CHAPTER 1

INTRODUCTION

1. GENERAL INTRODUCTION

1.1. Statement and significance of problem

Mosquitoes are the most important vectors of parasitic and viral infections to people worldwide (Vineetha and Murugan, 2009). The main vectors of medical importance are *Anopheles* spp. (malaria, lymphatic filariasis, and Japanese encephalitis), *Culex* spp. (lymphatic filariasis, Japanese encephalitis), *Aedes* spp. (dengue/dengue hemorrhagic fever, yellow fever, lymphatic filariasis) and *Mansonia* spp. (lymphatic filariasis) (Rozendaal, 1997; Mourya et al., 1989). Up until now, there has been no vaccination available for the prevention of mosquito-borne diseases, except yellow fever and Japanese encephalitis. The elimination and control measures of diseases have focused on the management of mosquito vectors. Vector controls in Thailand have been carried out by many methods such as applying larvicides, adulticides and repellents to reduce or stop the spreading of diseases (Gratz, 1999; Kittayaong et al., 2006; Pethuan et al., 2007).

The application of mosquito repellents on the skin, clothing and bed net is one of the best tools for protecting and reducing human contact with vector and nuisance arthropods (Gupta and Rutledge, 1994; Curtis, 1992; Fradin, 2001). Repellent not only affords advantages in protection against a wide range of vectors (WHO, 1995), but it also is the primary means of arthropod-borne disease prevention available in areas where vector control is not practical (Gupta and Rutledge, 1994; Copeland et al., 1995). The best-known chemical insect repellent is *N,N*-diethyl-m-toluamide, now called *N,N*-

diethyl-3-methylbenzamide (DEET) (Fradin and Day, 2002). DEET is used as an active ingredient in commercial insect repellent. Formulated by the United States Department of Agriculture for the U.S. Army in 1946 for protection against biting insects and control of disease transmission, it was registered subsequently in 1957 by the United States Environmental Protection Agency (U.S. EPA, 1997) for use in the general population (Sax and Lewis, 1989). DEET has been used as a broad-spectrum repellent against mosquitoes, ticks, chiggers, biting flies, fleas and other insects that can transmit disease (Davis, 1985; Jensenius et al., 2005). In addition, other chemical repellents such as dimethyl phthalate (DMP), para-methane 3-8, diol (PMD), ethyl butylacetylaminopropionate (IR3535) and, Picaridin (KBR3023) have been reported as effective substances against mosquitoes and other biting insects (Barnard and Xue, 2004; Karunamoorthi and Sabesan, 2010). However, although they are efficient, adverse reactions associated with their application have been reported. Complications from accidental DEET ingestion by drinking DEET solutions, exposure via inhalation or contact with eyes or overdosing or spraying into the eye also have occurred (Zadikoff, 1979; Tenenbein, 1987; Veltri et al., 1994; Petrucci and Sardini, 2000; Briassoulis et al., 2001; Bell et al., 2002; ATSDR, 2012). Case reports of children exposed to DEET were made from 1961 to 2002. The most frequent symptoms found were lethargy, convulsions, seizures, involuntary movements, tremors and, headaches (de Garbino and Laborde, 1983; Osimitz and Grothaus, 1995; Osimitz and Murphy, 1997; Braissoulis et al., 2001). Furthermore, this compound has an unpleasant odor, oily feel, toxic reactions and, high skin penetration, and it can damage plastic and, synthetic fabric (Qiu et al., 1998).

For these reasons, many researchers need to find new synthetic insecticides from a plant origin, which is effective, environmentally friendly and easily biodegradable. In many parts of the world, plant products have been used traditionally as repellents against insects, primarily for avoiding nuisance biting (Curtis et al., 1991). Up to now, numerous plants have been reported as potential natural sources of insect repellents (Sharma et al., 1993; Phasomkusolsil and Soonwera, 2010; Tawatsin et al., 2001; Barnard and Xue, 2004; Miot et al., 2004; Govindarajan and Sivakumar, 2011; Solomon et al., 2012).

