Chapter 1. Introduction

1.1. General

Biodegradation of wood caused by termites is recognized as one of the most serious problems for wood utilization, causing greater than $20 billion (US) annually in damage, control and repair costs worldwide (Su, 2002). In Malaysia, the cost of termite control was estimated at US $ 10-12 million for year 2003 and the total repair cost was 3-4 times higher (Lee, 2002a). Among the different genera of termites, *Coptotermes* sp was responsible for more than 90% of total damages in buildings and structures in West Malaysia (Lee, 2002b). Subterranean termites from the genus *Coptotermes* are significant wood destroying pests in the world (Sajap et al., 2005).

Termites are small insects, white, tan, or black which can cause damage to the wood structure. Termites are insects belonging to the order Isoptera, an ancient group of insects that dates back more than 100 million years. There are more than 2,500 different types of termites in the world. However, most of this diversity can be lumped into four distinct groups: dampwood, drywood, underground, and builder of the hill. Termites become a problem when they consume structural lumber. Every year thousands of U.S. housing units require treatment to control termites. Termites can also damage utility poles and other wooden structures. Termites are pests in California which include drywood, dampwood, and underground species. Termites are the most important pests which cause damage to wooden construction and products in tropical and subtropical countries and they are social insects with long lifespan in the living earth (Yeoh, 2007). These pests cause serious damage to wooden structures and posts and can also attack stored food, books and furniture (Lewis, 2001).

Zingiberaceae is a family of gingers, comprises about 1,200 species of which about 1,000 occur in tropical Asia. The richest area is the Southern Asian region, a floristically distinct region that includes Malaysia, Indonesia, Brunei, Singapore and Philippines, with 24 genera and about 600 species (Larsen, et al., 1999). Among the genera which are commonly used in traditional medicine are *Zingiber, Amomum, Curcuma*, *Aplinia* and etc. Some species in the genus *Alpinia* has showy and fragrant inflorescences. The genus *Alpinia* is found throughout the tropical Pacific region. *Alpinia galanga* or commonly known called greater galangal is widely used as condiment and in traditional medicine.

The family Asteraceae or Compositae is the largest family of flowering plants, in terms of number of species (Jeffrey, 2007). The genera *Tanacetum*, *Chrysanthemum* and *Pulicaria* contain species with insecticidal properties. *Chrysanthemum* is a genus (*Chrysanthemum*) of about 30 species of perennial flowering plants in the family Asteraceae, native to Asia and northeastern Europe (Liu, et al., 2012).

The two most effective control options for subterranean termites are soil treatment and baiting (Su and Scheffrahn, 2000). Soil treatments are typically made with large volumes of liquid termiticides that are either neurotoxins or inhibitors of mitochondrial respiration. Recent studies in Malaysia showed that field colonies *C. curvignathus and C. gestroi* can be effectively eliminated by using bait containing hexaflumuron (Sajap et al., 2000 and Sajap et al., 2002). It is also known that termites damage a variety of materials ranging from paper fabrics to even non-cellulosic materials such as asbestos, asphalt bitumen, lead, and metal foils (Bultman et al., 1979). Phytophagous insects use plant volatiles to recognize their host plants. Therefore, the use of essential oils as a non-host volatile emission to repel insect pests is a viable alternative for control (Mauchline et al., 2005). The high toxicity of biocides and unacceptable environmental consequences have resulted in severe restrictions. Use of synthetic pesticides for control has led to many

problems such as toxicity to non-target organisms and exposure to pesticides and residue in food (Arnason et al., 1989). In the search for alternatives, the use of natural plant products for the protection of wood is appealing (Nunes et al., 2004).

Chemical control has been a successful method of preventing termite attack, but the effects of these chemicals can create various problems for our health and to the environment. Firstly, the usage of synthetic chemicals can cause health hazards to both humans and animals that come into direct contact. Secondly, synthetic chemicals can cause environmental pollution which brings biochemical changes to the environment and also living organisms. Moreover, the synthetic chemicals can kill both beneficial and harmful insects (Lewis, 1997).

In recent years it has become evident, as a result of public opinion and environmental laws, that new and safer alternatives to traditional synthetic pesticides are both desirable and mandated. Under these conditions, the biological method has become the suitable alternative method for synthetic chemicals (David et al., 2011). Secondary metabolites with no known function in photosynthesis, growth or other fundamental aspects of plant physiology provide a new source of natural pesticide and antifeedant (Arnason et al., 1989: Coats, 1994). Green plants are widely used in traditional cultures worldwide and increasing drastically in most developed and developing countries as natural alternatives to synthetic chemicals (Ramesh et al., 2011). Biological methods include botanicals (essential oil, seed, bark, leaf, fruit, root, wood, resin), as well as fungal, bacterial, and nematode can be used for termite control singly and in combination (Verma et al., 2009).

The three essential oils obtained from clove, *Syzgium aromaticum*, West African black pepper (WABP), *Piper guineense*, and ginger, *Zingiber officinale* significantly reduced the percentage of *Callosobruchus maculatus* adults that emerged from the bambara

groundnut cultivars in the F_1 generation and the number of adult offspring that developed in the cultivars during the 3-month storage period (Ajayi and Lale, 2000). As a result of increased resistance for control a higher concentration of termiticide need to be sprayed at shorter intervals. However this may lead to environmental pollution and food poisoning.

1.2. Objective of Study

The objectives of this study are:

i. To identify the antifeeding activity and active compounds in *Chrysanthemum indicum* and *Alpinia galanga* extract against *Coptotermes gestroi, Coptotermes curvignathus* and *Macrotermes carbonarius*.

ii. To determine the acute toxicity that can cause optimal antifeedant effect against *C. gestroi*, *C. curvignathus* and *M. carbonarius*.

iii. To determine the effectiveness of the identified bioactive compound towards termites in the field.

iv. To compare the effectiveness of the identified bioactive compound with commercial termiticide (Chlorpyrifos).

Chapter 2. Literature review

2.1. Termites

2.1.1. Termites

Termites are the most successful social insects on the planet as they have long lifespan and they are most important pest causing damage to wooden construction and other wood products in most tropical and subtropical countries (Yeoh, 2007). Consequently the control of termites is inevitable and necessary (Salem, 2008). Generally, termites belong to the order Isoptera and referred to as ‘white ants’; however their morphology are different from ants and other social Hymenopterans such as bees and wasps (Gedeon, 2006). The original word of Isoptera is from the Greek word ‘isos’ which means equal and ‘pteron’ means wing and also refers to two pairs of identical adult wings (Harris, 1957).

Subterranean termites are eusocial insects, living in large communities of several hundreds to millions individuals in the soil or above ground that are connected to soil. They also have a great influence on the ecosystem. From the economic point of view termites can be very destructive, since they feed and destroy various structures or materials that man utilizes such as buildings, furniture and fabrics or they can be beneficial in assisting the conversion of dead trees and other plant products to substances that can be utilized by plants, supply materials for food chains and soil engineering (Gedeon, 2006).

2.1.2. Termite colony structure

Termites live in large communities and divided into four different castes: the reproductive (king and queen), soldiers and workers. Each caste is different in their morphology and behavior but they live together in cooperation otherwise the colony will be destroyed (Collins, 1984). Termites build a stout mound under good conditions, may attain a large

size and a height of almost 2 meters. The size of the nest mound varies with location probably due to availability of food. Large colonies are included with a number of supplementary reproductive castes, producing eggs or replacing the founding queen (Gedeon, 2006). The parent termites are the king and the queen. The queen's role is to lay eggs and also in pheromone regulation of each caste in a colony (Gedeon, 2006). Mature colonies of termites which are usually 6 to 7 years old would naturally contain more than 60, 000 workers, for example *Reticultermes flavipes* in Virginia (Dini, 2010). These large subterranean termite colonies often become decentralized over time and occupy multiple nesting sites interconnected by a network of underground tunnels (Dini, 2010).

2.1.3. Termite morphology and life cycle

Most termites are identified based on the soldier's morphology. Soldiers and workers can be either male or female and they are wingless. Soldiers represent one-tenth of the population and their major role is to defend the colony (Gedeon, 2006). The soldiers also have nasus, an elongated projection of fontanelle for their defense purpose by squirting irritating chemical substance through it (Collins, 1984). The soldiers possess a frontal gland which contains a milky sticky fluid, whose secretion can be toxic or repellent to intruders for defense purpose. The fluid gives the color to body and head. It also has four segments in tarsi and two segments at abdominal cerci (Yeoh, 2007).

The worker caste plays a major role in the survival of the colony. The job task is to collect food, process it, feed other castes and construct the mound or nest (Harris, 1957). The soldiers and workers do not have eyes but they are not totally blind as they are able to sense lights. Both soldier and worke castes exhibit dimorphism. Winged reproductive or alates of both sexes are produced in mature colony in large amounts.

Termites are hemimetabolus in their life cycle. Newly hatch nymphs or larvae will undergo first instar and they are translucent white and very active. They were fed with nutrient-rich salivary secretions produced by their parents (Gedeon, 2006). Termites normally undergo a number of instars until they achieve their mature form as sterile workers or soldiers depending on the colony needs. Usually at the beginning of a colony, all larvae become workers, then occasionally the larvae undergo additional instar by developing large head and jaws of different shape becoming soldiers (Harris, 1957). The worker termites will mature in their third instar while soldiers mature on their fifth instar. The colony grows slowly for many years by enlarging their nests and building activities as the number of individuals increased (Gedeon, 2006).

2.1.4. Termite control and management

Preventing subterranean termites from infesting a structure is an important management strategy (Yeoh, 2007). Chemicals were often applied at higher rates than required to control and the break down products are not environmentally friendly. Subterranean termite control aims to lower the pest population to an economic level. The current control includes chemical and physical barriers which may cause environmental problems, toxic chemicals entering food chains and then transferred to human (Gedeon, 2006).

Antifeedant activity is a method that reduces the consumption ability of termites. Antifeedant activity is a behavior modifying substance that deters feeding through a direct action on the taste organs. The definition excludes chemicals that suppress feeding by acting on the central nervous system following ingestion and absorption in termites (Adeyemi et al., 2010). Entomopathogenic fungus *Metarhizium anisopliae* shows influence in antifeeding activity and is toxic to subterranean termite *Coptetermes gestroi* (Paimin et al., 2000). Toxicity caused by natural plants is effective to suppress the regenerative

activity in termites (Ravi et al., 2010). Plants such as *Polygonam hydropiper* and *Progesterone parviflorus* cause mortality and repellent activity in the termite *Reticulitermes hesperus* (Grace et al., 1987). Moreover, monoterpenoids isolated from *Flourensia cernua* effectively control the termites (Tellez et al., 2001). Besides that, *Cupressus nootkatensis* and *Thuja plicata* also show high antifeedant and toxic activities against termites (Hennon et al., 2007). Terthiophene composition from *Echinops* species shows 100% mortality in *Coptotermes formosanus* (Fokialakis et al., 2006).

2.1.5. Development and efficacy of termicides

Insecticides are classified based on their chemical structures and divided into two main groups, which is, organic insecticides and inorganic insecticides. The organic insecticides are divided into synthetic insecticides and botanical insecticides. The organic synthetic is further divided into four classes, which are, Chlorinated hydrocarbonsor organochlrones, Organophosphate, Carbamates and Pyrethroids (Lee et al., 2006). Even though those termiticides are still effective for the control of termite, yet new termiticides are being introduced to replace old ones such Organochlorine and organophosphates now known as Chlorpyrifos and pyrethroid such as cypermethrin (Vongkaluang, 2005).

2.1.6. Chemical control

Chemical barriers are used in soil as a method to control and prevent termite and this control method has a high environmental impact because of large amounts of insecticides applied in open areas (Su and Schefrahn, 1998). Two groups of termiticides applied into soil are repellent termiticides and non-repellent. The repellent type is Chlorpyrifos and the non-repellent are Fipronil, Imidacloprid and Indoxacarb (Gedeon, 2006).

Termite-susceptible wood can be turned into a termite resistant material by treating with chemical toxicant (wood preservatives) that inhibits feeding by termites such as Chromated Copper Arsenate (CCA), Ammoniacal Copper Quat compound (ACQ), and Disodium Octoborate Tetrahydrate (DOT) are used as wood preservatives and these methods are most toxic to termites when ingested and also discourage new kings and queens from establishing colonies (Su and Schefrahn, 1998).

Space fumigation involves the use of toxic gas inside the structure sealed around and isolated area or object infested with termite. This method should be handled with care because it is extremely harmful to humans, animals and also plants (Pearce, 1997).

Baiting system has an advantage of not contaminating the soil with chemicals. In this method, non-toxic baits are placed near colonies of termites later to be replaced by baits which are toxic (Pearce, 1997).

2.1.7. *Coptotermes gestroi*

Coptotermes gestroi, the Asian subterranean termite is a small species of termite that lives underground. Both this species and the Formosan subterranean termite, (*Coptotermes formosanus*) are destructive pests native to Asia, but have spread to other parts of the world including the United States (Rudolf and Nan, 2011). In Asia, this species is known as the Philippine milk termite (Menandro, 2009).

The termite species *Coptotermes havilandi* was determined by Kirton and Brown (2003) to be identical to *Coptotermes gestroi* so, following the principle of priority, the older name is now used (Hou, 2009).

These termites are voracious feeders and will consume wood, cardboard and paper and sometimes even fabric (Venite, 2013). They feed on all sorts of cellulose containing materials and will drill holes in such materials as rubber, plastic and Styrofoam in their

search for food. They also attack living trees by consuming the heartwood which weakens the tree and can bring it down in a storm. They live underground and enter buildings through cracks, expansion joints and utility conduits. *Coptotermes gestroi* sometimes form foraging tubes along the surface of the ground and the outside surfaces of structures. They eat structural timbers from the inside outwards, leaving a thin film of surface wood which may display a blistered appearance (Chantuma, 2009). In Singapore and Malaysia, this species is responsible for 80% to 90% of the damage caused to man-made structures by insects and it is the commonest species of termite found in built up areas.

2.1.8. *Coptotermes curvignathus*

Termite is one of the common pests of oil palm planted on peat in Malaysia and Indonesia (Lim and Silek, 2001) *Coptotermes curvignathus* has been identified as the major palm killer especially the immature palm. It has also been reported to attack *Acacia mangium* rubber (*Hevea brasiliensis)* and other fruit trees such as coconut and mango (Khoo et al., 1991) *Coptotermes curvignathus* is the largest in size and most aggressive among the oriental *Coptotermes* spp. (Thapa, 1981). It has been noted to damage fresh tissue rather than scavenging and feeding on woody material. It is easy to identify as it secretes a milky white liquid from its frontal fontanelle when in defense.

Thus, termite control in plantations has resorted to more environmental friendly and more target specific methods. Baiting system has been widely used nowadays with slow acting toxicant or chitin synthesis inhibitor such as hexaflumuron (Tsunoda et al., 1998; Su et al., 2000) incorporated. Hoe *et al*. (2009) explored the use of entomopathogenic fungi such as *Metarhizium anisopliae* var. *anisopliae* to control *Coptotermes curvignathus*. The baiting system capitalizes on the feeding behavior of termite, trophallaxis that involves grooming and exchange of secretions or liquid food between individuals (Pearce, 1997).

Thus when the pest feeds on the bait, the chemical will be passed on from one to another and in times will gradually reduce the pest population. The success of baiting system rest on the knowledge of the behavior of the respective pest (Su et al., 1991).

2.1.9. *Macrotermes carbonarius*

Macrotermes carbonarius is the only open foraging *Macrotermes* species and also the only black species (Neoh and Lee, (2009). It is found in South East Asia and parts of Indochina, but its range is patchy and it is only locally abundant. This means where it occurs, it is common, but where it is not found, there is no trace of them, even over vast swaths of what would be considered suitable habitat. It mainly occurs in lowland rainforest areas, but can be found in crop/agricultural plantations (Neoh and Lee, (2009).

