UNIVERSITI PUTRA MALAYSIA

IN VITRO ACARICIDAL ASSESSMENT OF BETEL LEAVES (*Piper betle*) AQUEOUS AND ETHANOL EXTRACTS ON BROWN DOG TICK (*Rhipicephalus sanguineus*)

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "***IN VITRO* ACARICIDAL ASSESSMENT OF BETEL LEAVES, *Piper betle* AQUEOUS AND ETHANOL EXTRACTS ON BROWN DOG TICK *Rhipicephalus sanguineus***" by Muhammad Hafiz bin Shamsi and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project.

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DEDICATION

"Behold in the creation of the heavens and earth,

And the alternation of night and day there are

Indeed signs for men of understanding"

Surah 'Ali 'Imran 3:190

Alhamdulillah, this thesis is dedicated to my family, friends and the one I love, whom had accompanied me in my journey of becoming a veterinarian.

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Alhamdulillah, for giving the strength and patience in completing this task. I would like to express my deepest gratitude to Associate Professor Dr Hassan Hj. Mohd Daud, my supervisor for his patient guidance, care, and help to complete this project. Dr. Nur Mahiza Md Isa, my co-supervisor for her willingness to spend some time and giving some constructive suggestions to help me in this project.

To my family. Especially the strongest women I ever known in my life, my mum, and dad for building me up for what I am, thank you sincerely. Brothers through thick and thin, Miss Nurfatin Shakira Zaini the apple of my eyes. I would like to extend my gratitudes to those who have help me in completing this project, Staff of Aquatic Lab, FPV UPM, Dr. Diyana, Dr Akmal, and Pn Latifah for the guidance in the laboratory work. Staff of the Animal Aquatic Health Unit, UPM and Dr Fuad, for the help and advice. Staff of Institute of Tropical Research and Tropical Products, UPM for the willingness to share with me the knowledge about the world of herbs and plants.

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LIST OF ABBREVIATIONS

°C	Degree Celcius
%	Percentage
cm	Centimeter
g	Gram
min	Minute
mL	Milliliter
mm	Millimeter
rpm	Revolutions per minute
g/vol	Gram per volume
v/v	Volume over volume

ABSTRAK

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

PENILAIAN *IN VITRO* KESAN AKARI EKSTRAK AKUA DAN ETANOL DAUN SIRIH (*Piper betle*) TERHADAP KUTU ANJING (*Rhipicephalus sanguineus)*

Oleh

MUHAMMAD HAFIZ BIN SHAMSI

2017

Penyelia: Profesor Madya Dr Hassan Hj. Mohd Daud

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Kutu anjing kelabu, *Rhipicephalus sanguineus*, boleh dijumpai seluruh dunia menghisap darah anjing dan malahan juga pada mamalia lain dan manusia, sambil pada masa yang sama menyebarkan pelbagai jenis penyakit. Terdapat pelbagai jenis kimia sintetik bagi menangani masalah jangkitan kutu ini. Oleh itu matlamat utama kajian ini dijalankan adalah untuk mengkaji cara semulajadi untuk membunuh kutu ini. Untuk itu daun sireh (*Piper betle*) telah dipilih kerana diketahui mengandungi pelbagai jenis fitokimia yang bioaktif. Kutu anjing telah dikutip dari anjing

geladangan di Unit Kawalan Vektor, Dewan Bandaraya Kuala Lumpur menggunakan penyepit dan disimpan di dalam bekas khas yang telah diubahsuai. Daun sireh yang segar dipetik dari Taman Pertanian Universiti, Universiti Putra Malaysia. Daun dikeringkan menggunakan ketuhar udara panas dan kemudian di hancurkan menggunakan pengisar dapur. Daun yang telah dikisar telah direndam dalam larutan ethanol dan air, ditapis dan dikeringkan menggunakan penyejat putar untuk mendapatkan pati ekstrak herba. Dua jenis eksperimen berbeza telah dijalankan bagi menguji kesan ekstrak terhadap kutu yang dilarutkan dari 5^0 ke 5^{-4}. Keputusan menunjukkan bahawa kedua-dua jenis eksperimen bagi larutan etanol mempunyai kesan ketara sebagai racun akari dengan kadar dos maut dari kadar cairan satu ke lima kali. Larutan ekstrak etanol mempunyai kadar kematian yang lebih tinggi di mana 26 dari 50 ekor kutu berjaya dibunuh (52%), manakala, bagi larutan ekstrak akua hanya mampu membunuh 8 ekor dari 50 ekor kutu (16%). Sebagai penutup, ekstrak daun sirih menunjukkan potensi untuk dijadikan racun akari bagi *R. sanguineus.*

Kata kunci: Ekstrak, *Piper betle,* racun akari, *Rhipicephalus sanguineus*

ABSTRACT

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

***IN VITRO* ASSESSMENTS OF BETEL LEAVES (*Piper betle*) AQUEOUS AND ETHANOL EXTRACTS ON BROWN DOG TICK (*Rhipicephalus sanguineus*)**

By

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2017

Supervisor: Assoc. Professor Dr Hassan Hj. Mohd Daud

Co-Supervisor: Dr. Nur Mahiza Md Isa

The brown dog tick, *Rhipicephalus sanguineus*, is found worldwide with high tendency to feed on dogs and other mammals and human while spreading various types of pathogens. Various synthetic chemicals are available to treat the infestation. The main objective of this study was to find a natural alternative as a potential anti-tick compound. Naturally, betel leaves was chosen as it has many ascribed phytochemicals. The brown dog ticks were collected from stray dogs compounded at Vector Control Unit, Kuala Lumpur City Municipal, using forceps and kept alive in modified

specimen containers. Fresh *P. betle* leaves were collected from the University's Agriculture Park, Universiti Putra Malaysia. The leaves were dried in hot-air oven and grounded using bench-top grinder. Later the powder was soaked in ethanol and water dilutions, filtered and dried using the rotary evaporator to obtain the crude extract. Two different set of experiments were set-up, whereby ticks were exposed to both of the extracts prepared in dilutions ranging from 5^0 to 5^{-4} . Results from both experiments showed significant effect of the betel leaves as acaricide with the lethal dose from one to five dilutions of the pure extract. The ethanol extract have higher efficacy with 26 out of 50 ticks died (52%), meanwhile for the aqueous extract only 8 ticks died out of 50 (16%). In conclusion, the betel leaves ethanolic extract showed the potential to be used as acaricides for *R. sanguineus.*

Keywords: Acaricides, extract, *Piper betle*, *Rhipicephalus sanguineus*

1.0 INTRODUCTION

The brown dog tick, *Rhipicephalus sanguineus* (Latereille, 1806) is an arthropod, which make it of great significant in human medical and veterinary aspects. It feeds on blood for survival, and as it feeds it causes direct damage to the host and able to transmit various agents of diseases (Oliver, 1989). Examples of the pathogenic agents are *Babesia vogeli, B. gibsoni*, *Hepatozoon canis*, *Rickettsia conorii*, *R. rickettsii*, *Erlichia canis*, *Anaplasma platys* (Dantas-Torres, 2008; Fourie et al., 2013; Nicholson, Allen, McQuiston, Breitschwerdt, & Little, 2010).