Plant substances such as flavonoids, terpenoid, volatile oils and so on have created considerable attention in recent years, due to their diverse phytochemical properties that repel mosquitoes (Carroll and Loye, 2006; Wanga et al., 2008; Dhandapani and Kadarkarai, 2011). They can be extracted from many parts of the plants, that contain active components, including bark, leaves, flowers, roots, fruit, seeds, etc. (Dremova et al., 1971; Ansari et al., 2005; Noosidum et al., 2008). The well-known mosquito repellents that derive from plants are citronella and eucalyptus oils. Citronella and eucalyptus have been used commercially by human communities in many parts of the world against arthropod vectors and pests (Dethier, 1947; Trongtokit et al., 2005; Kim et al., 2005; Müller et al., 2008; Yang and Ma, 2005; Mandal, 2011).

Recently, commercial repellent products containing plant-based ingredients have gained increasing popularity among consumers (Maia and Moore, 2011). Therefore, finding new plant-based repellents is important in developing alternative products for personal protection from mosquito attacks and in helping to interrupt mosquito-borne disease transmission.

2. LITERATURE REVIEW

2.1. Mosquitoes

Mosquitoes are classified into the family Culicidae, Suborder Nematocera, order Diptera, class Insecta of Phylum Arthropod. Over 3,500 species of the Culicidae have already been described. They are generally divided into three subfamilies (Knight and Stone, 1997)

1. Toxorhynchitinae: *Toxorhynchites* spp.
2. Anophelinae (Anophelines): *Anopheles* spp., *Bironella* spp., and *Chagasia* spp.
3. Culicinae (Culicines): *Aedes* spp., *Culex* spp., *Mansonia* spp., *Armigeres* spp., *Haemagogus* spp., *Sabethes* spp., and *Psorophara* spp.

A total of 3,529 species of Culicidae are currently recognized (Harbach, 2013), which contains 43 genera. This study focused on the examination of two genera, *Aedes* of the Culicines, a potential vector of dengue viruses and *Anopheles* of the Anophelines a potential vector of malaria

The mosquitoes are a family of small, like other true flies, Culicids exhibits "complete metamorphosis" consisting of four stage starts as an egg then goes to an larva then goes to a pupa then is an adult (Figure 1.1). The events that characterize the immature life history of a mosquito are hatching, larval development, pupation, and adult emergence. The larva differs from the adult in various aspects such as anatomy, habitat, and food. Transformation to the adult occurs during the non-feeding pupal stage (Clements, 1992; Beaty and Marquardt, 1997).