Macrotermes carbonarius forages mainly at night and occasionally during the day, up till early afternoon, depending on the surrounding environment and weather. In exposed areas, they will rarely emerge during daylight hours (Inon et al., 2012).

The major workers fan out from subterranean tunnels, moving in columns to specific locations to chew up dead leaves, grasses, twigs, branches, and any other relatively soft plant matter. They do not attack human made structures or trees. Foraging locations are not fixed, and changed daily in most cases. While *Macrotermes carbonarius* builds large mounds up to around 2 meters high, a major proportion of their nest (and population) is located below ground level. Their mound walls are extremely thick in most places, and used to house their fungus gardens and nurseries (Judith and Karl, 1999).

As one would expect for an open air forager, *Macrotermes carbonarius* soldiers are aggressive, and readily rush out to confront any intruders if their nest or foraging columns are disturbed, just like ants would. This behavior is actually more akin to ants, rather than termites, as most termites are shy and adopt passive defense methods. Their soldiers can

deliver a painful bite with their large mandibles, but they do not sting, unlike many types of ants (Neoh and Lee, (2011).

2.2. *Alpinia galanga*

2.2.1. The Zingiberaceae Family

Zingiberaceae is a family of gingers, comprises about 1200 species of which about 1000 occur in tropical Asia. The richest area is the Southern Asian region, a floristically distinct region that includes Malaysia, Indonesia, Brunei, Singapore and Philippines, with 24 genera and about 600 species (Matsuda et al., 2003). It belongs to Kingdom of Plantae, Order of Zingiberales, Family of Zingiberaceae, Genus of Alpinia and Species of *Alpinia galanga*. The common names for *A. galanga* is Greater galangal in English, Lengkuas in Malay, Hong dou kou in Chinese, Arattai in Tamil and Kha in Thai.

2.2.2. Distribution and Morphology of *Alpinia galanga*

Alpinia galanga bears underground stems called rhizomes which have strong aromatic smell with conspicuously nodes and internodes (Jirawan, 2005). The rhizomes branch out into different pieces, each of which is from 1 1/2 to 3 inches in length, and seldom more than 3/4 inch thick. Each piece of the rhizome is usually cylindrical in shape, and these are often cut while in a fresh state, each piece of the rhizome is marked at short intervals by the presence of a narrow and whitish color body, which gives rise to raised rings, the legacy of scars produced by former scaly leaves growing along the rhizome.

The rhizomes are characterized externally by a dark reddish-brown color, and cuttings of the inner rhizome are characterized by the presence of a dark center surrounded by a wider and paler layer on the outer rim, that also darkens considerably when the

rhizome is dried during processing. The rhizomes of galanga have a strong aromatic odor, and a spicy or pungent taste (Farnsworth and Bunyapraphatsara, 1992).

2.2.3. Pharmacological activity

Alpinia galanga was used for various purposes including as a stomachache (Ibrahim et al., 2004; Jirovetz et al., 2003), antibacterial (Janssen and Scheffer, 1985; Jirovetz et al., 2003), antifungal (Janssen and Scheffer, 1985), antitumor (Yang and Eilerman, 1999), antiulcer (Yang and Eilerman, 1999), antiallergic (Matsuda et al., 2003), antioxidant (Juntachote and Berghofer, 2005) and food condiment (Ibrahim et al., 2004; Jirovetz et al., 2003).

Alpinia galanga was found to be effective in treatment of allergy and the isolated compounds inhibit the release of antigen IgE mediated in passive cutaneous anaphylaxis reactions in mice (Matsuda et al., 2003). Methanolic extract of *Alpinia galanga* showed potent inhibitory activity against human immunodeficiency virus type-1 (HIV-1) and human cytomegalovirus (HCMV) (Tewtrakul et al., 2003). The ether extract of *A. galanga* are more potent than the ethyl acetate in antibacterial activity and significantly effective on *Staphylococcus aureus* and *Klebsiella pneumonia* (Elsamma et al., 1996). 1,8-Cineole from the ethanol extract of *Alpinia galanga* was discovered to have antibacterial activity against *Staphylococcus aureus.* The antimicrobial activity is due to the composition of 1,8-cineole, 4-allyphenyl acetate and a-bisabolene (Tachakittirungrod and Chowwanapoonpohn, 2007).

Alpinia galanga can inhibit a wide variety of human pathogenic fungi, zoonotic dermatophytes and yeast-like *Candida albicans*. The ethanolic extracts exhibited antifungal activity against *Trichophyton longifusus, Colletotrichum musae* and *Fusarium oxysporum, Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton concentricum, Rhizopus stolonifer* and *Aspergillus niger* (Chudiwal et al., 2010).

Alpinia galanga have anti-inflammatory and analgesic effects towards rheumatic condition. It acts as beneficial therapeutics for treating inflammatory immune disorders and inhibits the carrageenan-induced paw inflammation. Not only that, it shows drastic significant effect on reducing symptoms of osteoarthritis (Chudiwal et al., 2010)

Pompimon et al., (2009) reported the effects of p-hydroxycinnamaldehyde from *A. galangal* acetone extracts on human chondrocytes, Osteoarthritis (OA) is the most common form of arthritis and affects millions of people worldwide and patients have traditionally been treated with non-steroidal anti-inflammatory drugs (NSAIDs), but these are associated with significant side effects.

Mahae and Chaiseri (2009) studied antioxidant activities and antioxidative components in extracts of *A. galanga*. They found that 50% ethanol in water has antioxidant activity when compared with two other samples based on a water extract and the essential oil which were determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) methods. The ethanolic extracts showed the highest DPPH free radical scavenging ability as well as the highest ORAC value when compared to the water extract and the essential oil.

The ethanolic extract of *A. galanga* is reported to possess hypolipidemic activity in rats when 20mg/day extract for a period of 4 weeks was given to rats. This caused reduction in the serum and tissue levels of total cholesterol, triglycerides, and phospholipids significantly increased the serum levels of high density lipoproteins (HDL) in rats. Effects of extract on lipid profile exhibited the efficacy of *A. galanga* in lowering the risk of arteriosclerosis (Achuthan and Padikkala 1997).

2.2.4. Other effects

For different countries, galanga is used differently. In most South East Asian countries dried galanga is employed only in the absence of fresh galanga whereas in Indonesia slices or powder of the fresh or dried rhizome are used frequently. The rhizome is used against rheumatism, bronchial catarrh, bad breath, and ulcers whooping colds in children, throat infections, to control incontinence, fever and dyspepsia (Lee and Houghton, 2005). The root has been used in Europe as a spice for over a thousand years, having probably been introduced by the Arabian or Greek physicians, but it has now largely gone out of use except in Russia and India. The rhizomes have been used as flavors in native dishes and ingredients in many traditional medicines to treat various ailments, such as stomach disorders and skin diseases. In India the rhizomes have many applications in traditional medicines such as for skin diseases, indigestion, colic, dysentery, enlarged spleen, respiratory diseases, mouth and stomach cancer. It is used as a body deodorizer and halitosis remedy (Gupta, 2010).

2.2.5. Toxicities

Composition of *Alpinia oxyphylla* reduce the fecundity and food consumption of Formosan subterranean termites (Boue and Raina., 2003). Constituents of *Zingiber officinale* have antifeedant activity against *Spilosoma oblique* (Agarwal et al., 2001). Besides this, compositions of *Curcuma* spp. have insecticidal activity to control stored-product pests (Stoll, 2000). Zingiberaceae essential oils were proven to be toxic to few parasitoids (Duangsamorn et al., 2000). So far no antifeedant activity studies have been done against termite using *Alpinia galanga.*

2.3. *Chrysanthemum indicum*

2.3.1. Anti-inflammatory, antigout and antithrombotic activity

Chrysanthemum indicum extract prepared from the inflorescence or bud showed anti-inflammatory activity with the butanol soluble fraction showing more activity than other fractions (Cheng et al., 2005). The butanol fraction possessed anti-inflammatory, humoral and cellular immunomodulatory and mononuclear phagocytic activities, which we attributed due to the presence of flavonoids in the plant (Cheng et al., 2005). At a dose of 150 mg/kg, p.o., the butanol soluble fraction of the herb caused significant inhibition of auricle edema in mice. Delayed-type hypersensitivity reaction induced by 2,4-dinitro-fluorobenzene was significantly enhanced by butanol extract (150 & 300 mg/kg, p.o.) as was antibody generation by splenic cells of mice and IgM levels in mice sera in response to sheep red blood cells in cyclophospamide induced mice (Cheng et al., 2005). 70% ethanolic extract from *C. indicum* also exhibit anti-inflammatoty effect in mice skin (Cheng et al., 2005).

Inhibition of xanthine oxidase activity (extract IC_{50}, 22 µg/ml; allopurinol as positive control IC_{50}, 1.06 µg/ml) was exhibited by the methanol extract of the flower of *C. indicum*, thus providing a basis for the use of this medicinal plant for gout treatment (Kong et al., 2000). Partial evidence for the empiric and traditional use of *C. indicum* in the treatment or prevention of thrombosis was provided by the observation that the aqueous extract was 10-12 times more potent on PAF-induced aggregation of human platelet rich plasma compared to ADP aggregation of rat platelet rich plasma (Arodogan et al., 2002).

2.3.2. Antimicrobial activity

The essential oil showed antimicrobial activity against many microorganisms which was attributed to their content of camphor and borneol, and the lower amounts of α-terpineol, terpinen-4-ol, ρ-cymene and linalool (Shunting et al., 2005). The oil from fresh flowers was believed to possess a strong antimicrobial effect because of its high percentage of 1,8-cineol (30.41%) and camphor (23.52%) (Shunting et al., 2005). Antibacterial activities of the essential oils against *Staphylococcus aureus* and *Escherichia coli* were shown by disk diffusion tests (Arodogan et al., 2002).

2.3.3. Other effects

The methanolic extract from the flowers was found to show inhibitory activity against rat lens aldose reductase (Yoshikawa et al., 1999). The methanolic extract and ethyl acetate soluble portion from the flowers showed inhibitory activity against nitric oxide production in lipopolysaccharide-activated macrophages with potent inhibitory activity shown by the acetylenic compounds and flavonoids from the ethyl acetate-soluble portion (Yoshikawa et al., 2000).

The water extract of the flower has a coronary vasodilating action and a renal vasoconstricting action in the open chest dog with the pharmacological profile of the water extract to be in part, similar to that of adenosine (Kato et al., 1986). Intravenous administration of the aqueous extract (5-20 mg/kg) produced a decrease in aortic blood pressure and increased to the values above the pre-injection level. A two-fold increase in coronary blood flow was elicited by the aqueous extract (13.8mg/kg) and by adenosine (29.5µg/kg) (Kato et al., 1986).

2.3.4. Toxicities

Sesquiterpene lactones from the crude extract of dried flowers gave strong reactions on epicutaneous application to guinea pigs sensitized with an extract of *C. indicum*. One of the allergens was identified as a sesquiterpene lactone of the guaianolide type which was identical to arteglasin-A derived from *Artemisia douglasiana* bess (Hausen and Schulz, 1976).

Chapter 3. Materials and methods

3.1.1. Plant materials

The leaves of the *Chrysanthemum indicum* (Plate 1) were collected from the Chrysanthemum flower garden, Cameron Highlands, Pahang. The rhizomes of the *Alpinia galanga* (Plate 2) were bought from a vegetable farm in Hulu Langat, Selangor. The leaves and rhizomes were dried in the shade for 3 days at ambient temperature. The plants were authenticated by Professor Dr. Halijah Ibrahim, Institute of Biological Sciences, Faculty of Science, University of Malaya. Voucher specimens were deposited at the herbarium, Rimba Ilmu, Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia. Specimen number of *A. galanga* is HI 1423 and *C. indicum* is HI 1424.

Plate 1. *Chrysanthemum indicum*.

Plate 2. Rhizome of *Alpinia galanga.*

3.1.2. Commercial termiticides

Commercial termiticide was purchased from the chemical company Wesco Agencies (M) Sdn. Bhd. The commercial termiticide that was used in this study contain 21.2% of chlorpyrifos (Plate 3).

Plate 3. Commercial termiticide, Chlorpyrifos

3.2. Termites

The termites, *Coptotermes gestroi* were collected from rotten wood in Taman Kemacahaya, Cheras, Malaysia. *Coptotermes curvignathus* was collected from living oil palm tree whereas *Macrotermes carbonarius* was collected from a mound in an Oil Palm Plantation, Labu, Negeri Sembilan, Malaysia. All the termites were collected from the same colony, respectively. The termites were kept in 70% ethanol. The termites species were identified based on morphology of soldiers using books, Termites of Peninsular Malaysia (Tho, 1992) and Termites of Sabah (Thapa, 1981). For the confirmation of the termites, the termites were sent to Dr. Shawn Cheng, Forest Research Institute of Malaysia (FRIM), Kepong, Malaysia. The specimens were deposited in the Entomology Lab, FRIM. Specimen number of *C. gestroi* is ENT 130, *C. curvignathus* is ENT 131 and *M. carbonarius* is ENT 132.

3.2.1. Identification of Termites

Subfamily: Coptotermitinae Holmgrem

Genus: Coptotermes Wasmen

Key to species

Soldiers

1. Large species, head length to side base of mandibles 1.45-1.65; head width 1.25-1.38 mm.…….**curvignathus** Holmgren

   Smaller species, head length to side base of mandibles 0.95-1.32 mm ……2

2. Mandibles nearly straight, apices slightly bend inwards……..……3

   Mandibles sabre-shaped, strongly curved inwards from middle; head length to side base mandibles 1.12-1.25 mm, width 1.00-1.07 mm……...**sepangensis** Krishna

3. Large species, head length to side base of mandibles 1.25-1.32 mm; width 0.97-1.03 mm …..…….……**borneensis** Oshima

   Smaller species, head length to side base of mandibles 0.95-1.05 mm, width 0.85-0.93 mm ……..…….**kalshoveni** Kemner

(Thapa, 1981)

Subfamily: Macrotermitinae Kemner

Genus: Macrotermes Holmgren

Key to species

Major Soldiers

1. Tip of labrum short, triangular; lateral lobes of thorax rounded….....**gilvus** Hagen

   Tip of labrum trilobed, median lobe greatly produced anteriorly; lateral lobes of thorax angular…….…….2

2. Head wider width 3.90-4.30 mm, head length to side base of mandibles 4.65-5.00 mm…….....**malaccensis** Haviland

   Head narrower, width less than 3.50 mm; head length to side base of mandibles 4.00-4.65 mm……...…….3

3. Mandibles hooked apically……...4

   Mandibles sabre-shaped; head length to side base of mandibles 4.20-4.35 mm, width 3.37-3.40 mm…….……..**latignathus** new species

4. Large species; head length to side base mandibles 4.50-4.60 mm, width 3.30-3.40 mm; sides of head gradually narrowed anteriorly…..**beaufortensis** new species

   Smaller species; head length to side base of mandibles 4.00 mm, width 2.90 mm; sides of head slightly narrowed anteriorly….……**probeaufortensis** new species

(Thapa, 1981)

Minor Soldiers

1. Tip of labrum short, triangular; lateral lobes of thorax rounded…..**gilvus** Hagen

   Tip of labrum trilobed, median lobe greatly produced anteriorly; lateral lobes of thorax angular……..…….2

2. Mandibles strongly incurved apically or sabre shaped.....……...3

   Mandibles weakly incurved apically; head length to side base of mandibles 2.47-2.70 mm, width 1.92-2.05 mm……..…...**latignathus** new species

3. Head wider width 2.30-2.45 mm, head length to side base of mandibles 2.85-3.10 mm…….…….....**malaccensis** Haviland

   Head narrower, width up to 2.25 mm…….4

4. Head sides weakly convex; head length to side base mandibles 2.70-2.95 mm, width 2.10-2.25 mm……**probeaufortensis** new species

   Head sides straight and gradually narrowed anteriorly; head length to side base of mandibles 2.60-2.85 mm, width 1.90-2.10 mm.…..…. **beaufortensis** new species

(Thapa, 1981)

***Coptotermes gestroi* Oshima**

Superficially, soldiers of *Coptotermes gestroi* resemble those of *Coptotermes formosanus*. Both species have a large opening on the forehead called the fontanelle. When viewed from above, both also share tear drop-shaped heads. Microscopic examination of the fine hairs on the head reveals diagnostic differences. *Coptotermes gestroi* soldiers have one pair of hairs near the rim of the fontanelle, while in *Coptotermes formosanus*, two pairs originate around the fontanelle. Additionally, the lateral profile of the top of the head just behind the fontanelle shows a weak bulge in *Coptotermes gestroi* that is absent in *Coptotermes formosanus*. Measurements in millimeters of soldiers:

Length of head to base of mandibles	1.14 (1.02-1.20)
Width of head at base of mandibles	0.45 (0.42-0.50)
Maximum width of head	0.91 (0.80-1.02)

(Tho, 1992)

***Coptotermes curvignathus* Holmgren**

The soldiers of *C. curvignathus* are distinctive in being large as well as in having strongly incurved mandibles. No other species in this region comes within the size range of *C. curvignathus*. Measurements in millimeters of soldiers:

Length of head to base of mandibles	1.68 (1.51-1.85)
Width of head at base of mandibles	0.71 (0.65-0.82)
Maximum width of head	1.34 (1.28-1.57)

(Tho, 1992)

***Macrotermes carbonarius* Hagen**

Macrotermes carbonarius is coloured black and is free ranging in its foraging habit. It is also a very large species, the head capsule length being greater than 4.2 mm and 3.0 mm in the major and minor soldier respectively. These characteristic make it impossible to mistake for any other species in the country. *M. carbonarius* is confined to the lowlands below 160 metres. Not common on hilly terrain, steep slopes or riparian areas below the flood line. Otherwise common to all low-lying flat land throughout the country, especially in lowland depterocarp forest and coastal forests. Also occurs in rubber, coconut, teak and other tree crop plantations and is common around rural habitation.