It can feeds on other mammals including domestic animals and humans, which likely to happen when there is no dog or food source around and is able to complete its entire life cycle indoors (Lord, 2008). Thus, the infestation of the tick could increase drastically with just a few tick presence in the house or in the kennel especially after the walk about in the field (Dantas-Torres, 2008; Dantas-Torres, Figueredo, & Brandão-Filho, 2006; Lord, 2008; Nicholson et al., 2010).

Currently, there are various methods being used to control the tick flare, such as chemical controls using spot-on formulations, impregnated collars, shampoos, sprays, dips and powders, containing various active pesticides compounds e.g. Fipronil, amitarz, carbaryl, and pyrethroids (Dantas-Torres, 2008; Fourie *et al*., 2013; Williams *et al*., 2015). However, the increase trend of misuse of the pesticides brings a lot of concern as it could cause environmental pollution and toxicity to humans and other non-target organisms (Dantas-Torres, 2008; Dantas-Torres et al., 2006). Several issues, importantly the abuse in the pesticide usage have caused significant increased

in the pesticide resistance, thus creating new alternative of chemical control is extremely difficult and expensive (World Health Organization, 2006).

Alternatively, the idea is to use the common and readily available tropical herbs in Malaysia as a new source of cheap, effective, and environmental friendly to combat this problem. The betel leaves, *Piperaceae betle* was selected as it has been proven traditionally to have anti-microbial, anti-oxidative, anti-haemolytic and also anti-parasitic properties (Pin *et al*., 2006; Chakraborty & Shah, 2011; Wendy *at el.*, 2014; Jawale & Society, 2016; Syahidah *et al*., 2017).

Thus, the main objective of this study is:

To assess the effects of the betel leaves aqueous and ethanol extracts on the *Rhipicephalus sanguineus* as pesticide.

The hypothesis for this study is:

Betel leaves extract has potential effect on the *Rhipicephalus sanguineus* as acaricides.

2.0 LITERATURE REVIEW

2.1 Tick

There are approximately 825 tick species out of the 35,000 described species of acarines, which all of the tick species are obligate blood feeders with almost 90% of them are host specific and normally do not include humans and their livestock (Morand, Krasnov, & Poulin, 2006) . The rest of the 10% have a great significance due to their parasitic behaviour and the ability to transfer pathogenic agents to humans and other vertebrate. Tick are vectors of more kinds of microorganisms more than any other single arthropod taxon, including mosquitoes (Estrada-Peña & Estrada Peña, 2015).

The tick could be further divides into two main large families, which are the Argasidae (soft ticks) and the Ixodidae (hard ticks). The two families have different life cycles, morphological and physiological traits in which used to separate both of them.

The family Ixodidae (Ixodid ticks or hard ticks) includes a few species of concern, belonging to the genera Amblyomma (*A. cajennense*), Dermacentor (*D. andersoni*), Ixodes (*I. ricinus*), Haemaphysalis (*H. leporispalustris*), and Rhipicephalus (*Rhipicephalus sanguineus*). The name hard tick comes from the presence of sclerotized dorsal plate, which could enhance the ability of transmitting pathogens. They take quite sometimes during feeding on various vertebrae host, and some have high affinity towards human as host. Their bites usually painless initially and might be unnoticed for hours and could extend to days (Parola *et al*., 2005).

The soft tick does not have a suctum, and their mouthparts are prognathous located anterioventrally and with a leathery integument they can rapidly expends, making the nymph and adults able to engorge up to 10 times of their body mass within hours or even minutes. Many physiological processes differ between the two families like the way they digest blood (Hajdušek *et al.,* 2013). Hard tick in the otherhand, excrete the excess of water from the blood meal back into the host via the salivary gland while the soft tick use their coxal organs, a specialised ultrafiltration organ on the coxae 1 (Estrada-Peña & Estrada Peña, 2015).

.2.2 Basic Biology and Ecology of Ticks

All ticks need host, and they have three development stages besides the eggs starting from the larva, the nymph, and the adult tick. The feeding cycles are differ according to the family of the tick, for example Ixodid tick requires a few days to feed, and argasid tick only takes a few minutes to feed, usually during the night hours when the host is resting (Estrada-Peña & Estrada Peña, 2015). Figure 1, represent a diagram of the lifecyle of the ixodid tick from the larva to the adult. Ixodid ticks feed huge amount of blood once in each of their active stage, where the immature stages (larvae and nymph) will feed on the small host like the rats and birds, while the adults will feed on the larger host exmple the carnivours and ungulates. However, it is not a must for all the tick species as some of the tick speciess have preference towards a particular host.

The tick will probe the skin of the host with their moutparts after they find a suitable feeding place on the host. Then they will secretes sement like substances

which it acts as an hold to the skin for feeding. After a few hours of attachment, the process of multiple peristaltic movements begins producing saliva into the feeding cavity which have various active compounds which helps to sustain the blood flow into the feeding cavity, lysis of the surrounding cell of the feeding cavity, and also evasion of the host immune response. Approximately 24 hours after the tick feeding, the trasmission of the disease would likely to occour, but it might starts sooner (Nuttall and Labuda 2004; Voordouw 2015).

The tick will detached and dropped to the ground after a blood meal. Then they will moult while the engorged adult female will lay thousand of eggs instead of moulting around the decaying vegetation which give protection and have high relative humidity for its survival.