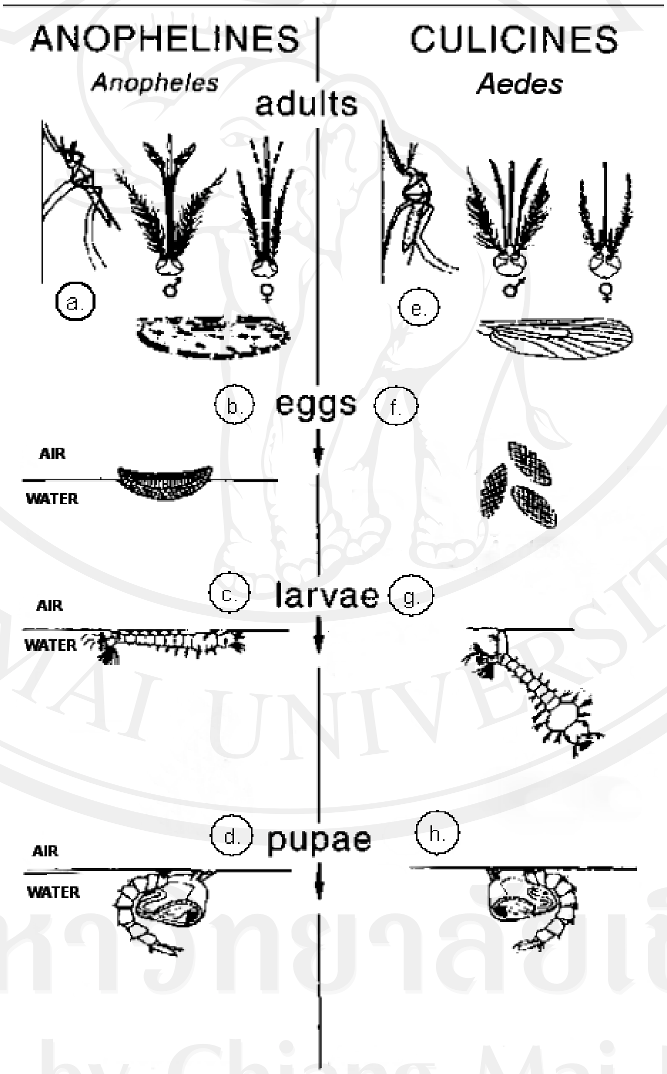

Figure 1.1 The life-cycles of *Anopheles* and *Aedes* mosquitoes
(Warrell and Gilles, 2002)

- ***Aedes* spp. are mosquito member of**

Phylum	Arthropoda,
Class	Insecta,
Order	Diptera,
Suborder	Nematocera,
Family	Culicidae,
Subfamily	Culicinae,
Genus	*Aedes*.

Egg stage

After taking a blood meal, female *Aedes* produce on average 100 to 200 eggs per batch. The females can produce up to five batches of eggs during a lifetime. The number of eggs is dependent on the size of the bloodmeal. Eggs are laid on damp surfaces in areas likely to temporarily flood, such as tree holes and man-made containers like barrels, drums, jars, pots, flower vases, buckets, plant saucers, discarded bottles, tanks, tins, tyres, water cooler, etc., and a lot more places where rain-water collects or is stored. The female *Aedes,* lays her eggs separately unlike most species. Not all eggs are laid at once, but they can be spread out over hours or few days, depending on the availability of suitable substrates. Eggs will most often be placed at varying distances above the water line. The female mosquito will not lay the entire clutch at a single site, but rather spread out the eggs over several sites.

The eggs of *Aedes* mosquito (Figure 1.1f) are smooth, long, ovoid shaped, and roughly one millimeter long. When first laid, eggs appear white but within minutes turn a shiny black. In warm climates eggs may develop in as little as two days, whereas in cooler temperate climates, development can take up to a week. Laid eggs can survive for very long periods in a dry state, often for more than a year.

Larva stage

After hatching of the eggs, the larvae (Figure 1.1g) feed upon small aquatic organisms, algae and particles of plant and animal material in water-filled containers (CDC, 2012b). Most of the larval stage is spent at the water's surface, although they will swim to the bottom of the container if disturbed or when feeding. Larvae are often found around the home in puddles, tires, or within any object holding water. Larval development is temperature dependent. The larvae pass through four instars, spending a short amount of time in the first three, and up to three days in the fourth instar. Fourth instar larvae are approximately eight millimetres long. Males develop faster than females, so males generally pupate earlier. If temperatures are cool, *Aedes* spp. can remain in the larval stage for months so long as the water supply is sufficient.

Pupa stage

After the fourth instar, the larvae enter the pupal stage (Figure 1.1h). Mosquito pupae are mobile and respond to stimuli. Pupae do not feed and take approximately two days to develop. Adults emerge by ingesting air to expand the abdomen thus splitting open the pupal case and emerge head first.

Adult stage

Aedes adult (Figure 1.1e) are differs in characteristic white scale ornamentation from many other Culicine species. The anterior half of the head has large convex kidney shaped compound eyes laterally (Andrew and Bar, 2013). The legs are long and jointed structures. The coxae have white scale patches in both male and female (Christophers, 1960; Huang, 1979). Male and female adults feed on nectar of plants; however, female mosquitoes need blood in order to produce eggs. *Aedes aegypti* is a day biting mosquito. This species is most active for approximately 2 hr after sunrise and several hours before sunset, but it can bite at night in well-lit areas. This mosquito can bite people without being noticed because it approaches from behind and bites on the ankles, and elbows. *Ae*. *aegypti* prefers biting people but it also bites dogs, and other domestic animals, mostly mammals. Only females bite to obtain blood in order to lay eggs. The life span for adult mosquitoes is around three weeks (CDC, 2012b).