(Tho, 1992)

3.3. Extraction methods

3.3.1. Methanolic Extraction

Extraction method used in this study was modified from the method described by Schlüter and Seifert (1988). A total of 100 g dried leaves and dried rhizomes were soaked in 1000 ml of methanol for 24 hours respectively. The mixture was filtered, and the filtrate was extracted into a 20 ml concentrated extract by using a rotary evaporator at 40 °C. The concentrated extract was served as stock extract.

3.3.2. Extraction of essential oil

Two kilogram of fresh rhizomes (Plate 4) and 2 kg of leaves (Plate 5) were cut into small pieces and subjected to steam-distillation at 96 °C for 3 hours using a Clevenger-type apparatus (Plate 6); the extracted oil was dried over anhydrous sodium sulfate. The concentrated essential oil was served as stock extract.

Plate 4. Fresh rhizomes of *Alpinia galanga.*

Plate 5. Fresh leaves of *Chrysanthemum indicum.*

Plate 6. Fresh rhizomes of *Alpinia galanga* and leaves of *Chrysanthemum indicum* in Clevenger apparatus.

3.4. Dual choice bioassay using crude extract

3.4.1. Crude methanolic extraction

Two paper discs (4.0 cm diameter, ~19.5 mg dry weight) were placed in Petri dishes (9 cm diameter). One disc treated with 25 µl of *A. galanga* methanolic extract was left to dry then weighed and moistened with 15 µl of distilled water (Messer et al., 1990). For control, a disk was treated with 25 µl of methanol. Both papers were placed in petri dish, respectively. Ten termites were placed in the treated petri dish. Ten replicates were used. The termites were exposed to treated and control paper disk for a consecutive 3 days at the laboratory conditions (26.4 ± 0.2 ◦C, 63.2 ± 0.6% RH).

At the end of the experiment, the paper disks were re-weighed to calculate the paper consumed by the termites. Mean weight of paper consumed by termite was counted and percentage of antifeedant activity was calculated according to the formula of Bentley et al. (1984):

$$\text{Antifeedant activity} = \frac{\text{Consumption in control disc} - \text{Consumption in treated disc}}{\text{Consumption in control disc}} \times 100$$

The results were statistically analyzed using ANOVA. The bioassay was repeated with methanolic extract of *C. indicum.*

3.4.2. Essential oil

Two paper discs (4.0 cm diameter, ~19.5 mg dry weight) were placed in Petri dishes (9 cm diameter). One disc treated with 25 µl of *A. galanga* essential oil was left to dry then weighed and moistened with 15 µl of distilled water (Messer et al., 1990). For control, a disk was treated with 25 µl of hexane. Both papers were placed in each petri dish, respectively. Ten termites were placed in the treated petri dish. Ten replicates were used. The termites were exposed to treated and control paper disk for a consecutive 3 days at the laboratory conditions (26.4 ± 0.2 ◦C, 63.2 ± 0.6% RH).

At the end of the experiment, the paper discs were re-weighed to calculate the paper consumed by the termites. Mean weight of paper consumed by termite was counted and percentage of antifeedant activity was calculated according to the formula of Bentley et al. (1984):

$$\text{Antifeedant activity} = \frac{\text{Consumption in control disc} - \text{Consumption in treated disc}}{\text{Consumption in control disc}} \times 100$$

The results were statistically analyzed using ANOVA. The bioassay was repeated with essential oil of *C. indicum.*

3.5. Isolation of crude extracts using column chromatography

The essential oil was isolated by using the chromatography method using silica gel column (Plate 7). Firstly, a small amount of glass wool was put into the end of the pipette. Then silica gel 60 (230-400 mesh) which eluted hexane was filled into the pipette. The experiment of chromatography was started by pouring the hexane solution into the prepared pipette until it went through the whole gel. This is with the purpose of cleaning and compacting the gel without air bubble. Then, about 2 ml of the essential oil was poured into the cleaned silica gel in the pipette. The purified solution that went through the silica gel was then collected in a bottle. Ten ml of the purified fraction was collected and labelled as fraction 1. These purified fractions were collected each in different glass vial and labelled as fraction 2 to fraction 8. The entire fractions were then kept in the refrigerator (5ºC).

3.6. Dual choice bioassay using fractions from column chromatography

One disk treated with 25 µl of fraction 1 from *A. galanga* essential oil and the disk was left to dry then weighed and moistened with 15 µl of distilled water (Messer et al., 1990). For control, a disk was treated with 25 µl of hexane. Both papers were placed in petri dish, respectively. Ten termites were placed in the treated petri dish. Ten replicates were used. The termites were exposed to treated and control paper disk for a consecutive 3 days at the laboratory conditions (26.4 ± 0.2 ◦C, 63.2 ± 0.6% RH). The experiment was also repeated with fraction 2 to fraction 8.

At the end of the experiment, the paper disks were re-weighed to calculate the paper consumed by the termites. Mean weight of paper consumed by termite was counted and percentage of antifeedant activity was calculated according to the formula of Bentley et al. (1984):

$$\text{Antifeedant activity} = \frac{\text{Consumption in control disc} - \text{Consumption in treated disc}}{\text{Consumption in control disc}} \times 100$$

The results were statistically analyzed using ANOVA. The bioassay was repeated with fractions from essential oil of *C. indicum.*

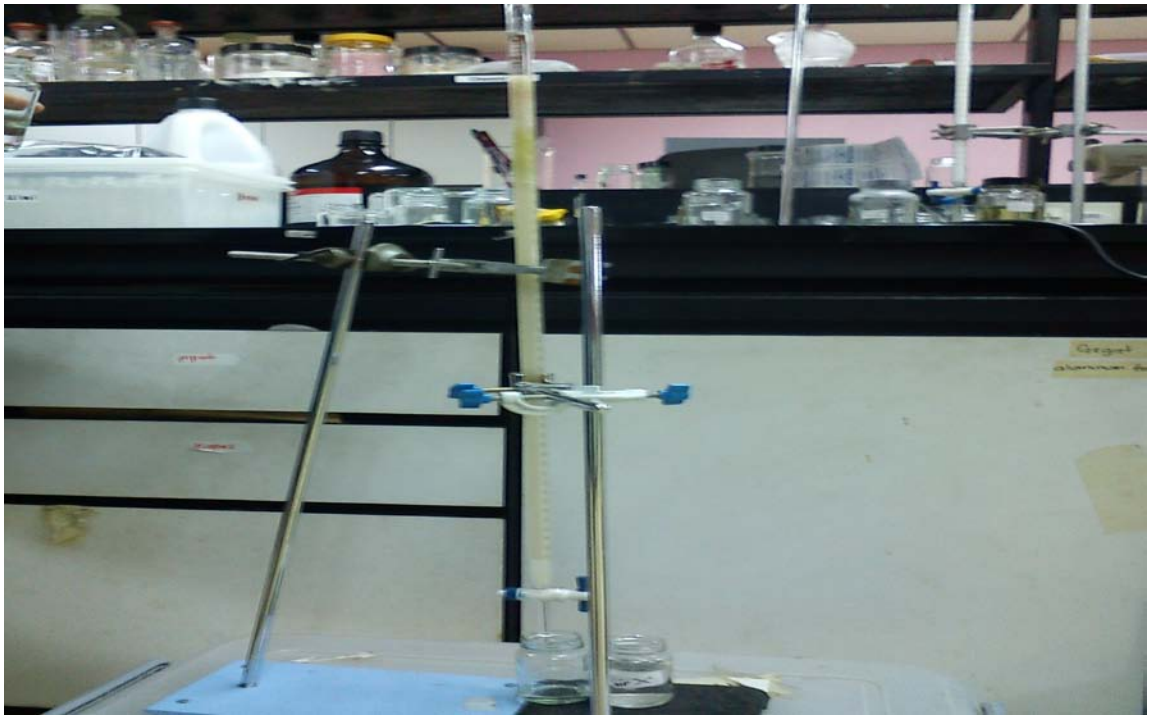


Plate 7. Separation of crude extract by column chromatography

3.7. Thin Layer Chromatography Analysis (TLC)

The fraction which gave positive response in the feeding bioassay was analysed using TLC plate silica gel F254 (Plate 8). Diethyl ether and methanol of 70:30 solvent mixtures were used. The separation that occurred on the TLC plate was observed under UVGL-58 UV light of short wave 254nm / long wave 366nm. A spot of extract was made by Drummond Microcaps® disposable pipette, containing 5 µl of extract. Retardation factor (Rf) values were determined. The fractions with similar Rf were combined and made into a concentrate using rotary evaporator. All fractions were kept in refrigerator until further analysis.

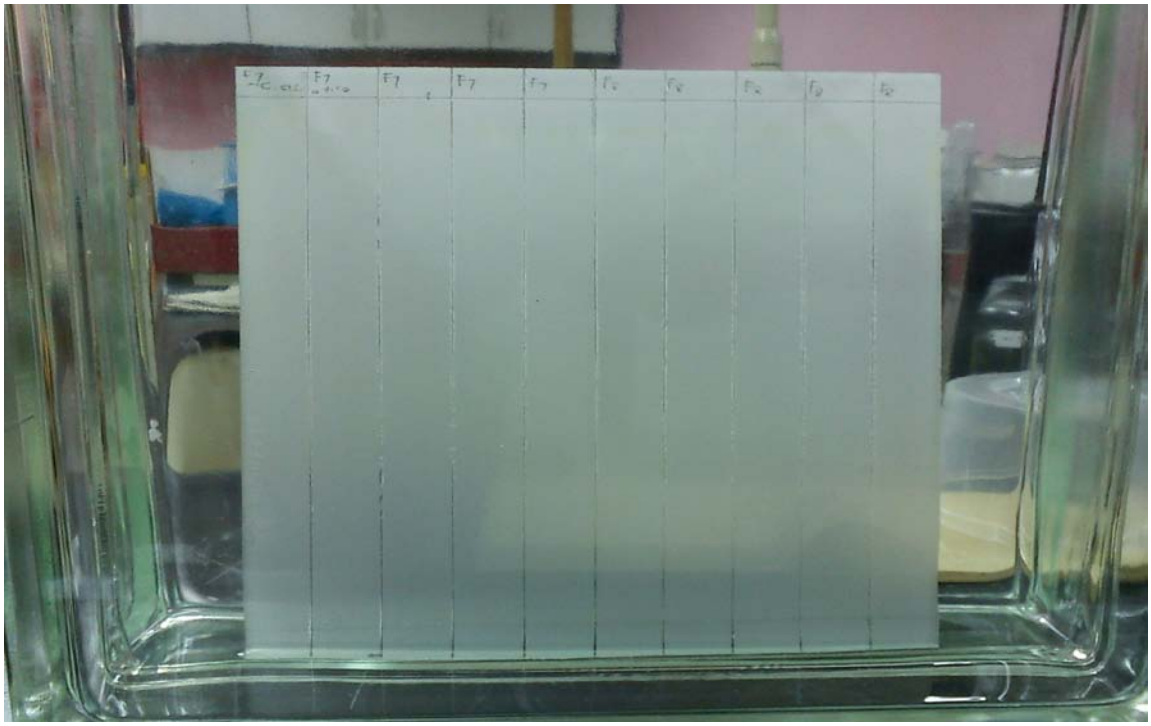


Plate 8. Separation of positive fraction TLC

3.8. Dual choice bioassay using fractions from Thin Layer Chromatography (TLC)

One disc was treated with 25 µl of spot 1 fraction from *A. galanga* essential oil and the disc was left to dry then weighed and moistened with 15 µl of distilled water (Messer et al., 1990). For control, a disc was treated with 25 µl of hexane. Both papers were placed in petri dish, respectively (Figure 1). Ten termites were placed in the treated petri dish. Ten replicates were used. The termites were exposed to treated and control paper disk for a consecutive 3 days at the laboratory conditions (26.4 ± 0.2 ◦C, 63.2 ± 0.6% RH). The experiment was also repeated with spot 2 to spot 4.

At the end of the experiment, the paper disks were re-weighed to calculate the paper consumed by the termites. Mean weight of paper consumed by termite was counted and percentage of antifeedant activity was calculated according to the formula of Bentley et al. (1984):

$$\text{Antifeedant activity} = \frac{\text{Consumption in control disc} - \text{Consumption in treated disc}}{\text{Consumption in control disc}} \times 100$$

The results were statistically analyzed using ANOVA. The bioassay was repeated with spots from essential oil of *C. indicum.*

3.9. Identification of components using GCMS

The essential oil composition was determined by GCMS. Retention times and mass spectral data were compared with the MS instrument library and NIST library. Relative percentages of the major components were calculated by integrating the registered peaks. GCMS experiments were performed on an ion trap GCQ-Plus (Finnigan, Thermo-Quest, Austin, TX, USA) instrument with MS program using a silica capillary column Rtx®-5MS (30 m x

0.25 mm ID, 0.25 mm). The carrier gas was helium (40 cms^{-2}). The port temperature was set to 200 °C in splitless mode with 1.0 µl injection volume. The initial temperature was maintained at 40 °C for 2 min, and then increased to 210 °C at 2 °C min^{-1}, and maintained at this temperature for up to 120 min.

3.10. Dual choice bioassay using synthetic compound

One disc was treated with 25 µl of 100 ppm of 1, 8-cineol and the disk was left to dry then weighed and moistened with 15 µl of distilled water (Messer et al., 1990). For control, a disk was treated with 25 µl of hexane. Both papers were placed in petri dish, respectively (Figure 1). Ten termites were placed in the treated petri dish. Ten replicates were used. The termites were exposed to treated and control paper disk for a consecutive 3 days at the laboratory conditions (26.4 ± 0.2 ◦C, 63.2 ± 0.6% RH). The experiment was also repeated with 200 ppm, 500 ppm and 1000 ppm of 1,8-cineol.