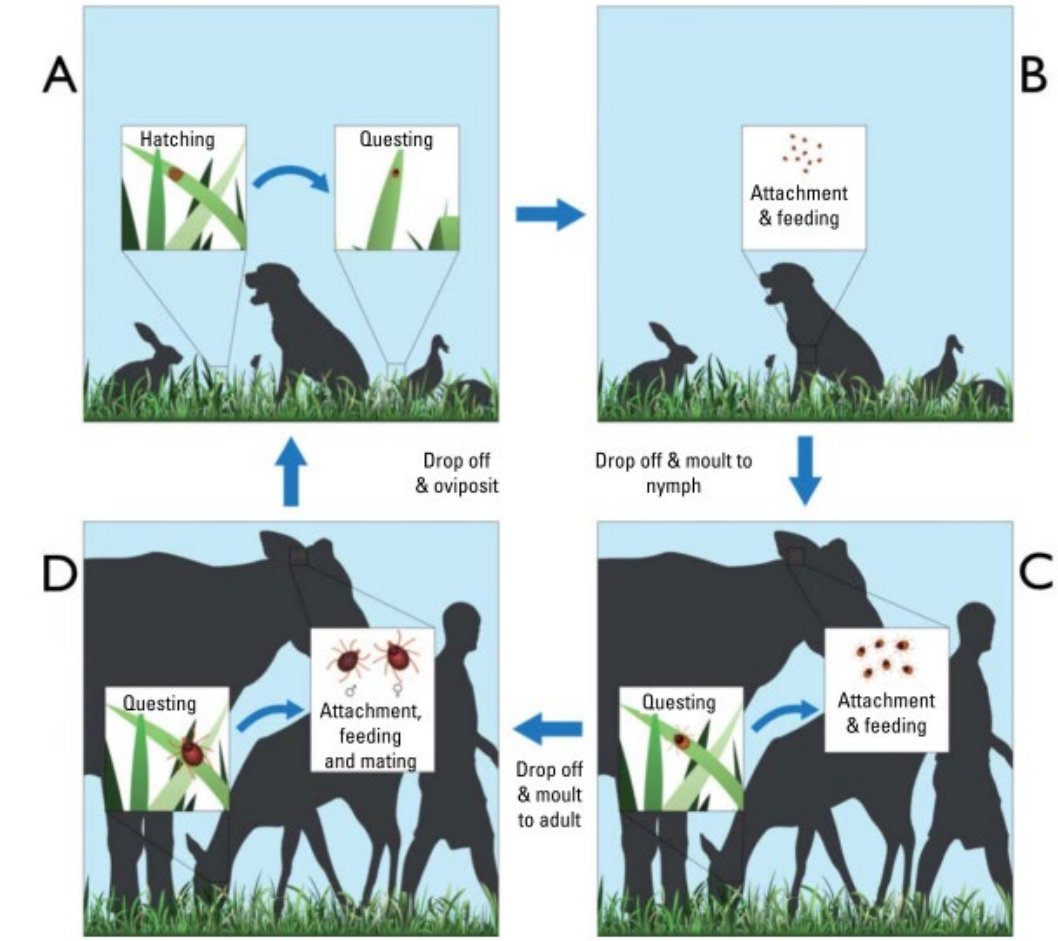

Figure 1: The life cycle of the three hosts tick

2.3 *Rhipicephalus sanguineus*

Under the genus *Rhipicephalus* they approximately 79 species, which includes five more species that in the genus *Boophilus* (Barker and Murrell, 2004). The *Rhipicephalus* tick are usually inornate, small, with few sexual dimorphism. *R. sanguineus,* the other name for brown dog tick are the common ectoparasite of domestic dog worldwide and it primarily choose the dog as the host, although it also reported feeding on wild and domestic animals, including humans (Dantas-Torres, Figueredo and Brandão-Filho 2006).

Table 1
Common names of the *R. sanguineus*[a]

Common name	Language	Country
Tropical dog tick	English	South Africa
Tique sanguine	French	Canada
La garrapata del perro	Spanish	Panama
Kennel tick	English	United Kingdom
Hondeteek or hondenteek	Dutch	Netherlands
Hondehokbosluis or hok-bosluis	Afrikaans	South Africa
Garrapata parda del perro	Spanish	Argentina
Garrapata marrón del perro	Spanish	Argentina
Garrapata café del perro	Spanish	Chile
Carrapato vermelho do cão	Portuguese	Brazil
Brown dog tick	English	United States and South Africa
Braune Hundszecke or Braune Hundezecke	German	Germany

[a] Adapted from Ticksbase (http://www.icttd.nl).

Figure 2: Common name of R. sanguineus

2.4 Veterinary and Medical Significance

R. sanguineus are known to carry and transmits pathogens of various agents of diseases (Oliver, 1989). Examples of the agents are the *Babesia vogeli*, *B. gibsoni, Hepatozoon canis, Rickettsia conorii, R. rickettsii, Erlichia canis, Anaplasma platys* (Dantas-Torres, 2008; Fourie et al., 2013; Nicholson et al., 2010).

Table 2
Alphabetical list of pathogens that are or may be transmitted by *R. sanguineus* ticks

Pathogen	Associated disease	Reference
Anaplasma marginale[b]	Bovine anaplasmosis	Parker and Wilson (1979)
Anaplasma platys[a] (formerly *Ehrlichia platys*)	Canine cyclic thrombocytopenia	Simpson et al. (1991)
Babesia caballii[b]	Equine babesiosis	Enigk (1943)
Babesia canis	Canine babesiosis	Regendanz and Muniz (1936)
Babesia gibsoni	Canine babesiosis	Sen (1933)
Cercopithifilaria grassi (formerly *Dipetalonema grassi*)	Canine filariosis	Bain et al. (1982)
Coxiella burnetii	Q fever	Mantovani and Benazzi (1953)
Dipetalonema dracunculoides	Canine filariosis	Bain (1972); Olmeda-García et al. (1993)
Ehrlichia canis	Canine monocytic ehrlichiosis	Groves et al. (1975)
Hepatozoon canis	Canine hepatozoonosis	Nordgren and Craig (1984)
Leishmania infantum[a] (syn. *Leishmania chagasi*)	Canine visceral leishmaniasis	Blanc and Caminopetros (1930)
Mycoplasma haemocanis (formerly *Haemobartonella canis*)	Canine haemobartonellosis	Seneviratna et al. (1973)
Rangelia vitalli[a]	Nambiuvu or peste de sangue	Loretti and Barros (2005)
Rickettsia conorii	Mediterranean spotted fever	Brumpt (1932)
Rickettsia rickettsii	Rocky Mountain spotted fever	Parker et al. (1933)
Theileria equi[b] (formely *Babesia equi*)	Theileriosis	Enigk (1943)

[a] Despite the evidence indicating that *R. sanguineus* can be a vector of these pathogens, further research is needed to prove it.
[b] *R. sanguineus* ticks seldom bite hosts other than dogs and thus its role in the transmission of these pathogens in nature is probably minor.

Figure 3: List of pathogens transmitted by R. sanguineus

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2.5 Tick Saliva and the Susceptibility of Dogs to *R. sanguineus* Infestation

In ticks, the salivary glands are the main organs responsible for osmoregulation where they excrets approximately 70% fluids containing water and ions. They also contain some of the powerful immunomodulatory characteristic that maintain the long period of blood-feeding (Hajdušek *et al*., 2013). The saliva ingredients able to supress the host immune system and inflammatory response containing the vasodilators, anesthetic, anti-inflammatry, antihemostatic and immunosupressive molecules like the anit histamines (Nuttal and Labuda, 2004). There also a risk of tick-borne pathogen transmission and establishment due to the immunomodulatory effects of the tick saliva (Bishop, Mejia, P.rez De Le.n, Tabachnick, & Titus, 2006) as it impares the T-cells proliferation and its microbicidal activity of the macrophages. After the attachment of the tick on the skin of the host, the pathogens will able to survive and proliferate form the impared local immune respose of the host (Nava *et al*., 2015).