- ***Anopheles* spp. are mosquito member of**

Phylum Arthropoda,

Class Insecta,

Order Diptera,

Suborder Nematocera,

Family Anophelidae,

Subfamily Anophelinae,

Genus *Anopheles*.

Egg stage

After obtaining a full blood meal, *Anopheles* females can lay 50 to 200 eggs per opposition. Eggs are laid singly directly on water and are unique in having floats on either side. The eggs of *Anopheles* are elongated, small (approximately 0.5 × 0.2 mm), brown-blackish, boat-shaped eggs (Figure 1b).

Females deposit eggs individually on the surface of the water. Preferred oviposition sites include fresh water streams, ponds, and lakes with aquatic vegetation (Carpenter and Lacasse, 1955). Eggs are not resistant to drying and hatch two to three days after oviposition, although hatching may take up to 2-3 weeks in colder climates (CDC, 2012a). In situations where water is in relatively short supply, the eggs may remain in damp mud for days.

Larva stage

Mosquito larvae have a well-developed head with mouth brushes used for feeding, a large thorax, and a segmented abdomen. They have no legs. In contrast to other mosquitoes, *Anopheles* larvae lack a respiratory siphon and for this reason position themselves so that their body is parallel to the surface of the water. The larvae lie horizontally at the surface of the water where they feed on small organic particles (Figure 1.1c).

Larvae breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently. The larvae spend most of their time feeding on algae, bacteria, and other microorganisms in the surface microlayer. They dive below the surface only when disturbed. Larvae swim either by jerky movements of the entire body or through propulsion with the mouth brushes. Larvae develop through four stages, or instars, after which they metamorphose into pupae. Fourth instar larvae are approximately six to eight millimetres long. The larvae occur in a wide range of habitats but most species prefer clean, unpolluted water. Larvae of *Anopheles* mosquitoes have been found in fresh- or salt-water marshes, grassy ditches, mangrove swamps, rice fields, the edges of streams and rivers, and small, temporary rain pools. Many species prefer habitats with vegetation. Others prefer habitats that have none. Some breed in open, sun-lit pools while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants (O'Malley 1992; CDC, 2012a).

Pupa stage

The pupa is comma-shaped when viewed from the side (Figure 1.1d). The head and thorax are merged into a cephalothorax with the abdomen curving around underneath. As with the larvae, pupae must come to the surface frequently to breathe, which they do through a pair of respiratory trumpets on the cephalothorax. Pupae do not feed, after a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges. The duration from egg to adult varies considerably among species and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as little as 5 days but usually take 10-14 days in tropical conditions (CDC, 2012a).

Adult stage

Anopheles mosquitoes can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the wings. The slender bodies and long with 3 sections: head, thorax and abdomen, two sensory palps, long wing and legs, no scales on the abdomen and forward-projecting proboscis (needle-shaped) used for feeding. Three pairs of legs and a pair of wings are attached to the thorax. Adult *Anopheles* can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting (Figure 1.1a). Adult mosquitoes usually mate within a few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate. Males live for about a week, feeding on nectar and other sources of sugar. Females will also feed on sugar sources for energy but usually require a blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed. This process depends on the temperature but usually takes 2-3 days in tropical conditions. Once the eggs are fully developed, the female lays them and resumes host seeking. The cycle repeats itself until the female dies. Females can survive up to a month (or longer in captivity) but most probably do not live longer than 1-2 weeks in nature. Their chances of survival depend on temperature and humidity, but also their ability to successfully obtain a blood meal while avoiding host defenses (CDC, 2012a).

2.2 Mosquito control

Mosquito control is the process of actively reducing the number of mosquitoes. Comprehensive mosquito control can use one or more approaches that target different environments and life stages of the mosquito. The most important reason to control mosquitoes is to reduce the likelihood of diseases such as dengue hemorrhagic fever, West Nile virus, malaria, lymphatic filariasis, Japanese encephalitis being transmitted to people through mosquito bites. Throughout history, no insect has been a more significant contributor to human discomfort, disease, and death than the mosquito. Even mosquitoes that do not transmit disease can be bothersome in their biting behavior. In severe instances nuisance mosquitoes can be economically detrimental to businesses, and reduce the quality of life for residents (MVCAC, 2005).