At the end of the experiment, the paper disks were re-weighed to calculate the paper consumed by the termites. Mean weight of paper consumed by termite was counted and percentage of antifeedant activity was calculated according to the formula of Bentley et al. (1984):

$$\text{Antifeedant activity} = \frac{\text{Consumption in control disc} - \text{Consumption in treated disc}}{\text{Consumption in control disc}} \times 100$$

The results were statistically analyzed using ANOVA. The bioassay was repeated with 100 ppm, 200 ppm, 500 ppm and 1000 ppm concentrations of identified synthetic compound, farnesene and the commercial termiticide, chlorpyrifos.

3.11. Determination of Effective Dose 50 (ED_{50})

An ED_{50} was determined according to staircase method (Randhawa, 2009) using 10 termites and increase doses of essentials oils, synthetic compounds and commercial termiticide, chlorpyrifos. ED_{50} is the dose of essential oils, synthetic compounds and chemical termicide that is effective to cause antifeedant activity in 50% of the termite exposed. Four doses were choose for determination of ED_{50}. After 24 hours, the mean weight of paper consumed by termite was counted and percentage of antifeedant activity was calculated according formula from Bentley et al. (1984):

$$\text{Antifeedant activity} = \frac{\text{Consumption in control disc} - \text{Consumption in treated disc}}{\text{Consumption in control disc}} \times 100$$

The percentage of antifeedant activity for all tested dosage were pooled and subjected to probit analysis. The 50% effective dose (ED_{50}) was obtained by using probit analysis in computer software SPSS (version 12).

3.12. Field application of synthetic active compound

Blocks of rubber wood with a measurement of 22 (long) x 5 (wide) x 2.5 (thick) cm were dried overnight in the oven at 60° C and weighed. Two field applications were conducted, one was for two weeks period and another one was for sixteen weeks period. Rubber wood was used in this study as *C. gestroi* significantly prefers to feed on rubber wood (Yeoh and Lee, 2007). They were dip-treated overnight with synthetic chemical in plastic trays whereas the controls were dip-treated with hexane alone. Each test consists of a treated and a control wooden block. The extract and chemical treated blocks (Plate 10) were air-dried and introduced into *C. gestroi* infested area in Taman Kemacahaya, Cheras (Plate 9). The

test was replicated 5 times for each concentration. Each pair of wooden blocks was placed at 5 meters distance. Four different concentrations (500 ppm, 1000 ppm, 2000 ppm and 5000 ppm) were used in this test. After two weeks and sixteen weeks, the blocks were removed, cleaned, dried overnight and weighed to determine weight loss (Plate 11 and 12). Weight loss in each block was recorded as wood consumed by termite. Mean weight of wood consumed by termite was counted and percentage of antifeedant activity was calculated according to the formula of Bentley et al. (1984):

$$\text{Antifeedant activity} = \frac{\text{Consumption in control disc} - \text{Consumption in treated disc}}{\text{Consumption in control disc}} \times 100$$

The results were statistically analyzed using ANOVA

Plate 9. Treated and untreated blocks were inserted into termite infested area.

Plate 10. Treated and untreated blocks before expose to termite infested area.

Plate 11. Treated and untreated blocks after 2 weeks of exposure to termite infested area.

Plate 12. The blocks after 2 weeks of exposure to termite infested area.

Chapter 4. Results

4.1. Identification of Termites

Each of the respective species was documented using microscope fitted camera. Measurements in millimeters of *C. gestroi* soldiers (Plate 3):

Length of head to base of mandibles	1.18 mm
Width of head at base of mandibles	0.47 mm
Maximum width of head	0.98 mm

Plate 13. *Coptotermes gestroi.*

Measurements in millimeters of *C. curvignathus* soldiers (Plate 4):

Length of head to base of mandibles	1.12 mm
Width of head at base of mandibles	0.48 mm
Maximum width of head	0.97 mm

Plate 14. *Coptotermes curvignathus.*

Measurements in millimeters of *M. carbonarius* soldiers (Plate 5):

Length of head to base of mandibles	3.39 mm
Width of head at base of mandibles	1.02 mm
Maximum width of head	2.68 mm

Plate 15. Minor soldier of *Macrotermes carbonarius.*

4.2. Dual choice bioassay using crude methanolic extract and essential oil of *A. galanga*

Table 1 shows the mean weight of paper disc treated with methanolic extract of *A. galanga* consumed by *C. gestroi, C. curvignathus* and *M. carbonarius* in dual choice bioassay after 3 days exposure.

Methanolic extract of *Alpinia galanga* was not antifeedant to *Coptotermes gestroi* and the percentage of antifeedant activity (PA) was -1.95% ($P>0.05$). Similarly, *Coptotermes curvignathus* and *M. carbonarius* consumption of paper was not significant. The PA value of *A. galanga* methanolic extract towards *C. curvignathus* was -0.55% ($P>0.05$). The PA value against *M. carbonarius* was -2.66% ($P>0.05$).

However, PA value of *A. galanga* essential oil against *C. gestroi* was 53.51% ($P<0.05$) showing a significant reduction in paper consumption after 24 hours. Similarly, PA value of essential oil to *C. curvignathus* and *M. carbonarius* were 53.45% and 52.31% ($P<0.05$), respectively. Similar outcome were received for 48 and 72 hours observation periods respectively.

Table 1: Mean weight of paper disc treated with methanolic extract and essential oil of *A. galanga* consumed by *C. gestroi*, *C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite Species	Extract	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	Methanolic ext.	7.31±0.42[a]	7.17 ± 0.28[a]	-1.95[a]	12.40±0.69[a]	12.21±0.61[a]	-1.56[a]	15.41±0.89[a]	15.77±0.99[a]	2.28[a]
	Essential oil	3.25±0.22[b]	6.99 ± 0.35[a]	53.51[b]	5.10±0.24[b]	12.20±0.58[a]	58.20[b]	8.10±0.65[b]	15.85±0.75[a]	48.90[b]
C. curvignathus	Methanolic ext.	7.34 ±0.36[a]	7.30 ± 0.29[a]	-0.55[a]	12.36±0.84[a]	12.24±0.68[a]	-0.98[a]	15.56±0.98[a]	15.70±1.07[a]	0.89[a]
	Essential oil	3.24±0.23[b]	6.96 ± 0.32[a]	53.45[b]	5.22±0.18[b]	12.28±0.76[a]	57.49[b]	8.16±0.55[b]	15.98±0.74[a]	48.94[b]
M. carbonarius	Methanolic ext.	7.31±0.34[a]	7.12 ± 0.25[a]	-2.66[a]	12.76±0.69[a]	12.26±0.62[a]	-4.08[a]	15.49±0.92[a]	15.70±1.07[a]	1.34[a]
	Essential oil	3.30±0.24[b]	6.92 ± 0.34[a]	52.31[b]	5.14±0.27[b]	12.04±0.59[a]	57.31[b]	8.16±0.55[b]	15.98±0.74[a]	48.94[b]

Means with the same letter are not significantly different between groups. ANOVA ($P>0.05$).
Means with different letter are significantly different between groups. ANOVA ($P\leq0.05$).

4.3. Dual choice bioassay using different concentrations of *A. galanga* essential oil

Alpinia galanga essential oil that was tested in this study was antifeedant to *Coptotermes gestroi, Coptotermes curvignathus* and *Macrotermes carbonarius* and the concentration–response analyses were significant (Table 2). Regardless of time there was no significant antifeedant activity in 500 ppm of *A. galanga* essential oil showed against *Coptotermes gestroi, Coptotermes curvignathus* and *Macrotermes carbonarius* whereas there was a significant antifeedant activity in 1000 ppm, 2000 ppm and 5000 ppm of *A. galanga* essential oil. The antifeedant activity of the essential oil extracted from *A. galanga* was significantly influenced by the concentration applied. Essential oil extracted from the *A. galanga* showed the same antifeedant activity to *C. curvignathus* and *M. carbonarius.* When applied at concentrations ranging from 500 to 5000 ppm during 24 hours of exposure the highest percentage of antifeedant (PA) values for this oil was 53.10%. The same tendency was observed with essential oil against *C. curvignathus* and *M. carbonarius*: the PA values with this essential oil at concentrations of 500 to 5000 ppm were 54.05% and 54.39%, respectively. Similar outcome were received for 48 and 72 hours observation periods respectively.

Table 2: Mean weight of paper disc treated with different concentration of *A. galanga* essential oil consume by *C. gestroi*, *C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite Species	Extract	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	500 ppm	6.99 ± 0.37^{a}	7.09 ± 0.35^{a}	1.41^{a}	12.02 ± 0.57^{a}	12.08 ± 0.56^{a}	0.50^{a}	15.85 ± 0.60^{a}	15.74 ± 0.75^{a}	-0.70^{a}
	1000 ppm	4.57 ± 0.40^{b}	6.99 ± 0.35^{a}	34.62^{b}	8.10 ± 0.49^{b}	12.20 ± 0.58^{a}	33.61^{b}	12.87 ± 0.47^{b}	15.85 ± 0.75^{a}	18.80^{b}
	2000 ppm	3.30 ± 0.24^{c}	6.90 ± 0.38^{a}	52.17^{c}	5.09 ± 0.22^{c}	12.22 ± 0.58^{a}	58.35^{c}	8.14 ± 0.70^{c}	15.95 ± 0.75^{a}	48.97^{c}
	5000 ppm	3.25 ± 0.22^{c}	6.93 ± 0.26^{a}	53.10^{c}	5.08 ± 0.24^{c}	12.34 ± 0.51^{a}	58.83^{c}	8.21 ± 0.61^{c}	16.01 ± 0.75^{a}	48.72^{c}
C. curvignathus	500 ppm	6.90 ± 0.37^{a}	7.12 ± 0.41^{a}	3.09^{a}	12.04 ± 0.72^{a}	12.02 ± 0.42^{a}	-0.17^{a}	15.84 ± 0.58^{a}	15.60 ± 0.82^{a}	-1.53^{a}
	1000 ppm	7.10 ± 0.34^{a}	7.06 ± 0.32^{a}	-0.57^{a}	12.00 ± 0.45^{a}	12.14 ± 0.72^{a}	1.15^{a}	15.86 ± 0.69^{a}	15.88 ± 0.74^{a}	0.13^{a}
	2000 ppm	3.32 ± 0.24^{c}	6.90 ± 0.38^{a}	51.88^{c}	5.20 ± 0.16^{c}	12.38 ± 0.76^{a}	58.00^{c}	8.16 ± 0.92^{c}	16.08 ± 0.74^{a}	49.25^{c}
	5000 ppm	3.18 ± 0.19^{c}	6.92 ± 0.29^{a}	54.05^{c}	5.08 ± 0.26^{c}	12.36 ± 0.64^{a}	58.90^{c}	8.38 ± 0.36^{c}	16.04 ± 0.75^{a}	47.76^{c}
M. carbonarius	500 ppm	7.08 ± 0.37^{a}	7.06 ± 0.36^{a}	-0.28^{a}	12.02 ± 0.48^{a}	12.14 ± 0.72^{a}	0.99^{a}	15.98 ± 0.67^{a}	15.88 ± 0.74^{a}	-0.63^{a}
	1000 ppm	7.18 ± 0.36^{a}	7.02 ± 0.34^{a}	-2.28^{a}	12.34 ± 0.34^{a}	11.94 ± 0.19^{a}	-3.35^{a}	15.66 ± 0.68^{a}	15.20 ± 0.60^{a}	-3.03^{a}
	2000 ppm	3.24 ± 0.23^{c}	6.86 ± 0.40^{a}	52.77^{c}	5.00 ± 0.59^{c}	12.18 ± 0.59^{a}	58.95^{c}	8.20 ± 0.76^{c}	15.40 ± 0.60^{a}	46.75^{c}
	5000 ppm	3.12 ± 0.13^{c}	6.84 ± 0.23^{a}	54.39^{c}	5.18 ± 0.18^{c}	12.24 ± 0.59^{a}	57.68^{c}	8.24 ± 0.34^{c}	15.40 ± 0.49^{a}	46.49^{c}

Means with the same letter are not significantly different between groups. ANOVA (P>0.05).
Means with different letter are significantly different between groups. ANOVA (P≤0.05).

4.4. Dual choice bioassay using crude methanolic extract and essential oil of *C. indicum*

Methanolic extract of *Chrysanthemum indicum* was not antifeedant to *Coptotermes gestroi* and the percentage of antifeedant activity (PA) was -2.47% (P>0.05). *Coptotermes curvignathus* and *M. carbonarius* consumption of paper also showed that methanolic extract was not significant too. The PA value of *C. indicum* methanolic extract towards *C. curvignathus* was -4.45% (P>0.05). The PA value against *M. carbonarius* was -2.35% (P>0.05).

However, PA value of *C. indicum* essential oil against *C. gestroi* was 53.50% (P<0.05) and it shows a significant reduction in paper consumption after 24 hours. Similarly, PA value of essential oil to *C. curvignathus* and *M. carbonarius* were 55.15% and 52.26% (P<0.05), respectively. Similar results were observed for 48 and 72 hours of exposure (Table 3).

Table 3. Mean weight of paper disc treated with methanolic extract and essential oil of *C. indicum* consumed by *C. gestroi*, *C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite species	Extraction	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	Methanolic ext.	7.05 ± 0.58^{a}	6.88 ± 0.29^{a}	-2.47^{a}	11.97 ± 0.59^{a}	11.95 ± 0.65^{a}	-0.17^{a}	15.47 ± 0.81^{a}	16.06 ± 0.95^{a}	3.67^{a}
	Essential oil	3.32 ± 0.32^{b}	7.14 ± 0.29^{a}	53.50^{b}	4.17 ± 0.45^{b}	12.11 ± 0.54^{a}	65.57^{b}	6.19 ± 0.72^{b}	15.79 ± 0.73^{a}	60.80^{b}
C. curvignathus	Methanolic ext.	7.06 ± 0.63^{a}	6.74 ± 0.32^{a}	-4.45^{a}	11.90 ± 0.63^{a}	11.94 ± 0.53^{a}	0.34^{a}	15.70 ± 0.74^{a}	16.14 ± 0.96^{a}	2.73^{a}
	Essential oil	3.22 ± 0.27^{b}	7.18 ± 0.26^{a}	55.15^{b}	4.12 ± 0.38^{b}	12.10 ± 0.39^{a}	65.95^{b}	6.46 ± 0.71^{b}	16.12 ± 0.38^{a}	59.93^{b}
M. carbonarius	Methanolic ext.	6.96 ± 0.71^{a}	6.80 ± 0.29^{a}	-2.35^{a}	11.80 ± 0.78^{a}	12.00 ± 0.66^{a}	1.67^{a}	15.58 ± 0.70^{a}	15.86 ± 0.82^{a}	1.77^{a}
	Essential oil	3.38 ± 0.29^{b}	7.08 ± 0.32^{a}	52.26^{b}	4.44 ± 0.47^{b}	12.20 ± 0.64^{a}	63.61^{b}	6.20 ± 0.68^{b}	15.98 ± 0.42^{a}	61.20^{b}

Means with the same letter are not significantly different between groups. ANOVA ($P>0.05$).
Means with different letter are significantly different between groups. ANOVA ($P\leq0.05$).

4.5. Dual choice bioassay using different concentrations of *C. indicum* essential oil

Chrysanthemum indicum essential oil that was tested in this study was antifeedant to *Coptotermes gestroi, Coptotermes curvignathus* and *Macrotermes carbonarius* and the concentration–response analyses were significant (Table 4). Regardless of time there was no significant antifeedant activity in 500 ppm of *C. indicum* essential oil showed against *C. gestroi, C. curvignathus* and *M. carbonarius* whereas there was a significant antifeedant activity in 1000 ppm, 2000 ppm and 5000 ppm of *C. indicum* essential oil. The antifeedant activity of the essential oil extracted from *C. indicum* was significantly influenced by the concentration applied. Essential oil extracted from the *C. indicum* showed the same antifeedant activity to *C. curvignathus* and *M. carbonarius.* When applied at concentrations ranging from 500 to 5000 ppm during 24 hours of exposure, the highest percentage of antifeedant (PA) values for this oil was 53.10%. The same tendency was observed with essential oil against *C. curvignathus* and *M. carbonarius*: the PA values with this essential oil at concentrations of 500 to 5000 ppm were 54.05% and 54.39%, respectively. Similar results were observed for 48 and 72 hours exposure (Table 4).