Dogs with the recurrent cycles of attachment of the tick unable to develop a delayed-type hypersensitivity (DTH) response indicating the deficient of cell-mediated immune response torawds the tick infestations. Meanwhile, guiena pigs was able to produce the strong cell-mediated immune response on multiple successive infestations from the *R. sanguineus* ticks which suitable to show the example of the resistant host (Szabó *et al.*, 2003). Mainly, neutrophils could be seen with sequential histopathology of the feeding sites of the *R. sanguineus* of the dog, meanwhile the guiena pigs react with mononuclear cells which are more specific like the eosinophils and basophils (Ferreira *et al.*, 2003). It showed that the dogs do not develop resistance like the guiena pigs do, suggesting that the *R. sanguineus* might have evolve its salivary immunomodulatory factors to accustomed to the dogs immune response for survival (Ferreira *et al.,* 2003).

Some dog breeds also have shown higer susceptibility towards *R. sanguineus* infestations then others like amount of adult tick feeding on English Crocker Spaniel dogs was significantly higher then the number of adult tick feeding on the mongrel dogs (Louly *et al.,* 2007). This indicated that there are more information needed to be obtained for us to access the susceptibility of different dog breeds.

2.6 Controls

There are multiple method can be used as control for the tick population which includes the use of chemical and non-chemical stratergies. Talking about the tick control, we need to consider that only approximately 5% of the tick are on the host

body meanwhile the rest is in the environment. Thus, the combination of method is ideal to control both the tick on the dog itself and also the environment.

2.7 Chemical controls

There are various methods was devised to control the tick flare which include the chemical control using spot-on formulations, impregnated collars, shampoos, sprays, dips and powders, with various active compounds like the Fipronil, amitarz, carbaryl, and pyrethroids which are the commonly used pesticides (Dantas-Torres, 2008; Fourie et al., 2013; Williams et al., 2015). However, the current trend of the misuse of the pesticides brings a lot of concern as it could cause environmental pollution and toxicity to humans and other non-target organisms (Dantas-Torres 2008; Dantas-Torres, Figueredo and Brandão-Filho 2006). It also brings a lot of issues as the abuse in the pesticide usage have cause significant increase in the pesticide resistance, thus creating new alternative of chemical control is extremely difficult and expensive (World Health Organization, 2006).

As about 95% of the tick are in environment, it also need to be treated by using acaricides where the dog lived. The environmenntal treatment could be effective providing when the restricted areas are treated. Few factors of concern are the level of environmental infestation, presence of infestations in the areas next to the treated one, residual effect of the acaricide, and the environment condition. The usage of acaricides on the environment have its own concerns in which the improper use can cause environmental pollution and toxicity to humans and also non-target organisms (Dantas-Torres, 2008; World Health Organization, 2006). Acaricide resistance is also

one of the serious issue in concern to the missuse of the acaricides expecially on the long-term use (Labarrthe, 1994; World Health Organization, 2006).

2.8 *Piper betle*

Piper betle (Piperaceae) leaves is commonly use as traditional herbs and the crops is grown extensively in India, Sri Langka, Malaysia, Thailand, Taiwan, and other South Asian countries. It is reported to have various uses such as aromatic, antibacterial, antipruritic, antiseptic, bronchodilator, expectorant, protection against intestinal parasites, anti-platelet, antifungal and anti-oxidant (Akter *et al.* 2014; Chakraborty and Shah 2011; Hoque *et al*. 2011; Jawale and Society 2016; Syahidah *et al*. 2017).

The common names are betel (English), paan (Indian), phlu (Thai) and Sirih (Bahasa Indonesia). According to Syahidah *et al.* (2017), the main consituents of the leaves are the volatile oils e.g. phenols, betel-phenol, chavibetol, chavicol, cadinene and hydroxychavicol, with claims for their medicinal properties such as the anti-tumour, anti-fertility, anti-parasitic, digestive, antacid, decongestant, carminative, stimulant, antipyretic, anti-inflammatory, anti-allergic, antiseptic, hepatoprotective, radio-protective, anti-platelet, antifungal, nematocidal and anti-oxidant.

2.9 Ethanol Extract of the Betel Leaves

Various active compounds have been found in the ethanol extract of the betel leaves, such as tanins, anthraquinones, flavanoides, alkaloides, terpernoids, saponins,

cardica glycosides, glycosides, reducing sugars and phlobatanins which have huge potential in health aspects and disease management (Kumari, 2015).

3.0 MATERIALS AND METHODS

3.1 Study Animals

The brown dog ticks collected from stray dogs kept at the Vector Control Unit under Kuala Lumpur City Municipal, Kuala Lumpur using forceps and kept in modified specimen containers with multiple small holes, size ~1 mm for aeration and maintained in the room temperature (27ºC; 70% humidity) , until further used. Both female and male adult unfed tick selected to provide a uniform data.

3.2 Preparation of Herb

Piper betle leaves were obtained from the University's Agriculture Park, Universiti Putra Malaysia (UPM), Serdang, Selangor. Fresh and healthy leaves were collected in the morning and the evening and after which were cleaned with running tap water to remove dirt particles and soil. The leaves were dried using hot-air oven (Memmert Laboratory Oven, Schwabach, Germany) at 40 ºC for 4 days. Then the dried leaves were chopped into smaller pieces and grounded into powder using the bench-top grinder (Panasonic, MY333). The herbal powder was kept in the airtight glass bottle at room temperature prior to extraction.

3.3 Preparation of Ethanol Extraction

The ethanol extraction were prepared using slightly method as described by Syahidah *et al.* (2017), using 500g of the herbal powder added into 1500mL of 80% ethanol in Schott's bottle wrapped with aluminium foil. The solution was let to stand

for 4 days in room temperature, before being filtered through Whatman no 42 filter paper and dried using rotary evaporator at 50 ºC with the rotation of 150 rpm. The crude extract obtained was kept in -20 ºC until further use. The yield percentage was determined using the formula as described by Syahidah *et al*., (2017**).**

$$\% \text{ Yield} = \frac{W^2 - W^1}{W^0} \text{ X } 100$$

Where:

W^2 is the weight of the extract and container

W^1 is the weight of the empty container

W^0 is the weight of the initial dried sample

3.4 Preparation of Aqueous Extract

The method of aqueous extraction was adapted from Jenie & Apriyantono (2008) with slight modification. The dried herbal powder was mixed with deionised distilled water with the ratio of 1:10 (g/vol) and homogenised using magnetic stirrer for 24 hours with 150 rpm. It was then filtered using Whatman no 42 membrane filter paper and dried using rotary evaporator at 50ºC with the rotation of 150 rpm. The was crude extract collected into a closed container and stored in -20ºC until further use.