Most mosquito control programs reduce mosquito populations through a multi-faceted approach known as Integrated Pest Management (IPM). A mosquito has four life stages -egg, larva, pupa, and adult. In the life cycle of the mosquito, only the adult stage does not require standing water. An IPM program targets each life stage of the mosquito, but is intended to eliminate as many mosquitoes as possible before they emerge as biting adults. Mosquito control strategies have changed considerably over the past few decades. Diesel oil and inorganic poisons such as Paris green (copper-aceto-arsenite) were the basic tools of early mosquito larva control efforts.

2.3 Plant-derived repellents

In searching for potential plant-based repellents, numerous plant materials, including plant extracts and essential oils, have been evaluated for repellency against many arthropods of medical importance. Repellency of *Achillea millefolium* (yarrow) extract, birch/pine tar, citronella, clove, eucalyptus, geranium, lavender, lily of the valley and peppermint oils against *Ae. aegypti* in laboratory and field tests predominantly with *Ae. communis* and *Ae. cinereus* showed that ethanol extract of yarrow and oils exhibited less repellent activity than DEET and *N,N*-diethyl mandelic acid amide (DEM) (Thorsell et al., 1998).

The essential oils of *Cymbopogon commutatus*, *C. martinii*, *C. pendulus*, *C. nardus* and two hybrid varieties were evaluated for repellent properties against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* in both laboratory and field conditions. The result showed that all grass species gave complete protection from mosquito bites for 4 hr, whereas *C. nardus* provided protection for as much as 8.0-10.0 hr (Tyagi et al., 1998). Ansari et al., 2000 reported the repellent actions of *Dalbergia sissoo* oil against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* under laboratory conditions. It was demonstrated that the protection (91.6±2%) obtained from sissoo oil was comparable to that from commercial mylol oil (93.8±1.2%) consisting of di-butyl and dimethyl phthalates. The repellent activity of methanol extract from *Ferronia elephantum* leaves was tested on *Ae. aegypti* in the laboratory, and at concentrations of 1.0 and 2.5 mg/cm^2 it was found to give 100% protection for up to 2.14±0.16 hr and 4.00±0.24 hr, respectively. The total percentage protection of *F. elephantum* was 45.8% and 59.0% for 10 hr at 1.0 mg/cm^2 and 2.5 mg/cm^2 concentration, respectively (Venkatachalam and Jebanesan, 2001). The volatile oils extracted by steam distillation from turmeric (*Curcuma longa*), kaffir lime (*Citrus hystrix*), citronella grass (*Cymbopogon winterianus*) and hairy basil (*Ocimum americanum*) were evaluated in a mosquito cage and large room conditions for effects of repellency against three mosquito vectors: *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus* (Tawatsin et al., 2001). The oils from turmeric, citronella grass and hairy basil, especially with the addition of 5% vanillin, repelled the three mosquito species under cage conditions for up to 8 hr, while DEET alone provided protection for at least 8 hr against *Ae. aegypti* and *Cx. quinquefasciatus* and 6 hr against *An. dirus*. However, DEET with the addition of 5% vanillin gave protection against the three mosquito species for at least 8 hr. The results of large room evaluations confirmed the response for each repellent treatment obtained under cage conditions.

Methanol extracts and steam distillates of 23 aromatic medicinal plant species that derived from Kyungdong Market, Seoul, South Korea, were examined for repellent activity against female blood-starved *Ae. aegypti* and compared with DEET. At a dose of 0.1 mg/cm^2, the repellency of extracts from *Cinnamomum cassia* bark (91%), *Nardostachys chinensis* rhizome (81%), *Paeonia suffruticosa* root bark (80%) and *C. camphora* steam distillate (94%) was comparable to DEET (82%). The effective duration (≈ 1 hr) of *C. cassia* and *N. chinensis* extracts was comparable to DEET (Yang et al., 2004). The mosquito repellent activity of pine (*Pinus longifolia*) oil was evaluated by Ansari et al., (2005), who showed that it had strong repellent action against mosquitoes, providing 100% and 97% protection for 11 and 9 hr against *An. culicifacies* and *Cx. quinquefasciatus*, respectively. Electrically heated mats prepared from pine oil provided 94 and 88% protection against *An. culicifacies* and *Cx. quinquefasciatus* for 10 and 7 hr, respectively.