Table 4. Mean weight of paper disc treated with different concentration of *C. indicum* essential oil consumed by *C. gestroi*, *C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite species	Concentration	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	500 ppm	6.85 ± 0.43^{a}	7.02 ± 0.27^{a}	2.42^{a}	11.93 ± 0.36^{a}	11.98 ± 0.56^{a}	0.42^{a}	15.90 ± 0.34^{a}	15.66 ± 0.69^{a}	-1.53^{a}
	1000 ppm	4.56 ± 0.39^{b}	7.14 ± 0.29^{a}	36.13^{b}	5.74 ± 0.45^{b}	12.11 ± 0.54^{a}	52.60^{b}	7.46 ± 0.44^{b}	15.79 ± 0.73^{a}	52.75^{b}
	2000 ppm	2.39 ± 0.43^{c}	7.21 ± 0.33^{a}	66.85^{c}	4.17 ± 0.44^{c}	12.11 ± 0.54^{a}	65.57^{c}	6.38 ± 0.50^{c}	15.84 ± 0.76^{a}	59.72^{c}
	5000 ppm	2.40 ± 0.42^{c}	7.21 ± 0.28^{a}	66.71^{c}	4.18 ± 0.46^{c}	12.27 ± 0.49^{a}	65.93^{c}	6.13 ± 0.67^{c}	15.89 ± 0.61^{a}	61.42^{c}
C. curvignathus	500 ppm	6.88 ± 0.45^{a}	6.96 ± 0.34^{a}	1.15^{a}	11.94 ± 0.40^{a}	11.98 ± 0.72^{a}	0.33^{a}	15.86 ± 0.34^{a}	15.34 ± 0.83^{a}	-3.39^{a}
	1000 ppm	6.82 ± 0.47^{a}	7.08 ± 0.19^{a}	3.67^{a}	11.92 ± 0.36^{a}	11.98 ± 0.42^{a}	0.50^{a}	15.92 ± 0.38^{a}	15.98 ± 0.36^{a}	0.38^{a}
	2000 ppm	2.26 ± 0.38^{c}	7.22 ± 0.36^{a}	68.70^{c}	4.28 ± 0.52^{c}	12.04 ± 0.48^{a}	64.45^{c}	6.58 ± 0.52^{c}	16.22 ± 0.40^{a}	59.43^{c}
	5000 ppm	2.54 ± 0.49^{c}	7.32 ± 0.22^{a}	65.30^{c}	4.06 ± 0.36^{c}	12.34 ± 0.40^{a}	67.10^{c}	6.30 ± 0.59^{c}	16.14 ± 0.46^{a}	60.97^{c}
M. carbonarius	500 ppm	7.00 ± 0.44^{a}	7.08 ± 0.19^{a}	1.13^{a}	11.98 ± 0.43^{a}	11.92 ± 0.42^{a}	-0.50^{a}	16.02 ± 0.34^{a}	15.98 ± 0.36^{a}	-0.25^{a}
	1000 ppm	7.02 ± 0.44^{a}	7.00 ± 0.29^{a}	-0.29^{a}	12.08 ± 0.39^{a}	11.90 ± 0.65^{a}	-1.51^{a}	15.96 ± 0.36^{a}	15.74 ± 0.62^{a}	-1.40^{a}
	2000 ppm	2.30 ± 0.29^{c}	7.12 ± 0.38^{a}	67.70^{c}	4.34 ± 0.52^{c}	12.06 ± 0.68^{a}	64.01^{c}	6.58 ± 0.52^{c}	16.22 ± 0.40^{a}	59.43^{c}
	5000 ppm	2.66 ± 0.36^{c}	7.22 ± 0.26^{a}	63.16^{c}	3.98 ± 0.39^{c}	12.24 ± 0.63^{a}	67.48^{c}	6.30 ± 0.59^{c}	16.14 ± 0.46^{a}	60.97^{c}

Means with the same letter are not significantly different between groups. ANOVA ($P>0.05$).
Means with different letter are significantly different between groups. ANOVA ($P\leq0.05$).

4.6. Feeding Bioassay using *A. galanga* fractions from column chromatography

Table 5 shows the mean weight of paper disc treated with column chromatography fractions of *A. galanga* consumed by *C. gestroi, C. curvignathus* and *M. carbonarius* and the percentage of antifeedant activity in dual choice bioassay after 3 days exposure.

Fraction 4 of *A. galanga* essential oil that was tested in this study was antifeedant to *C. gestroi, C. curvignathus* and *M. carbonarius*. Regardless of time there was no significant antifeedant activity in other fractions of *A. galanga* essential oil against *C. gestroi*. Fraction 4 of *A. galanga* essential oil showed the same antifeedant activity to *C. curvignathus* and *M. carbonarius.* After 24 hours of exposure, the highest percentage of antifeedant (PA) values for this fraction 4 was 54.48%. The same tendency was observed with fraction 4 against *C. curvignathus* and *M. carbonarius*: the PA values with this fraction were 55.46% and 53.52%, respectively. Similar results were observed for 48 and 72 hours exposure.

Table 5. Mean weight of paper disc treated with fractions from *A. galanga* consumed by *C. gestroi*, *C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite Species	Fractions	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	Fraction 1	7.26 ± 0.42^{a}	7.21 ± 0.42^{a}	-0.69^{a}	12.37 ± 0.55^{a}	12.25 ± 0.66^{a}	-0.98^{a}	15.36 ± 0.84^{a}	15.82 ± 1.03^{a}	2.91^{a}
	Fraction 2	7.08 ± 0.51^{a}	6.83 ± 0.44^{a}	-3.66^{a}	12.06 ± 0.60^{a}	11.91 ± 0.74^{a}	-1.26^{a}	15.43 ± 0.74^{a}	15.89 ± 0.82^{a}	2.89^{a}
	Fraction 3	7.22 ± 0.47^{a}	7.21 ± 0.39^{a}	-0.14^{a}	12.37 ± 0.63^{a}	12.27 ± 0.70^{a}	-0.81^{a}	15.44 ± 0.73^{a}	16.09 ± 0.99^{a}	4.04^{a}
	Fraction 4	3.20 ± 0.25^{b}	7.03 ± 0.49^{a}	54.48^{b}	5.03 ± 0.21^{b}	12.25 ± 0.59^{a}	58.94^{b}	8.18 ± 0.65^{b}	15.70 ± 1.06^{a}	43.88^{b}
	Fraction 5	7.15 ± 0.42^{a}	6.86 ± 0.37^{a}	-4.23^{a}	12.43 ± 0.65^{a}	12.08 ± 0.66^{a}	-2.90^{a}	15.43 ± 0.78^{a}	15.75 ± 0.82^{a}	2.03^{a}
	Fraction 6	7.27 ± 0.47^{a}	7.25 ± 0.44^{a}	-0.28^{a}	11.91 ± 0.63^{a}	12.12 ± 0.79^{a}	1.73^{a}	15.57 ± 0.78^{a}	15.80 ± 0.78^{a}	1.46^{a}
	Fraction 7	7.11 ± 0.53^{a}	6.84 ± 0.43^{a}	-3.95^{a}	12.47 ± 0.57^{a}	12.11 ± 0.67^{a}	-2.97^{a}	15.51 ± 0.78^{a}	15.99 ± 0.85^{a}	3.00^{a}
	Fraction 8	7.19 ± 0.42^{a}	7.31 ± 0.37^{a}	1.64^{a}	11.95 ± 0.62^{a}	12.07 ± 0.70^{a}	0.99^{a}	15.38 ± 0.80^{a}	15.87 ± 0.83^{a}	3.09^{a}
C. curvignathus	Fraction 1	7.10 ± 0.43^{a}	7.30 ± 0.43^{a}	2.74^{a}	12.38 ± 0.68^{a}	12.24 ± 0.77^{a}	-1.14^{a}	15.48 ± 1.00^{a}	15.72 ± 1.16^{a}	1.53^{a}
	Fraction 2	7.06 ± 0.61^{a}	6.64 ± 0.46^{a}	-6.33^{a}	12.00 ± 0.73^{a}	11.86 ± 0.49^{a}	-1.18^{a}	15.62 ± 0.86^{a}	15.98 ± 0.87^{a}	2.25^{a}
	Fraction 3	7.08 ± 0.51^{a}	7.24 ± 0.45^{a}	2.21^{a}	12.24 ± 0.80^{a}	12.20 ± 0.83^{a}	-0.33^{a}	15.68 ± 0.70^{a}	16.18 ± 0.89^{a}	3.09^{a}
	Fraction 4	3.10 ± 0.18^{b}	6.96 ± 0.48^{a}	55.46^{b}	5.00 ± 0.26^{b}	12.30 ± 0.74^{a}	59.35^{b}	8.16 ± 0.79^{b}	15.62 ± 1.16^{a}	47.76^{b}
	Fraction 5	7.14 ± 0.43^{a}	6.80 ± 0.39^{a}	-5.00^{a}	12.34 ± 0.80^{a}	12.14 ± 0.87^{a}	-1.65^{a}	15.56 ± 0.96^{a}	16.02 ± 0.48^{a}	2.87^{a}
	Fraction 6	7.08 ± 0.41^{a}	7.30 ± 0.40^{a}	3.01^{a}	11.82 ± 0.78^{a}	12.04 ± 0.61^{a}	1.83^{a}	15.76 ± 0.86^{a}	15.94 ± 0.74^{a}	1.13^{a}
	Fraction 7	7.02 ± 0.60^{a}	6.70 ± 0.42^{a}	-4.78^{a}	12.48 ± 0.71^{a}	12.08 ± 0.80^{a}	-3.31^{a}	15.60 ± 0.96^{a}	16.04 ± 0.83^{a}	2.74^{a}
	Fraction 8	7.03 ± 0.43^{a}	7.40 ± 0.39^{a}	5.00^{a}	11.88 ± 0.75^{a}	12.04 ± 0.44^{a}	1.33^{a}	15.56 ± 0.94^{a}	16.12 ± 0.54^{a}	3.47^{a}
M. carbonarius	Fraction 1	7.42 ± 0.34^{a}	7.12 ± 0.44^{a}	-4.21^{a}	12.36 ± 0.38^{a}	12.26 ± 0.63^{a}	-0.82^{a}	15.24 ± 0.73^{a}	15.92 ± 1.01^{a}	4.27^{a}
	Fraction 2	7.10 ± 0.39^{a}	7.02 ± 0.38^{a}	1.39^{a}	12.12 ± 0.41^{a}	11.96 ± 0.99^{a}	-1.14^{a}	15.24 ± 0.61^{a}	15.80 ± 0.86^{a}	3.54^{a}
	Fraction 3	7.36 ± 0.38^{a}	7.18 ± 0.38^{a}	-2.51^{a}	12.50 ± 0.35^{a}	12.30 ± 0.64^{a}	-1.63^{a}	15.20 ± 0.74^{a}	16.00 ± 1.18^{a}	5.00^{a}
	Fraction 4	3.30 ± 0.27^{b}	7.10 ± 0.55^{a}	53.52^{b}	5.06 ± 0.15^{b}	12.20 ± 0.47^{a}	58.52^{b}	8.20 ± 0.56^{b}	15.78 ± 1.07^{a}	49.18^{b}
	Fraction 5	7.16 ± 0.42^{a}	6.92 ± 0.37^{a}	-3.47^{a}	12.52 ± 0.42^{a}	12.02 ± 0.45^{a}	-1.46^{a}	15.30 ± 0.65^{a}	15.48 ± 1.05^{a}	1.16^{a}
	Fraction 6	7.46 ± 0.44^{a}	7.20 ± 0.51^{a}	-3.61^{a}	12.00 ± 0.42^{a}	12.20 ± 1.00^{a}	1.64^{a}	15.38 ± 0.74^{a}	15.66 ± 0.87^{a}	1.79^{a}
	Fraction 7	7.20 ± 0.42^{a}	6.98 ± 0.43^{a}	-3.15^{a}	12.46 ± 0.39^{a}	12.14 ± 0.62^{a}	-2.64^{a}	15.42 ± 0.66^{a}	15.94 ± 0.96^{a}	3.26^{a}
	Fraction 8	7.36 ± 0.34^{a}	7.22 ± 0.36^{a}	-1.93^{a}	12.02 ± 0.44^{a}	12.10 ± 0.95^{a}	0.66^{a}	15.20 ± 0.69^{a}	15.62 ± 1.05^{a}	2.69^{a}

4.7. Feeding Bioassay using *C. indicum* fractions from column chromatography

Table 6 shows the mean weight of paper disc treated with column chromatography fractions of *C. indicum* consumed by *C. gestroi, C. curvignathus* and *M. carbonarius* and percentage of antifeedant activity in dual choice bioassay after 3 days exposure.

Fraction 5 of *C. indicum* essential oil that was tested in this study was antifeedant to *C. gestroi, C. curvignathus* and *M. carbonarius*. Regardless of time there was no significant antifeedant activity in other fractions of *C. indicum* essential oil against *C. gestroi*. Fraction 5 of *C. indicum* essential oil showed the same antifeedant activity to *C. curvignathus* and *M. carbonarius.* After 24 hours of exposure the highest percentage of antifeedant (PA) values for this fraction 5 was 19.97%. The same tendency was observed with fraction 5 against *C. curvignathus* and *M. carbonarius*: the PA values with this fraction 5 were 19.89% and 20.06%, respectively. Similar results were observed for 48 and 72 hours exposure.