3.5 Dilution Preparation

According to Marchiondo *et al.* (2013) on the guidelines evaluating the efficacy of paraciticides stated that for the dose determination studies, ideally required

four groups, each may be administered at 0, 0.5, 1 and 2 times of the anticipated dose. Thus the anticipated dose was taken from Syahidah *et al*. (2017) assuming that 1000mg was the lethal dose and the crude extract was diluted volume per volume (v/v) in five-fold dilutions ranging from $5^0, 5^{-1}, 5^{-2}, 5^{-3}$, and 5^{-4} by diluting 1 mL of crude extract with 4 mL deionised water, and kept in the 10 mL Bijou bottles. Similar dilutions were prepared for both water and ethanol extracts and kept in -20 ºC prior to use.

3.5. Evaluation on Dose Relation Effect

3.5.1 Experiment 1

Five adult unfed of mixed gender ticks were selected and put inside a petri dish containing a piece of filter paper measuring 3cm x 3cm, soaked with the herbal extract. The set-up was kept in the room temperature ~25 to 27 ºC and humidity at 70%. The abstract was observed for any immediate repellent and acaricidal effects as stated in the Guideline for the Testing and Evaluation of the Efficacy of Anti-parasitic Substances for the Treatment and Prevention Of Tick And Flea Infestation In Dogs And Cats (2007). The tick viability was also accessed based on the movement, normal posture and the leg coordination within the incubated period for various intervals of 10 sec, 5, 10, 30 minutes, 1, 2, 4, 8, 12, 24, 48, and 72 hours as stated in Marchiondo *et al.* (2013).

3.5.2 Experiment 2

Method used was adapted from Williams *et al.* (2015) where five adults, unfed of mixed gender ticks were selected and immersed in 2 mL of the prepared

concentrations in the Erlenmeyer flask for 3 minutes. The ticks were then collected from the flask, and dried using paper towel, before being transferred to a dry filter paper in a petri dish and covered. The maintenance and the observation method was done using the same method as described in Experiment 1.

		Ethanol Extract											
Concentration	Time	10 sec	5 min	10 min	30 min	1 hour	2 hours	4 hours	8 hours	12 hours	24 hours	48 hours	72 hours
1	Alive												
	Paralysed												
	Dead												
1:5	Alive												
	Paralysed												
	Dead												
1:25	Alive												
	Paralysed												
	Dead												
1:125	Alive												
	Paralysed												
	Dead												
1:625	Alive												
	Paralysed												
	Dead												

		Water Extract											
Concentration	Time	10 sec	5 min	10 min	30 min	1 hour	2 hours	4 hours	8 hours	12 hours	24 hours	48 hours	72 hours
1	Alive												
	Paralysed												
	Dead												
1:5	Alive												
	Paralysed												
	Dead												
1:25	Alive												
	Paralysed												
	Dead												
1:125	Alive												
	Paralysed												
	Dead												
1:625	Alive												
	Paralysed												
	Dead												

Figure 4: The observation table for both of the experiment showing the effect of Betel leaves extract on the ticks after exposure.

4.0 RESULTS

4.1 Overview

One hundred ticks were used for this experiment. A total of 34 ticks died after exposure to the betel leaves extracts as represented by the pie chart (Figure 5).

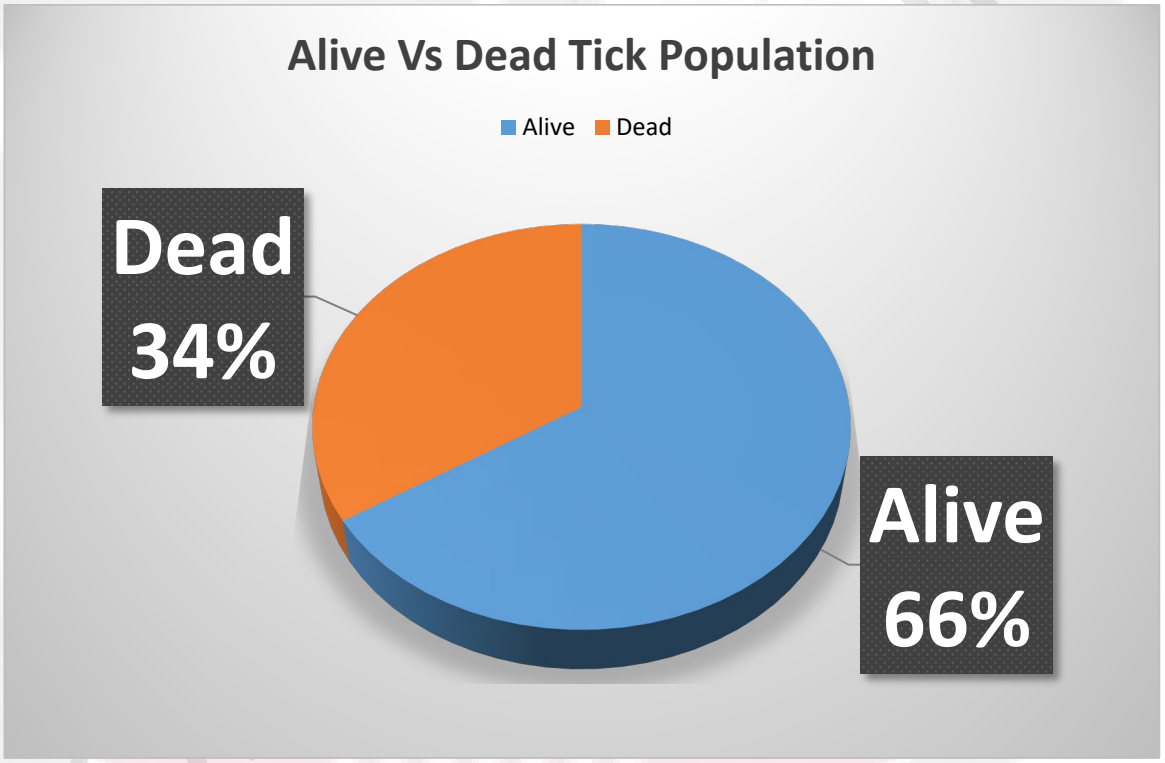

Figure 5: Pie chart showing the percentage of the alive and dead tick population at the end of experiment.

4.2 Comparison of the Acaricidal Effects of Aqueous Extract and Ethanol Extract

The bar chart (Figure 6) showed the number of tick that had died in both of the experiment after three days of the exposure to the extracts. Ethanol extract showed to

has higher mortality rate with 26 ticks died as compared to aqueous extract in which only eight ticks died.