Trongtokit et al., (2005) screened the repellent activity of 38 essential oils from plants at three concentrations (10%, 50% and undiluted) against *Ae. aegypti* under laboratory conditions using human subjects. The undiluted oils of *Cymbopogon nardus* (citronella), *Pogostemon cablin* (patchuli), *Syzygium aromaticum* (clove) and *Zanthoxylum limonella* (makaen) were the most effective, providing 2 hr of complete repellency, and clove oil gave the longest duration with 100% repellency (2-4 hr) against *Cx. quinquefasciatus* and *An. dirus*. The repellency of essential oils from 18 Thai plants against *Ae. aegypti*, *Ae. albopictus*, *An. dirus* and *Cx. quinquefasciatus* was compared with DEET and IR3535 under laboratory conditions by Tawatsin et al., 2006. The results showed that *An. dirus*, *Cx. quinquefasciatus*, and *Ae. albopictus* were more susceptible to all the essential oils (repellency 4.5-8 hr) than *Ae. aegypti* (repellency 0.3-

2.8 hr), whereas DEET and IR3535 provided excellent repellency against all four mosquito species (repellency 6.7-8 hr).

Choochote et al., (2007) reported the repellent activity of essential oils extracted from *Zanthoxylum piperitum*, *Anethum graveolens* and *Kaempferia galanga* against *Ae. aegypti* with median complete-protection times of 1, 0.5 and 0.25 hr, respectively. The protection times were increased significantly by incorporating 10% vanillin. The highest potential was established from Z. *piperitum* oil + 10% vanillin (2.5 hr). GC/MS analysis revealed that the main component of *Z. piperitum* fruit oil was limonene (37.99%), with minor amounts of sabinene (13.30%) and *β*-myrcene (7.17%). Gillij et al., (2008) reported the repellent activity of essential oils derived from *Acantholippia seriphioides, Achyrocline satureioides, Aloysia citriodora, Anemia tomentosa, Baccharis spartioides, Chenopodium ambrosioides*, *Eucalyptus saligna*, *Hyptis mutabilis*, *Minthostachys mollis*, *Rosmarinus officinalis*, *Tagetes minuta* and *T. pusilla*, all of which had varied efficacy depending on the geographic origin of the plant. While 90% of the essential oils from *A. satureoides* and *T. pusilla* demonstrated the lowest repellency, 12.5% of those from *B. spartioides*, *R. officinalis* and *A. citriodora* showed the longest repellent times. Comparisons of the principal components of each essential oil suggested that limonene and camphor were the main components responsible for the repellent effects.

Repellency of the ethanolic preparation of hexane-extracted *Apium graveolens* (celery seed) was investigated and compared with that of 15 commercial mosquito repellents including the most widely used, being DEET (Tuetun et al., 2005). It was revealed that 25% of hexane-extracted *A. graveolens*, with the addition of 5% vanillin, showed the highest repellency (5 hr). Furthermore, commercial repellents, except formulations of DEET, showed lower repellency than that of *A. graveolens* extract. Tuetun et al., (2008) investigated the laboratory repellent activity of celery-based products against female *Ae. aegypti* mosquitoes. The results demonstrated that G10 formula, the best AHE-developed product, provided remarkable repellency with a median protection time of 4.5 (4.5-5) hr, which was greater than that of ethanolic DEET solution (25% DEET, 3.5 hr) and comparable to that of the best commercial repellent, being Insect Block 28 (28.5% DEET, 4.5 hr).