Table 6. Mean weight of paper disc treated with fractions of *C. indicum* consumed by *C. gestroi*, *C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite Species	Fractions	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	Fraction 1	6.98 ± 0.45[a]	7.04 ± 0.36[a]	0.85[a]	11.89 ± 0.64[a]	12.04 ± 0.73[a]	1.25[a]	15.31 ± 0.80[a]	15.80 ± 1.04[a]	3.10[a]
	Fraction 2	7.33 ± 0.49[a]	7.15 ± 0.41[a]	-2.52[a]	12.47 ± 0.64[a]	12.16 ± 0.69[a]	-2.55[a]	15.57 ± 0.78[a]	15.76 ± 0.85[a]	1.21[a]
	Fraction 3	7.06 ± 0.43[a]	7.04 ± 0.41[a]	-0.28[a]	11.87 ± 0.72[a]	12.07 ± 0.80[a]	1.66[a]	15.57 ± 0.78[a]	15.95 ± 0.88[a]	2.38[a]
	Fraction 4	7.23 ± 0.51[a]	7.22 ± 0.39[a]	-0.14[a]	12.44 ± 0.68[a]	12.17 ± 0.70[a]	-2.22[a]	15.46 ± 0.79[a]	15.77 ± 0.84[a]	1.97[a]
	Fraction 5	5.73 ± 0.34[b]	7.16 ± 0.37[a]	19.97[b]	6.88 ± 0.51[b]	12.16 ± 0.47[a]	43.42[b]	8.18 ± 0.63[b]	15.82 ± 0.97[a]	48.29[b]
	Fraction 6	7.00 ± 0.58[a]	6.97 ± 0.48[a]	-0.43[a]	11.94 ± 0.76[a]	12.01 ± 0.74[a]	0.58[a]	15.43 ± 0.73[a]	15.96 ± 0.75[a]	3.32[a]
	Fraction 7	7.33 ± 0.45[a]	7.18 ± 0.37[a]	-2.09[a]	12.46 ± 0.65[a]	12.16 ± 0.65[a]	-2.47[a]	15.36 ± 0.79[a]	16.16 ± 0.80[a]	4.95[a]
	Fraction 8	7.00 ± 0.56[a]	6.97 ± 0.41[a]	-0.43[a]	11.88 ± 0.76[a]	11.99 ± 0.75[a]	0.92[a]	15.41 ± 0.80[a]	15.83 ± 0.75[a]	2.65[a]
C. curvignathus	Fraction 1	6.98 ± 0.47[a]	6.92 ± 0.37[a]	-0.87[a]	11.80 ± 0.81[a]	11.98 ± 0.52[a]	1.50[a]	15.40 ± 0.96[a]	15.72 ± 1.27[a]	2.04[a]
	Fraction 2	7.20 ± 0.57[a]	7.14 ± 0.47[a]	-0.84[a]	12.32 ± 0.90[a]	12.14 ± 0.80[a]	-1.48[a]	15.72 ± 0.93[a]	15.82 ± 0.90[a]	0.63[a]
	Fraction 3	7.08 ± 0.51[a]	6.90 ± 0.38[a]	-2.61[a]	11.74 ± 0.95[a]	12.02 ± 0.59[a]	2.33[a]	15.76 ± 0.86[a]	15.98 ± 0.88[a]	1.38[a]
	Fraction 4	7.08 ± 0.60[a]	7.22 ± 0.40[a]	1.94[a]	12.34 ± 0.90[a]	12.14 ± 0.83[a]	-1.65[a]	15.56 ± 0.96[a]	16.08 ± 0.55[a]	3.23[a]
	Fraction 5	5.72 ± 0.29[b]	7.14 ± 0.24[a]	19.89[b]	6.84 ± 0.54[b]	12.12 ± 0.44[a]	43.56[b]	8.16 ± 0.75[a]	15.70 ± 1.06[a]	48.03[b]
	Fraction 6	6.94 ± 0.73[a]	6.80 ± 0.46[a]	-2.06[a]	11.78 ± 1.00[a]	11.96 ± 0.49[a]	1.51[a]	15.68 ± 0.70[b]	16.06 ± 0.69[a]	2.37[a]
	Fraction 7	7.16 ± 0.52[a]	7.22 ± 0.44[a]	0.83[a]	12.36 ± 0.86[a]	12.14 ± 0.77[a]	-1.81[a]	15.46 ± 0.96[a]	16.20 ± 0.79[a]	4.57[a]
	Fraction 8	6.96 ± 0.68[a]	6.80 ± 0.38[a]	-2.35[a]	11.72 ± 1.01[a]	11.92 ± 0.53[a]	1.68[a]	15.60 ± 0.85[a]	16.14 ± 0.42[a]	3.35[a]
M. carbonarius	Fraction 1	6.98 ± 0.49[a]	7.16 ± 0.34[a]	2.51[a]	11.98 ± 0.49[a]	12.10 ± 0.95[a]	0.99[a]	15.22 ± 0.70[a]	15.88 ± 0.90[a]	4.16[a]
	Fraction 2	7.46 ± 0.42[a]	7.16 ± 0.39[a]	-4.19[a]	12.62 ± 0.38[a]	12.18 ± 0.65[a]	-3.61[a]	15.42 ± 0.67[a]	15.70 ± 0.89[a]	1.78[a]
	Fraction 3	7.04 ± 0.40[a]	7.18 ± 0.43[a]	1.95[a]	12.00 ± 0.47[a]	12.12 ± 1.04[a]	0.99[a]	15.38 ± 0.74[a]	15.92 ± 0.98[a]	3.39[a]
	Fraction 4	7.38 ± 0.40[a]	7.22 ± 0.43[a]	-2.22[a]	12.54 ± 0.46[a]	12.20 ± 0.64[a]	-2.79[a]	15.36 ± 0.69[a]	15.46 ± 1.01[a]	0.65[a]
	Fraction 5	5.74 ± 0.43[b]	7.18 ± 0.50[a]	20.06[b]	6.92 ± 0.53[b]	12.10 ± 0.54[a]	42.81[b]	8.20 ± 0.56[b]	15.94 ± 0.98[a]	48.56[b]
	Fraction 6	7.06 ± 0.47[a]	7.14 ± 0.48[a]	1.12[a]	12.10 ± 0.47[a]	12.06 ± 0.99[a]	-0.33[a]	15.18 ± 0.74[a]	15.86 ± 0.87[a]	4.29[a]
	Fraction 7	7.50 ± 0.33[a]	7.14 ± 0.34[a]	-5.04[a]	12.56 ± 0.44[a]	12.18 ± 0.61[a]	-3.12[a]	15.26 ± 0.69[a]	16.12 ± 0.90[a]	5.33[a]
	Fraction 8	7.04 ± 0.48[a]	7.14 ± 0.39[a]	1.40[a]	12.04 ± 0.48[a]	12.06 ± 0.99[a]	0.17[a]	15.22 ± 0.77[a]	15.52 ± 0.91[a]	1.93[a]

4.8. Feeding Bioassay using *A. galanga* spots from Thin Layer Chromatography (TLC)

Table 7 shows the mean weight of paper disc treated with TLC spots of *A. galanga* consumed by *C. gestroi, C. curvignathus* and *M. carbonarius* and percentage of antifeedant activity in dual choice bioassay after 3 days exposure.

Spot 4 of *A. galanga* essential oil that was tested in this study was antifeedant to *C. gestroi, C. curvignathus* and *M. carbonarius*. Regardless of time there was no significant antifeedant activity in other spots of *A. galanga* essential oil against *C. gestroi*. Spot 4 of *A. galanga* essential oil showed the same antifeedant activity to *C. curvignathus* and *M. carbonarius.* After 24 hours of exposure, the highest percentage of antifeedant (PA) values for this spot 4 was 54.01%. The same tendency was observed with spot 4 against *C. curvignathus* and *M. carbonarius*: the PA values with this spot 4 were 52.86% and 55.17%, respectively. Similar results were observed for 48 and 72 hours exposure.

Table 7. Mean weight of paper disc treated with *A. galanga* Thin Layer Chromatography spots consumed by *C. gestroi*, *C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite Species	Spot	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	Spot 1	7.31 ± 0.56[a]	7.18 ± 0.56[a]	-1.81[a]	12.26 ± 0.51[a]	12.12 ± 0.58[a]	-1.16[a]	15.50 ± 0.80[a]	15.79 ± 0.78[a]	1.84[a]
	Spot 2	7.11 ± 0.48[a]	6.91 ± 0.53[a]	-2.89[a]	12.02 ± 0.55[a]	11.87 ± 0.61[a]	-1.26[a]	15.53 ± 0.78[a]	16.05 ± 0.85[a]	3.24[a]
	Spot 3	7.20 ± 0.67[a]	7.24 ± 0.50[a]	0.55[a]	12.43 ± 0.64[a]	12.22 ± 0.64[a]	-1.72[a]	15.53 ± 0.77[a]	15.69 ± 0.82[a]	1.02[a]
	Spot 4	3.21 ± 0.44[b]	6.98 ± 0.27[a]	54.01[b]	4.99 ± 0.23[b]	12.29 ± 0.59[a]	59.40[b]	8.23 ± 0.61[b]	15.73 ± 1.02[a]	47.68[b]
C. curvignathus	Spot 1	7.50 ± 0.47[a]	7.12 ± 0.61[a]	-4.07[a]	12.32 ± 0.33[a]	12.08 ± 0.54[a]	-1.99[a]	15.28 ± 0.79[a]	15.70 ± 0.85[a]	2.68[a]
	Spot 2	7.10 ± 0.43[a]	7.10 ± 0.53[a]	0.00[a]	12.04 ± 0.60[a]	11.96 ± 0.81[a]	0.67[a]	15.34 ± 0.71[a]	16.00 ± 0.87[a]	4.13[a]
	Spot 3	7.32 ± 0.55[a]	7.20 ± 0.58[a]	-1.67[a]	12.54 ± 0.43[a]	12.22 ± 0.60[a]	-2.62[a]	15.42 ± 0.66[a]	15.38 ± 1.05[a]	-0.26[a]
	Spot 4	3.30 ± 0.51[b]	7.00 ± 0.32[a]	52.86[b]	4.98 ± 0.26[b]	12.20 ± 0.42[a]	59.18[b]	8.26 ± 0.58[b]	15.78 ± 1.05[a]	47.66[b]
M. carbonarius	Spot 1	7.12 ± 0.62[a]	7.24 ± 0.55[a]	1.66[a]	12.20 ± 0.68[a]	12.16 ± 0.66[a]	-0.33[a]	15.72 ± 0.83[a]	15.88 ± 0.79[a]	1.01[a]
	Spot 2	7.10 ± 0.58[a]	6.72 ± 0.51[a]	-5.65[a]	12.00 ± 0.57[a]	11.78 ± 0.40[a]	-1.87[a]	15.72 ± 0.88[a]	16.10 ± 0.93[a]	2.36[a]
	Spot 3	7.08 ± 0.81[a]	7.28 ± 0.48[a]	2.75[a]	12.32 ± 0.85[a]	12.22 ± 0.75[a]	-0.82[a]	15.64 ± 0.92[a]	15.96 ± 0.46[a]	2.01[a]
	Spot 4	3.12 ± 0.41[b]	6.96 ± 0.25[a]	55.17[b]	5.00 ± 0.23[b]	12.38 ± 0.76[a]	59.61[b]	8.20 ± 0.71[b]	15.68 ± 1.11[a]	47.70[b]

Means with the same letter are not significantly different between groups. ANOVA ($P>0.05$).
Means with different letter are significantly different between groups. ANOVA ($P\leq0.05$).

4.9. Feeding Bioassay using *C. indicum* spots from Thin Layer Chromatography (TLC)

Table 8 shows the mean weight of paper disc treated with TLC spots of *C. indicum* consumed by *C. gestroi, C. curvignathus* and *M. carbonarius* and percentage of antifeedant activity in dual choice bioassay after 3 days exposure.

Spot 2 of *C. indicum* essential oil that was tested in this study was antifeedant to *C. gestroi, C. curvignathus* and *M. carbonarius*. Regardless of time there was no significant antifeedant activity in other spots of *C. indicum* essential oil against *C. gestroi*. Spot 2 of *C. indicum* essential oil showed the same antifeedant activity to *C. curvignathus* and *M. carbonarius.* After 24 hours of exposure the highest percentage of antifeedant (PA) values for this spot 2 was 21.99%. The same tendency was observed with spot 2 against *C. curvignathus* and *M. carbonarius*: the PA values with this spot 2 were 21.41% and 22.56%, respectively. Similar results were observed for 48 and 72 hours exposure.

Table 8. Mean weight of paper disc treated with *C. indicum* Thin Layer Chromatography spots consumed by ten *C. gestroi*, *C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite Species	Spot	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	Spot 1	7.17 ± 0.42^{a}	7.12 ± 0.34^{a}	-0.70^{a}	12.41 ± 0.59^{a}	12.22 ± 0.61^{a}	-1.55^{a}	15.30 ± 0.79^{a}	15.89 ± 0.78^{a}	3.71^{a}
	Spot 2	5.57 ± 0.31^{b}	7.14 ± 0.29^{a}	21.99^{b}	6.75 ± 0.44^{b}	12.17 ± 0.54^{a}	44.54^{b}	8.21 ± 0.70^{b}	15.72 ± 1.10^{a}	47.77^{b}
	Spot 3	7.10 ± 0.30^{a}	7.07 ± 0.25^{a}	-0.42^{a}	11.88 ± 0.66^{a}	12.01 ± 0.71^{a}	1.08^{a}	15.50 ± 0.80^{a}	15.79 ± 0.78^{a}	1.84^{a}
C. curvignathus	Spot 1	7.30 ± 0.34^{a}	7.18 ± 0.33^{a}	-1.67^{a}	12.48 ± 0.28^{a}	12.20 ± 0.60^{a}	-2.30^{a}	15.20 ± 0.72^{a}	15.60 ± 0.98^{a}	2.56^{a}
	Spot 2	5.58 ± 0.40^{b}	7.10 ± 0.34^{a}	21.41^{b}	6.76 ± 0.48^{b}	12.22 ± 0.68^{a}	44.68^{b}	8.22 ± 0.61^{b}	15.84 ± 1.06^{a}	48.10^{b}
	Spot 3	7.06 ± 0.30^{a}	7.16 ± 0.22^{a}	1.40^{a}	12.00 ± 0.51^{a}	12.04 ± 0.92^{a}	0.33^{a}	15.28 ± 0.79^{a}	15.70 ± 0.85^{a}	2.68^{a}
M. carbonarius	Spot 1	7.04 ± 0.50^{a}	7.06 ± 0.37^{a}	0.28^{a}	12.34 ± 0.84^{a}	12.24 ± 0.71^{a}	-0.82^{a}	15.40 ± 0.93^{a}	16.18 ± 0.45^{a}	4.82^{a}
	Spot 2	5.56 ± 0.24^{b}	7.18 ± 0.26^{a}	22.56^{b}	6.74 ± 0.44^{b}	12.12 ± 0.43^{a}	44.39^{b}	8.20 ± 0.85^{b}	15.60 ± 1.24^{a}	47.44^{b}
	Spot 3	7.14 ± 0.34^{a}	6.98 ± 0.26^{a}	-2.29^{a}	11.76 ± 0.82^{a}	11.98 ± 0.54^{a}	1.84^{a}	15.72 ± 0.83^{a}	15.88 ± 0.79^{a}	1.01^{a}

Means with the same letter are not significantly different between groups. ANOVA ($P>0.05$).
Means with different letter are significantly different between groups. ANOVA ($P\leq 0.05$).

4.10. Feeding Bioassay using Synthetic Compound, 1,8-cineol

Table 9 shows the percentage of antifeeding activity of the synthetic compound, 1,8-cineol against *C. gestroi, C. curvignathus and M. carbonarius*. Regardless of time there was no significant antifeedant activity in 100 ppm of 1,8-cineol against *C. gestroi*. 1,8-cineol showed the same antifeedant activity to *C. curvignathus* and *M. carbonarius.* As shown in Table 9, after 24 hours of exposure the highest percentage of antifeeding activity (PA) of 61.38% was obtained in the 500ppm of 1,8-cineol against *C. curvignathus* among the termites examined. Next is *C. gestroi* with the PA value of 61.07% and followed by *M. carbonarius* with the PA value of 60.74%. Similar results were observed for 48 and 72 hours exposure.