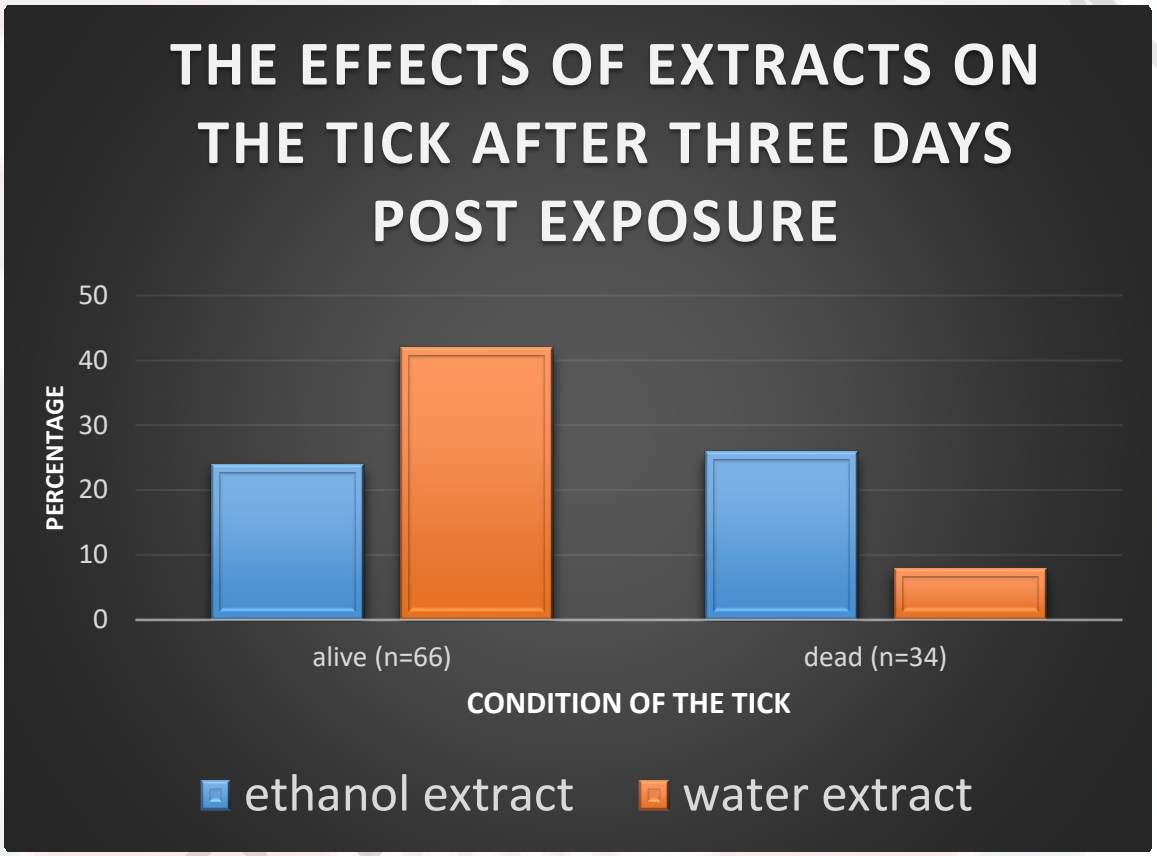

Figure 6: Bar chart of the effects of different types of herbal extracts on the tick survival after 3 days post exposure.

4.3 Assessment of the Extract Exposure Time on the Tick Mortality

Figure 7 represents duration of the extract exposure against number of tick mortality (cumulative) for both of the experiment for all of the concentrations. The chart indicated that the extract had some acute toxicity effect on the ticks, as the mortality could be seen on the first 5 minutes, especially in the pure and higher extract concentrations. Then the mortality of the tick started to spike up from the 30 minutes to 4 hours duration of the ethanol extract exposure where by 22 ticks died, and five

ticks died for water extract. After 48 hours of exposure, the mortality did not show much significant change.

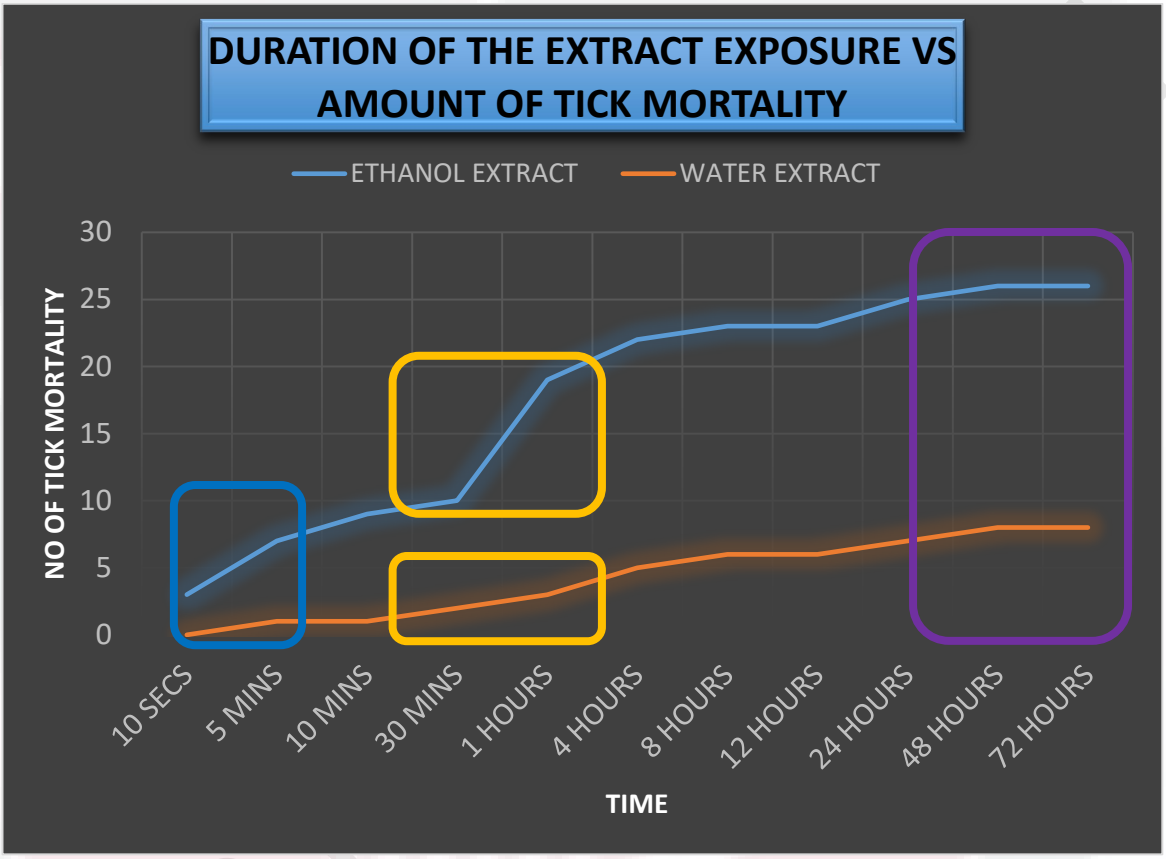

Figure 7: The relationship of the duration of the extract exposure and tick mortality

4.4 Assessment of the Effect of the Extract at Different Concentrations

After 12 hours of exposure to the ethanol extract in Experiment 1, 100% of the tick was killed in the pure concentration and in the 1:5 dilution factor after 4 hours of exposure. On the other hand, in the other concentrations, the ticks were still alive and healthy. For the Experiment 2 on the ethanol extract, both concentrated (pure) and 1: 5 dilution factor, gave 100% mortality after 1 hour post exposure, meanwhile for other dilutions the ticks remained alive.