Elango et al., (2010) tested the repellent properties of crude leaf ethyl acetate, acetone, and methanol extracts of *Aegle marmelos* (L.) Correa ex Roxb, *Andrographis lineata* Wallich ex Nees., *Andrographis paniculata* (Burm.f.) Wallich ex Nees., *Cocculus hirsutus* (L.) Diels, *Eclipta prostrata* L. and *Tagetes erecta* L. against *Cx. tritaeniorhynchus* Giles. It was found that the highest repellent activity was observed at 500 ppm in the methanol extracts of *A. marmelos* and ethyl acetate extracts of *A. lineata*, *C. hirsutus* and *E. prostrate*, and the mean complete protection time ranged from 120 to 150 min. The ethyl acetate extract of *A. lineata* showed 100% repellency for 120 min; while acetone extracts of *A. marmelos* and *C. hirsutus* and methanol extract of *T. erecta* showed complete protection for 90 min at 250 ppm.

Phasomkusolsil and Soonwera (2010) investigated insect bite protection and length of protection time with 30 repellents divided into 3 categories: plant oil, essential oil and essential oil with ethyl alcohol, which were tested against *Ae. aegypti*, *An. minimus* and *Cx. quinquefasciatus* under laboratory conditions. The combination of plant oils derived from Phlai (*Zingiber cassumunar*) and Sweet basil (*Ocimum basilicum*) were effective as repellents and feeding deterrents against *An. minimus* (205 min protection time at a biting rate of 0.9%), *Cx. quinquefasciatus* (165 min protection time at 0.9% biting rate) and *Ae. aegypti* (90 min protection time at 0.8% biting rate). Essential oil from citronella grass (*Cymbopogon nardus*) exhibited protection against *An. minimus* bites (130 min at 0.9% biting rate), *Cx. quinquefasciatus* (140 min at 0.8% biting rate) and *Ae. aegypti* (115 min at 0.8% biting rate).

Mandal (2011) evaluated the repellent activity of eucalyptus oil and *Azadirachta indica* seed oils against *Cx. quinquefasciatus*. At concentrations of 50% and 100% (v/v), *A. indica* oil provided 90.26% and 88.83% protection, respectively, while those of the eucalyptus oil yielded 93.37% and 92.04%, respectively, with a protection time of up to 240 min. Conti et al., (2012) evaluated the essential oils extracted from fresh air dried leaves of *Salvia dorisiana*, *S. longifolia* and *S. sclarea* (Lamiaceae) for their repellent activity against *Ae. albopictus*. It was demonstrated that *S. dorisiana* was the most effective oil at 0.2 and 0.4 μl/cm^2 of skin, and gave almost complete protection (with a protective efficacy of 90.99% and 95.62%, respectively) for 90 min. The main chemical constituent of *S. dorisiana* oil was oxygenated sesquiterpenes. The repellent properties of crude hexane, chloroform, benzene, acetone, and methanol extracts of the *Cassia* *tora* leaf were tested against *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi*. The methanol extract of *C. tora* gave 89.6% repellency for 210 min against *An. stephensi* followed by *Ae. aegypti* (87.6%) and *Cx. quinquefasciatus* (86.3%) (Amerasan et al., 2012).

Murugan et al., (2012) investigated effects of the ethanol extract of *Citrus sinensis* (orange peel) from India on repellent activity against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The repellency was highest at 450 ppm (mean complete protection time ranged from 150 to 180 min), with 100% repellency for 150 min, and at 350 ppm the complete protection time was 90 min. This study suggested that repellent activity of the ethanol extract of *C. sinensis* was dose dependent. Sritabutra and Soonwera (2013) investigated the formulation of 10% essential oils on repellent activity against *Ae. aegypti* and *Cx. quinquefasciatus*. The formulations of essential oil from clove oil in olive oil and in coconut oil gave the longest lasting protection time of 76.50 and 96.00 min, respectively against *Ae. aegypti*. The citronella grass oil in olive oil, citronella grass oil in coconut oil and lemongrass oil in coconut oil exhibited protection time against *Cx. quinquefasciatus* at 165.00, 105.00, and 112.50 min, respectively. Govindarajan et al., (2014) evaluated the repellent activity leaves and seeds of the plant *Delonix elata* against *An. stephensi*. Plant sample was extracted in various solvents including, hexane, benzene, chloroform, ethyl acetate and methanol. Among the tested solvents, both the leaf and seed methanol extracts showed maximum efficacy. The highest concentration of 5.0 mg/cm^2 provided over 210.00 and 180.00 min protection for the leaf and seed extracts, respectively.