Table 9. Mean weight of paper disc treated with synthetic compound, 1, 8-cineol consumed by *C. gestroi*, *C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite species	Concentration	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	100 ppm	5.05 ± 0.25^{a}	4.98 ± 0.24^{a}	-1.41^{a}	10.06 ± 0.37^{a}	9.41 ± 1.57^{a}	-6.91^{a}	15.06 ± 0.56^{a}	14.88 ± 0.77^{a}	-1.21^{a}
	200 ppm	2.53 ± 0.35^{b}	4.87 ± 0.40^{a}	48.05^{b}	4.63 ± 0.91^{b}	9.94 ± 0.50^{a}	53.42^{b}	7.28 ± 0.64^{b}	14.92 ± 0.76^{a}	51.21^{b}
	500 ppm	1.90 ± 0.25^{c}	4.88 ± 0.24^{a}	61.07^{c}	3.82 ± 0.43^{c}	9.88 ± 0.49^{a}	61.34^{c}	5.73 ± 0.67^{c}	14.65 ± 0.74^{a}	61.26^{c}
	1000 ppm	1.99 ± 0.23^{c}	4.84 ± 0.22^{a}	58.88^{c}	3.80 ± 0.53^{c}	9.75 ± 0.46^{a}	61.03^{c}	5.84 ± 0.64^{c}	14.59 ± 0.68^{a}	59.97^{c}
C. curvignathus	100 ppm	4.98 ± 0.32^{a}	5.02 ± 0.22^{a}	0.80^{a}	10.02 ± 0.42^{a}	9.96 ± 0.52^{a}	-0.60^{a}	15.02 ± 0.64^{a}	14.96 ± 0.77^{a}	-0.40^{a}
	200 ppm	5.02 ± 0.19^{a}	5.14 ± 0.21^{a}	2.33^{a}	10.12 ± 0.35^{a}	10.22 ± 0.44^{a}	0.98^{a}	15.12 ± 0.53^{a}	15.34 ± 0.67^{a}	1.43^{a}
	500 ppm	1.90 ± 0.29^{c}	4.92 ± 0.28^{a}	61.38^{c}	3.82 ± 0.52^{c}	9.86 ± 0.62^{a}	61.26^{c}	5.74 ± 0.78^{c}	14.78 ± 0.90^{a}	61.16^{c}
	1000 ppm	2.08 ± 0.28^{c}	4.76 ± 0.18^{a}	56.30^{c}	4.04 ± 0.49^{c}	9.56 ± 0.40^{a}	57.74^{c}	6.04 ± 0.74^{c}	14.32 ± 0.58^{a}	57.82^{c}
M. carbonarius	100 ppm	5.02 ± 0.19^{a}	4.94 ± 0.27^{a}	-1.62^{a}	10.12 ± 0.35^{a}	8.86 ± 2.12^{a}	-14.22^{a}	15.12 ± 0.53^{a}	14.80 ± 0.85^{a}	-2.16^{a}
	200 ppm	5.08 ± 0.32^{a}	4.60 ± 0.35^{a}	-10.43^{a}	10.00 ± 0.42^{a}	9.96 ± 0.52^{a}	-0.40^{a}	15.00 ± 0.64^{a}	14.96 ± 0.77^{a}	-0.27^{a}
	500 ppm	1.90 ± 0.22^{c}	4.84 ± 0.23^{a}	60.74^{c}	3.82 ± 0.40^{c}	9.90 ± 0.38^{a}	61.41^{c}	5.72 ± 0.62^{c}	14.52 ± 0.62^{a}	60.61^{c}
	1000 ppm	1.97 ± 0.16^{c}	4.92 ± 0.24^{a}	59.96^{c}	3.56 ± 0.50^{c}	9.94 ± 0.48^{a}	64.19^{c}	5.64 ± 0.52^{c}	14.86 ± 0.72^{a}	62.05^{c}

Means with the same letter are not significantly different between groups. ANOVA (P>0.05).
Means with different letter are significantly different between groups. ANOVA (P≤0.05).

4.11. Feeding Bioassay using Synthetic Compound, Farnesene

Table 9 shows the percentage of antifeeding activity of synthetic compound, farnesene against *C. gestroi, C. curvignathus and M. carbonarius*. Regardless of time there was no significant antifeedant activity in 100 and 200 ppm of farnesene against *C. gestroi*. Farnesene showed similar antifeedant activity to *C. curvignathus.* On the other hand, 100, 200 and 500 ppm of farnesene were not antifeedant to *M. carbonarius.* As shown in Table 10, after 24 hours of exposure the highest percentage of antifeeding activity (PA) of 59.25% was obtained in the 1000ppm of 1,8-cineol against *C. curvignathus* among the termites examined. Next is *C. gestroi* with PA value of 58.05% and followed by *M. carbonarius* with PA of 57.20%. Similar results were observed for 48 and 72 hours exposure.

Table 10. Mean weight of paper disc treated with synthetic compound, farnesene consumed by *C. gestroi, C. curvignathus* and *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite species	Concentration	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	100 ppm	4.98 ± 0.31^{a}	5.09 ± 0.21^{a}	2.16^{a}	9.88 ± 0.46^{a}	10.21 ± 0.38^{a}	3.23^{a}	14.99 ± 0.81^{a}	15.28 ± 0.59^{a}	1.90^{a}
	200 ppm	4.97 ± 0.24^{a}	5.28 ± 0.24^{a}	5.87^{a}	10.05 ± 0.38^{a}	10.49 ± 0.40^{a}	4.19^{a}	15.03 ± 0.53^{a}	15.68 ± 0.60^{a}	4.15^{a}
	500 ppm	2.41 ± 1.25^{b}	5.20 ± 0.26^{a}	53.65^{b}	4.09 ± 0.42^{b}	10.52 ± 0.48^{a}	61.12^{b}	6.13 ± 0.64^{b}	15.79 ± 0.73^{a}	61.18^{b}
	1000 ppm	2.19 ± 0.26^{b}	5.22 ± 0.29^{a}	58.05^{b}	4.28 ± 0.41^{b}	10.51 ± 0.52^{a}	59.28^{b}	6.29 ± 0.52^{b}	15.79 ± 0.73^{a}	60.16^{b}
C. curvignathus	100 ppm	5.20 ± 0.22^{a}	5.04 ± 0.26^{a}	-3.17^{a}	10.26 ± 0.26^{a}	10.08 ± 0.46^{a}	-1.79^{a}	15.52 ± 0.60^{a}	15.10 ± 0.72^{a}	-2.78^{a}
	200 ppm	4.76 ± 0.22^{a}	5.30 ± 0.28^{a}	10.19^{a}	9.50 ± 0.21^{a}	10.58 ± 0.42^{a}	10.21^{a}	14.46 ± 0.65^{a}	15.70 ± 0.64^{a}	7.90^{a}
	500 ppm	2.18 ± 0.48^{b}	5.26 ± 0.23^{a}	58.56^{b}	4.02 ± 0.47^{b}	10.72 ± 0.26^{a}	65.62^{b}	6.00 ± 0.73^{b}	16.12 ± 0.38^{a}	62.78^{b}
	1000 ppm	2.16 ± 0.36^{b}	5.30 ± 0.29^{a}	59.25^{b}	4.18 ± 0.46^{b}	10.70 ± 0.41^{a}	60.93^{b}	6.20 ± 0.35^{b}	16.12 ± 0.38^{a}	61.34^{b}
M. carbonarius	100 ppm	4.76 ± 0.22^{a}	5.14 ± 0.15^{a}	7.39^{a}	9.50 ± 0.21^{a}	10.34 ± 0.27^{a}	8.12^{a}	14.46 ± 0.65^{a}	15.46 ± 0.41^{a}	6.47^{a}
	200 ppm	5.20 ± 0.22^{a}	5.04 ± 0.26^{a}	-3.17^{a}	10.26 ± 0.26^{a}	10.08 ± 0.46^{a}	-1.79^{a}	15.52 ± 0.60^{a}	15.10 ± 0.72^{a}	-2.78^{a}
	500 ppm	5.06 ± 0.30^{a}	5.30 ± 0.28^{a}	4.53^{a}	10.30 ± 0.31^{a}	10.58 ± 0.42^{a}	2.65^{a}	15.36 ± 0.46^{a}	15.70 ± 0.64^{a}	2.17^{a}
	1000 ppm	2.20 ± 0.16^{b}	5.14 ± 0.30^{a}	57.20^{b}	4.38 ± 0.36^{b}	10.32 ± 0.58^{a}	57.56^{b}	6.58 ± 0.52^{b}	15.46 ± 0.88^{a}	57.44^{b}

Means with the same letter are not significantly different between groups. ANOVA ($P>0.05$).
Means with different letter are significantly different between groups. ANOVA ($P\leq0.05$).

4.12. Feeding Bioassay using Commercial Termiticide, Chlorpyrifos

Tables 11a, b and c show the percentage of antifeeding activity of commercial termiticide, chlorpyrifos against *C. gestroi, C. curvignathus and M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period. Regardless of the concentration there was a significant antifeedant activity in chlorpyrifos against *C. gestroi* at 24 hours of exposure (Table 11a). After 48 hours and 72 hours of exposure the antifeedant activity increased with the increased concentrations in *C. gestroi*. Similarly for the *C. curvignathus* and *M. carbonarius* the increment of the antifeedant activity is shown with the increased of concentrations after 24, 48 and 72 hours of exposure. As shown in Table 11b, after 24 hours of exposure the highest percentage of antifeeding activity (PA) of 98.28% was obtained in the 1000 ppm of chlorpyrifos in *C. curvignathus* among the termites examined.

Table 11a. Mean weight of paper disc treated with commercial termiticide, chlorpyrifos consumed by *C. gestroi* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite species	Concentration	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. gestroi	100 ppm	4.25 ± 0.25^{a}	4.88 ± 0.24^{a}	12.91^{a}	8.16 ± 0.37^{a}	9.32 ± 1.57^{a}	12.45^{a}	14.16 ± 0.56^{a}	14.77 ± 0.77^{a}	4.13^{a}
	200 ppm	2.53 ± 0.35^{b}	4.77 ± 0.40^{a}	46.96^{b}	4.62 ± 0.91^{b}	9.84 ± 0.50^{a}	53.05^{b}	9.28 ± 0.64^{b}	14.82 ± 0.76^{a}	37.38^{b}
	500 ppm	1.92 ± 0.25^{c}	4.78 ± 0.24^{a}	59.83^{c}	3.81 ± 0.43^{c}	9.78 ± 0.49^{a}	61.04^{c}	5.74 ± 0.67^{c}	14.55 ± 0.74^{a}	60.55^{c}
	1000 ppm	0.97 ± 0.23^{d}	4.74 ± 0.22^{a}	79.54^{d}	1.82 ± 0.53^{d}	9.65 ± 0.46^{a}	81.14^{d}	2.83 ± 0.64^{d}	14.49 ± 0.68^{a}	80.47^{d}

Means with the same letter are not significantly different between groups. ANOVA ($P>0.05$).
Means with different letter are significantly different between groups. ANOVA ($P\leq0.05$).

Table 11b. Mean weight of paper disc treated with commercial termiticide, chlorpyrifos consumed by *C. curvignathus* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite species	Concentration	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
C. curvignathus	100 ppm	4.94 ± 0.32^{a}	5.02 ± 0.22^{a}	1.59^{a}	8.04 ± 0.42^{a}	9.76 ± 0.52^{a}	17.62^{a}	14.01 ± 0.64^{a}	14.86 ± 0.77^{a}	5.72^{a}
	200 ppm	3.02 ± 0.19^{b}	5.04 ± 0.21^{a}	40.08^{b}	7.13 ± 0.35^{b}	10.02 ± 0.44^{a}	28.84^{b}	10.12 ± 0.53^{b}	15.24 ± 0.67^{a}	33.60^{b}
	500 ppm	1.92 ± 0.29^{c}	4.82 ± 0.28^{a}	60.17^{c}	3.80 ± 0.52^{c}	9.76 ± 0.62^{a}	61.07^{c}	5.72 ± 0.78^{c}	14.68 ± 0.90^{a}	61.04^{c}
	1000 ppm	0.08 ± 0.28^{d}	4.66 ± 0.18^{a}	98.28^{d}	1.03 ± 0.49^{d}	9.46 ± 0.40^{a}	89.11^{d}	2.04 ± 0.74^{d}	14.22 ± 0.58^{a}	85.65^{d}

Means with the same letter are not significantly different between groups. ANOVA ($P>0.05$).
Means with different letter are significantly different between groups. ANOVA ($P\leq0.05$).

Table 11c. Mean weight of paper disc treated with commercial termiticide, chlorpyrifos consumed by *M. carbonarius* in dual choice assay for 24, 48 and 72 hours exposure period.

Termite species	Concentration	Mean consumption of paper disc (mg)								
		24 hours			48 hours			72 hours		
		Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
M. carbonarius	100 ppm	5.01 ± 0.19^{a}	4.84 ± 0.27^{a}	-3.51^{a}	8.13 ± 0.35^{a}	8.76 ± 2.12^{a}	7.19^{a}	14.13 ± 0.53^{a}	14.70 ± 0.85^{a}	3.88^{a}
	200 ppm	4.08 ± 0.32^{b}	4.50 ± 0.35^{a}	10.37^{b}	7.02 ± 0.42^{b}	9.86 ± 0.52^{a}	28.80^{b}	8.01 ± 0.64^{b}	14.86 ± 0.77^{a}	46.10^{b}
	500 ppm	1.91 ± 0.22^{c}	4.74 ± 0.23^{a}	59.70^{c}	3.81 ± 0.40^{c}	9.80 ± 0.38^{a}	61.12^{c}	5.73 ± 0.62^{c}	14.42 ± 0.62^{a}	60.26^{c}
	1000 ppm	0.96 ± 0.16^{d}	4.82 ± 0.24^{a}	80.08^{d}	1.55 ± 0.50^{d}	9.75 ± 0.48^{a}	84.10^{d}	2.62 ± 0.52^{d}	14.73 ± 0.72^{a}	82.21^{d}

Means with the same letter are not significantly different between groups. ANOVA ($P>0.05$).
Means with different letter are significantly different between groups. ANOVA ($P\leq0.05$).

4.13. Determination of 50 % Effective Dose (ED_{50})

Table 12 shows the 50 % Effective Dose (ED_{50}) of 2 essential oils, 2 synthetic compounds and a chemical termicide. The essential oils extracted from the rhizomes of *A. galanga* and the leaves of *C. indicum* were found to cause antifeedant effect to *C. gestroi, C. curvignathus* and *M. carbonarius* adults (Table 11). On the basis of ED_{50} values, *C. gestroi* was more susceptible towards the essential oils extracted from *C. indicum* (ED_{50}= 4677 mg/kg) than the oils extracted from *A. galanga* (ED_{50}= 6607 mg/kg). Similarly, for *C. curvignathus* and *M. carbonarius,* essential oil from *C. indicum* (3467 and 1905 mg/kg) were more effective compared to *A. galanga* (4365 and 2512 mg/kg). In contrast, *C. gestroi* and *M. carbonarius* were more susceptible towards the synthetic compound, 1, 8-cineol (1202 and 259 mg/kg) than the synthetic compound, farnesene (1698 and 2455 mg/kg). However *C. curvignathus* was more susceptible towards farnesene compared to 1,8-cineol. On the other hand, bioassay with chemical termiticides, chlorpyrifos shows that *C. gestroi, C. curvignathus* and *M. carbonarius* were more susceptible to chlorpyrifos (504, 339 and 174 mg/kg) compared to the essential oils and the synthetic compounds.

Table 12: The 50% effective dose (ED_{50}) and regression line of essential oils, synthetic compounds and chemical termicide against three species of termites.

Termite	Essential Oils (mg/kg)				Synthetic Compound (mg/kg)				Termiticide (mg/kg)	
	A. galanga		*C. indicum*		1,8-cineol		Farnesene		Chlorpyrifos	
	ED50	Regression line	ED50	Regression line	ED50	Regression line	ED50	Regression line	ED50	Regression line
C. gestroi	6,607 (2027-11187)	Y=1.87x-2.139 R^2=0.803	4677 (1937-7418)	Y=2.103x-2.710 R^2=0.877	1202 (253-2152)	Y=1.732x-0.342 R^2=0.720	1698 (620-2776)	Y=2.015x-1.512 R^2=0.859	504 (440-527)	Y=0.727x-306.8 R^2=0.931
C. curvignathus	4365 (2493-6237)	Y=2.71x-4.871 R^2=0.909	3467 (2078-4857)	Y=2.88x-5.195 R^2=0.850	955 (491-1419)	Y=2.443x-2.292 R^2=0.915	933 (356-1511)	Y=2.044x-1.070 R^2=0.898	339 (299-358)	Y=1.105x-317.1 R^2=0.959
M. carbonarius	2512 (1087-3937)	Y=2.18x-2.411 R^2=0.831	1905 (1065-2746)	Y=2.678x-3.794 R^2=0.851	259 (199-716)	Y=2.208x-0.882 R^2=0.925	2455 (69-4841)	Y=1.533x-0.202 R^2=0.620	174 (158-189)	Y=2.192x-330.8 R^2=0.999

4.14. Identification of *A. galanga* components using GCMS

Figure 1 shows the chromatogram of *A. galanga* essential oil. The volatile constituents identified in the rhizome oils of *A. galanga* are given in Table 12. The results of this research showed that the rhizomes had 0.32 % yield from the fresh rhizomes.