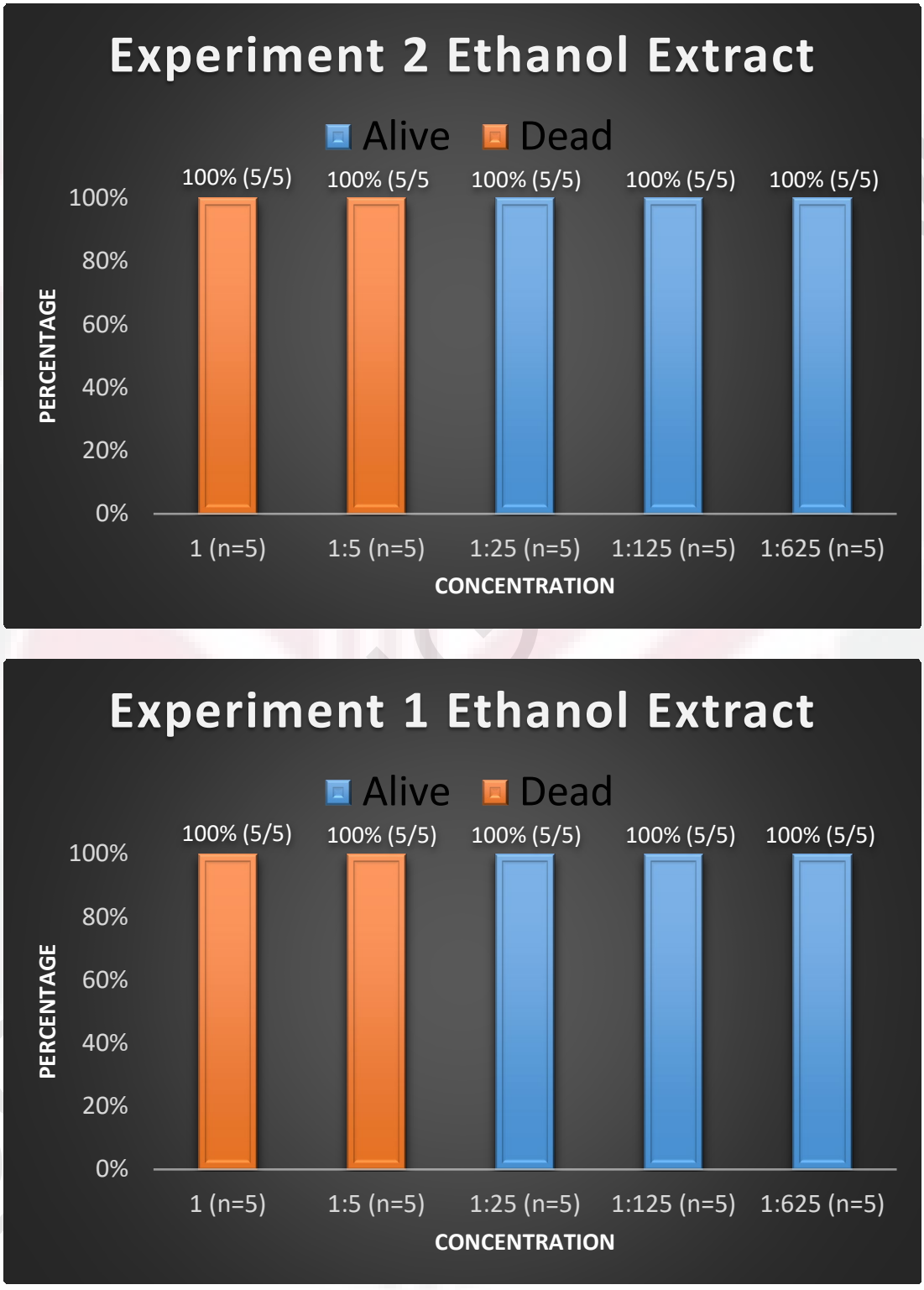

Figure 8: Percentage of tick mortality against the different concentrations of the ethanol extract in both of the experiments.

For the Aqueous extract in Experiment 1, 100% of the ticks have died in the pure concentration, and 20% of the tick population died in the 1:5 dilution factor after 2 hours of exposure. For the Experiment 2, for the aqueous extract, only one tick died in the pure concentrate after 12 hours of exposure, as tabulated in Figure 9.