Current studies, Karunamoorthi et al., (2014) investigated the repellent activity of *Juniperus procera* leaf oil against the malarial vector *An. arabiensis* at various concentrations. The tested concentrations of the essential oil of *J. procera* exhibited various degrees of repellency in terms of percentage of repellency and complete protection time against female *An. arabiensis* viz., 1.0, 1.5, 2.5 and 5.0 mg/cm^2 [64.10% (92 min)], [68.10% (125 min)], [72.20% (190 min)], and [80.60% (311 min)], respectively. The examined essential oil exhibited significant repellent properties and it has been identified that it could serve as a potent repellent against insect vectors of disease.

2.4 *Ligusticum sinense* Oliv. cv. Chuanxiong the most effective plant sample

Family: Umbelliferae or Apiaceae

English name: Chuanxiong rhizoma

Thai name or local name: โกฐหัวบัว (Juengprasert et al., 2011)

Part used: Rhizome

Botanical description: Irregular knotty and fist-like, 2-7 cm in diameter. Externally yellowish-brown, rough and shrunken, with many parallel and raised annulations, showing dent and subrounded stem scars on the top and numerous rootlet scars on the lower part and the nodes. Texture compact, uneasily broken, fracture yellowish-white or grayish-yellow, scattered with yellowish-brown oil dots. Cambium is an undulatering. Odour, strongly aromatic. Taste bitter, pungent and slight sweet, and with a numb aftertaste.

Habitat: China

Constituents: Chemical composition in Chuanxiong: volatile oils, lactones, alkaloids, oraganic acids, etc.

- **Volatile oils:** ethyl pentadecanoate, ethyl palmitate, ethyl heptadecanoate, ethyl isoheptadecanoate, ethyl octadecanoate, ethyl isooctadecanoate, methyl phenylacetate, sedanonic acid lactone, methyl pentadecanoate.
- **Lactones:** z-ligustilide, e-ligustilide**,** butyl phthalide, butylidene, senkyunolide, neocnidilide.
- **Alkaloids:** chuanxiongzine or tetramethyl pyrazine, perlolyrine, leucyl phenylalanine anhydride, adenine, l-valine-l-valine anhydride, choline trimethylamine.
- **Phenolic compounds:** ferulic acid, vanillin acid, caffeic acid, protocatechuic acid, 4-hydroxybenzoic acid.
- **Others:** spathulenol, sedanonic acid, folic acid, β-sitosterol, palmitic acid, linolenic acid, vitamin A, aliphatic oil, sucrose, heteropolysaccharides (e.g. LCP-1, LCP-2, LCP-3 and LCP-4)

Properties and uses: The pharmacological studies of Chuanxiong are mainly concentrated on hematology, cardiovascular system, cerebrovascular system, and so on.

- Inhibition of platelet aggregation and antithrombotic effect
- Anti-myocardial ischemia effect and increases of coronary blood flow
- Dilation of blood vessels and improve microcirculation
- Improving cerebral ischemia and brain damage
- Lung injury resistance

A. B.

Figure 1.2 *Ligusticum sinense* Oliv. cv. Chuanxiong

A: Chuanxiong tree (Juengprasert et al., 2011)

B: Chuanxiong rhizome

3. PURPOSES OG THE STUDY

3.1 To investigate repellent efficacy of plant products, including essential oils, ethanolic extracts and hexane extracts against mosquito vectors under laboratory conditions.

3.2 To investigate on repellent activity of the most effective sample under laboratory conditions.

3.3 To investigate on repellent activity of the most effective sample under field conditions.

3.4 To test physical and biological stability of the most effective plant sample under laboratory conditions.

3.5 To analyze the chemical composition of the most effective plant sample.

4. USEFULNESS OF THE STUDY

Nowadays, the public has turned its interest towards natural products for the control of mosquito vectors instead of synthetic compounds because they are a natural bioactive compound and safe for life, organisms, and the environment. Knowledge and information about plant extracts and their repellent activities derived from this study may be of remarkable value and benefit for combating mosquito-transmitted diseases. In addition, these findings may be of use in future development of new repellent formulations against mosquito vectors.