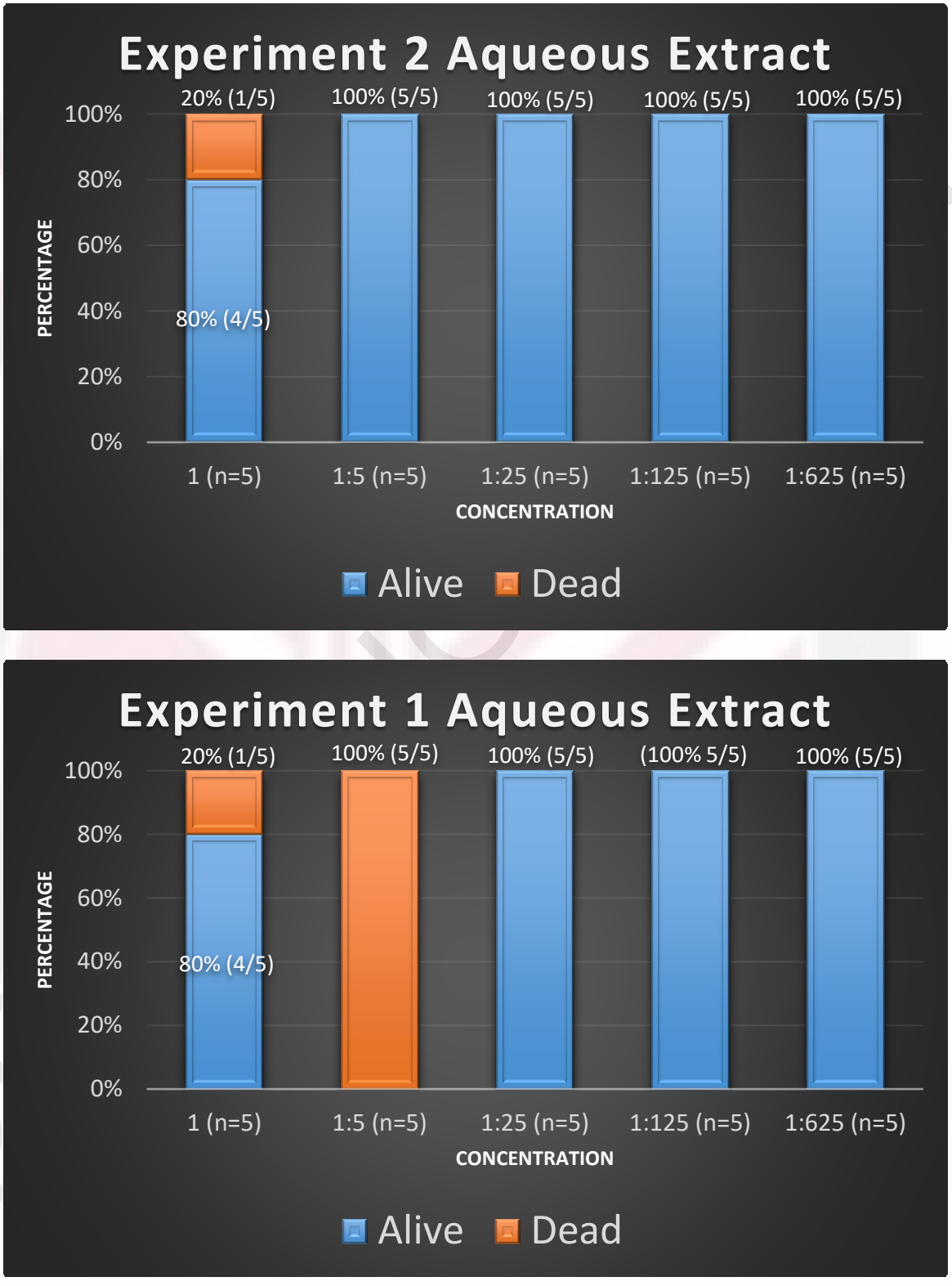

Figure 9: Percentage of tick mortality against the different concentrations of the aqueous extract in both of the experiments.

5.0 DISCUSSION

In this current study we observed that the ethanol extract showed higher efficacy as acaricides as compared to aqueous extract, as illustrated in Figure 5. This might be due to the nature of the extraction. According to Syahidah *et al.* (2017), alcohol extraction have been proven able to extract numerous bioactive compounds such as hydroxychavicol and eugenol which were the anti-bacterial compounds. Besides that, according to Akter *et al.* (2014) the betel leaves have potent antihelmintic activity in which could be due to the presence of several compounds like the alkaloid, polyphenol, flavonoid and terpene that interfered with the CNS function of the parasite and caused paralysis to the organism.

Thus, from the chart in Figure 7 it could be suggested that the duration of action for the herbal extract to have some acute acaricidal effect started from five minutes of administration to the maximum acaricides effect which were between 30 mins until 4 hours, and the minimum effect after two days post exposure. As shown in Figure 8 and 9, it was postulated that the lethal concentration dose for the ethanol extract was at the 1:5 dilution. Meanwhile for the aqueous extract, it seemed for both of the experiment, the lethal concentration dose suggested that only the pure extract concentration had a slight effect of the acaricides. The deviation of value in Experiment 1 in aqueous extract could has happened due to human error.

Thus, there are few suggestions as to improve the outcome of the experiment such as;

i) by carrying out chemical extraction to detect the bioactive ingredients in the leaves

ii) to use larger sample of ticks to give results that are more accurate

iii) to make early preparation as the herbal extraction processes required very long time

iv) devise more refine technique of extraction to bring out the best result

v) finally yet important, the methodology could be improvised to ensure that the human errors are reduced

6.0 CONCLUSION

In conclusion, this preliminary experiment has proved a significant acaricidal effect of the betel leaves ethanol extract for both dilutions i.e. for the pure and 1:5 serial dilutions. On the other hand there were only slight acaricidal effect for the aqueous extract even for the pure concentrate. For both of the extract, it seemed that the betel leaves could exhibit the acaricidal effect as fast as five minutes after application, with the maximum effect from the 30 minutes to 4 hours, while no significant changes after two days of exposure.

More testing and further refining needs to be done to fully understood the mechanism of the acaricidal effect of the betel leaves on the brown dog tick, and its efficacy on being the replacement for the commercial acaricides as a safe, effective and environmental friendly pesticide.

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8.0 APPENDICES

the different concentration versus the status of the tick

Concentration * Status Crosstabulation

			Status		
			Alive	Dead	Total
Concentration	Pure	Count	7	13	20
		% of Total	7.0%	13.0%	20.0%
	1 to 5 dilution	Count	5	15	20
		% of Total	5.0%	15.0%	20.0%
	1 to 25 dilution	Count	15	5	20
		% of Total	15.0%	5.0%	20.0%
	1 to 125 dilution	Count	19	1	20
		% of Total	19.0%	1.0%	20.0%
	1 to 625 dilution	Count	20	0	20
		% of Total	20.0%	0.0%	20.0%
Total		Count	66	34	100
		% of Total	66.0%	34.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	42.068[a]	4	.000
Likelihood Ratio	49.382	4	.000
Linear-by-Linear Association	35.294	1	.000
N of Valid Cases	100		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.80.

Chi square result water extract and ethanol vs the status of the tick

Crosstab

			Status		
			Alive	Dead	Total
Extract	Ethanol Extract	Count	24	26	50
		% of Total	24.0%	26.0%	50.0%
	Water extract	Count	42	8	50
		% of Total	42.0%	8.0%	50.0%
Total		Count	66	34	100
		% of Total	66.0%	34.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	14.439[a]	1	.000		
Continuity Correction[b]	12.879	1	.000		
Likelihood Ratio	15.005	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	14.294	1	.000		
N of Valid Cases	100				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 17.00.

b. Computed only for a 2x2 table

the method of experiment vs the status of the tick

Experiment * Status Crosstabulation

			Status		
			Alive	Dead	Total
Experiment	Experiment 1	Count	28	22	50
		% of Total	28.0%	22.0%	50.0%
	Experiment 2	Count	38	12	50
		% of Total	38.0%	12.0%	50.0%
Total		Count	66	34	100
		% of Total	66.0%	34.0%	100.0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.456[a]	1	.035		
Continuity Correction[b]	3.610	1	.057		
Likelihood Ratio	4.506	1	.034		
Fisher's Exact Test				.057	.028
Linear-by-Linear Association	4.412	1	.036		
N of Valid Cases	100				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 17.00.

b. Computed only for a 2x2 table