A total of 21 compounds were identified in the oil, comprising 87.8% of the total oil. The rhizome oil gave 7 monoterpenes (9.2%), 11 monoterpenoids (74.5%), one sesquiterpenes (3.2%) and two sesquiterpenoids (0.9%). Referring to the chromatogram, the highest peak was at retention time 17.021 minutes. 1, 8-cineole was identified as the most abundant compound with 61.9%.

1, 8-cineole was identified as the most abundant compound with area of 61.9% which is greater than the 40.5% reported in the oil of the same species obtained from Tenom, Sabah, Malaysia (Ibrahim et al., 2004). The high content of 1, 8-cineole may be due to the use of fresh rhizomes for extraction and the essential oil were analysed immediately with GC and GCMS. This study shows that *A. galanga* is a good source of 1, 8-cineole.

Figure 1. Chromatogram of *A. galanga* essential oil

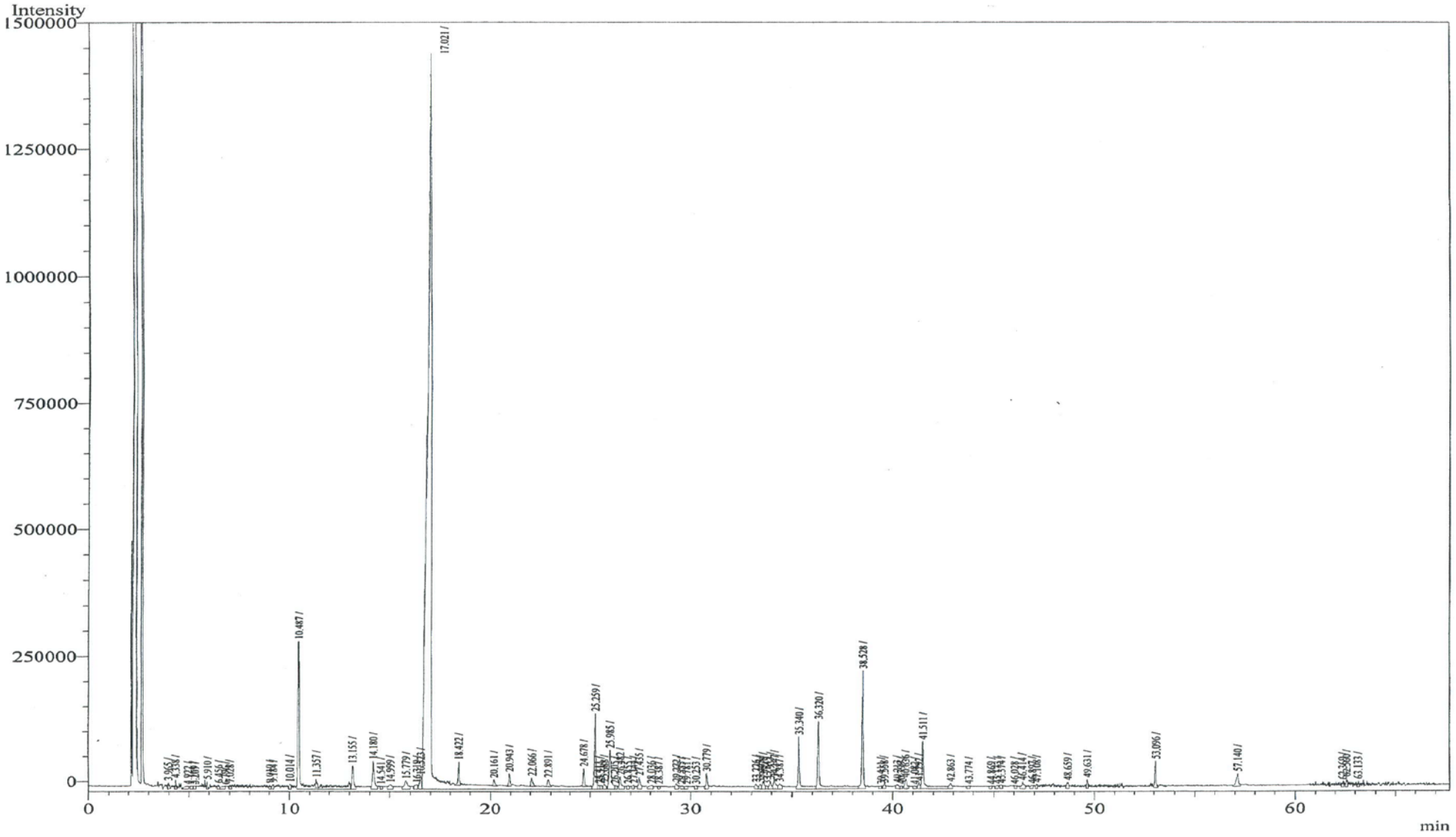


Table 13. Chemical composition of *A. galanga* essential oil.

Chemical Constituents	R.T	R.I.		Percentage (%)		Method of Identification	MW
		A. galanga	Reference	*A. galanga*	Jantan *et al.*		
α-pinene	10.487	926	939	5.7	2.0	MS	$C_{10}H_{16}$
Camphene	11.357	939	954	0.2	0.1	MS	$C_{10}H_{16}$
β-pinene	13.155	966	979	0.9	0.6	MS	$C_{10}H_{16}$
β-myrcene	14.180	982	991	1.0	0.1	MS, RI	$C_{10}H_{16}$
α-terpinene	15.779	1004	1017	0.6	t	MS	$C_{10}H_{16}$
1,8-cineole	17.021	1027	1031	61.9	40.5	MS, RI	$C_{10}H_{18}O$
ɤ -terpinene	18.422	1050	1060	0.6	0.3	MS, RI	$C_{10}H_{16}$
α-terpinolene	20.161	1078	1089	0.2	0.1	MS	$C_{10}H_{16}$
Linaloal	20.943	1091	1097	0.3	0.1	MS, RI	$C_{10}H_{18}O$
(E)-p-mentha-2,8-dien-1-ol	22.066	1110	1123	0.3	0.1	MS	$C_{10}H_{16}O$
cis-p-mentha-2,8-dien-1-ol	22.891	1126	1138	0.2	0.1	MS	$C_{10}H_{16}O$
4-terpineol	25.259	1170	1177	2.1	1.3	MS, RI	$C_{10}H_{18}O$
α-terpineol	25.985	1184	1189	1.6	1.1	MS, RI	$C_{10}H_{18}O$
trans-carveol	27.435	1212	1217	0.4	-	MS, RI	$C_{10}H_{16}O$
barnyl acetate	30.779	1280	1289	0.5	0.1	MS, RI	$C_{12}H_{20}O_2$
lavandulyl acetate	35.340	1377	1290	1.8	-	MS	$C_{12}H_{20}O_2$
methyl eugenol	36.320	1399	1404	3.2	1.5	MS, RI	$C_{11}H_{14}O_2$
trans-beta-farnesene	38.528	1451	1443	3.2	3.2	MS	$C_{15}H_{24}$
eugenol acetate	41.511	1522	1523	2.2	-	MS, RI	$C_{12}H_{14}O_3$
Farnesal	49.631	1736	-	0.2	-	MS	$C_{15}H_{24}O$
farnesyl acetate	53.096	1834	-	0.7	1.7	MS	$C_{17}H_{28}O_2$

Reference* = Adams 2001; Jantan *et, al.*, 2004.

MS = Mass Spectrometry, W9N11 2011.

RI = Retention Indices.

"-" = not reported

“t” = trace

4.15. Identification of *C. indicum* components using GCMS

Figure 2 shows the chromatogram of *C. indicum* essential oil. The volatile constituents identified from the leaf oil of *C. indicum* are given in Table 14. The results of this research showed that the leaves *C. indicum* had 0.21 % yield of fresh leaves.

A total of 21 compounds were identified in the oil, comprising 65.00% of the total oil. The leaf oil gave 7 monoterpenes (17.87%), 4 monoterpenoids (5.30%), 7 sesquiterpenes (35.87%) and two sesquiterpenoids (3.78%). Referring to the chromatogram, the highest peak was at retention time 39.672 minutes. Germacrene D was identified as most abundant compound with 14.07%.

The *C. indicum* with 14.07% of Germacrene D obtained in this study is greater than 10.60% reported in the oil of the same species obtained from Mt. Mireuk in Korea (Jung, 2009). The high content of Germacrene D may be due to the use of fresh leaves for extraction and the essential oil were analysed immediately with GC and GCMS.

Figure 2. Chromatogram of *C. indicum* essential oil

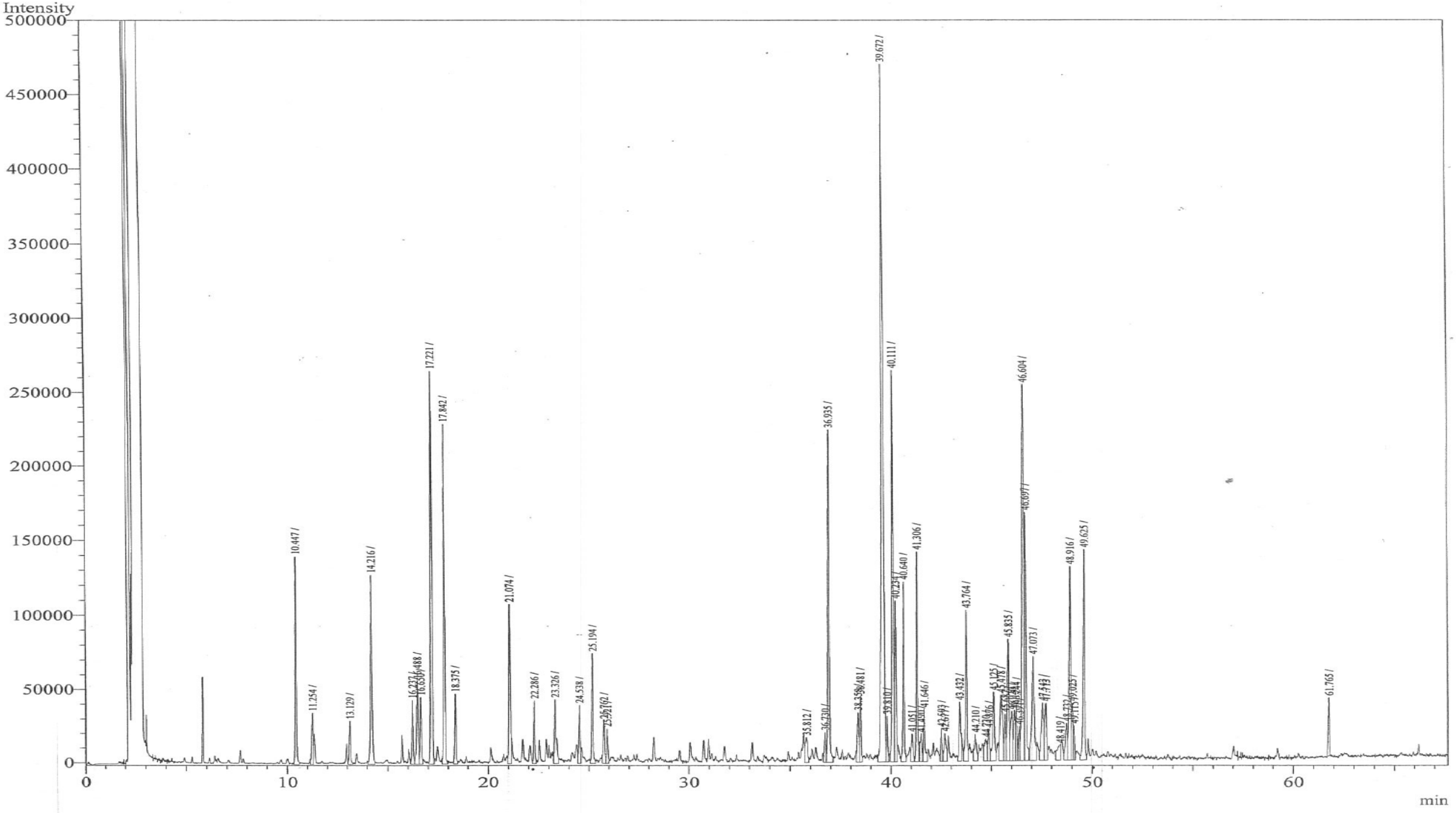


Table 14. Chemical composition of *C. indicum* essential oil.

Chemical Constituents	R.T.	R.I.		Percentage (%)		Method of Identification	MW
		C. Indicum	Reference	*C. Indicum*	Jung, 2009		
1,3-cyclopentadiene, 1,2,5,5-tetramethyl	10.447	925	-	2.55	-	MS	C_9H_{14}
α-pinene	11.254	937	939	0.67	4.40	MS, RI	$C_{10}H_{16}$
1,6-dimethylhepta-1,3,5-triene	14.216	981	-	2.86	-	MS	C_9H_{14}
p-Cymene	16.237	1014	1025	0.82	0.83	MS	$C_{10}H_{14}$
beta-phellandrene	16.488	1018	1030	1.13	1.40	MS	$C_{10}H_{16}$
cis beta-ocimene	17.221	1030	1037	5.46	1.10	MS, RI	$C_{10}H_{16}$
trans beta-ocimene	17.842	1040	1050	4.38	0.03	MS, RI	$C_{10}H_{16}$
Filifolone	21.074	1093	-	2.06	-	MS	$C_{10}H_{14}O$
Chrysanthenone	22.286	1115	1128	0.77	2.30	MS	$C_{10}H_{14}O$
Camphor	23.326	1134	1146	1.11	10.12	MS	$C_{10}H_{16}O$
4-Terpineol	25.194	1169	1177	1.36	3.40	MS, RI	$C_{10}H_{18}O$
Caryophyllene	36.935	1413	1419	4.64	5.10	MS, RI	$C_{15}H_{24}$
Germacrene D	39.672	1478	1485	14.07	10.60	MS, RI	$C_{15}H_{24}$
alpha-curcumene	39.810	1481	1481	0.55	0.21	MS, RI	$C_{15}H_{22}$
trans-alpha-Bergamotene	40.111	1488	1435	5.32	0.16	MS	$C_{15}H_{24}$
E,E-alpha-farnesene	40.234	1491	1506	1.94	0.30	MS	$C_{15}H_{24}$
beta-sesquiphellandrene	40.640	1500	1523	2.14	0.18	MS	$C_{15}H_{24}$
Caryophyllene oxide	43.764	1578	1583	2.18	0.78	MS, RI	$C_{15}H_{24}O$
tau-cadinol	46.244	1644	1640	0.81	3.00	MS, RI	$C_{15}H_{26}O$
beta-panasinsene	46.604	1653	1383	7.21	-	MS	$C_{15}H_{24}$
Mintsulfide	49.625	1736	1741	2.97	-	MS, RI	$C_{15}H_{24}S$

Reference* = Adams, 2001; Jung, 2009.

MS = Mass Spectrometry, W9N11 2011.

RI = Retention Indices.

"-" = not reported

4.16. Identification of positive active compounds from *A. galanga* and *C. indicum* after TLC

The major organic compound found in fraction 4 of *A. galanga* by GCMS was 1, 8-cineol as shown in the result (Figure 3). 1, 8-cineol was resolved as a single peak at retention time of 14.119 minutes. The percentage of area was 99.99 %.

The major organic compound found in fraction 5 of *C. indicum* by GCMS was farnesene as shown in result (Figure 4). Farnesene was resolved as the highest peak at retention time of 38.039 minutes. The percentage of area was 10.50 %. Isomers of farnesene also appeared at different retention time such as 34.824, 35.908, 36.571, and 37.187. The total percentage of area for farnesene was 26.80 %.

Figure 3: Chromatogram of positive active compound from *A. galanga* after TLC

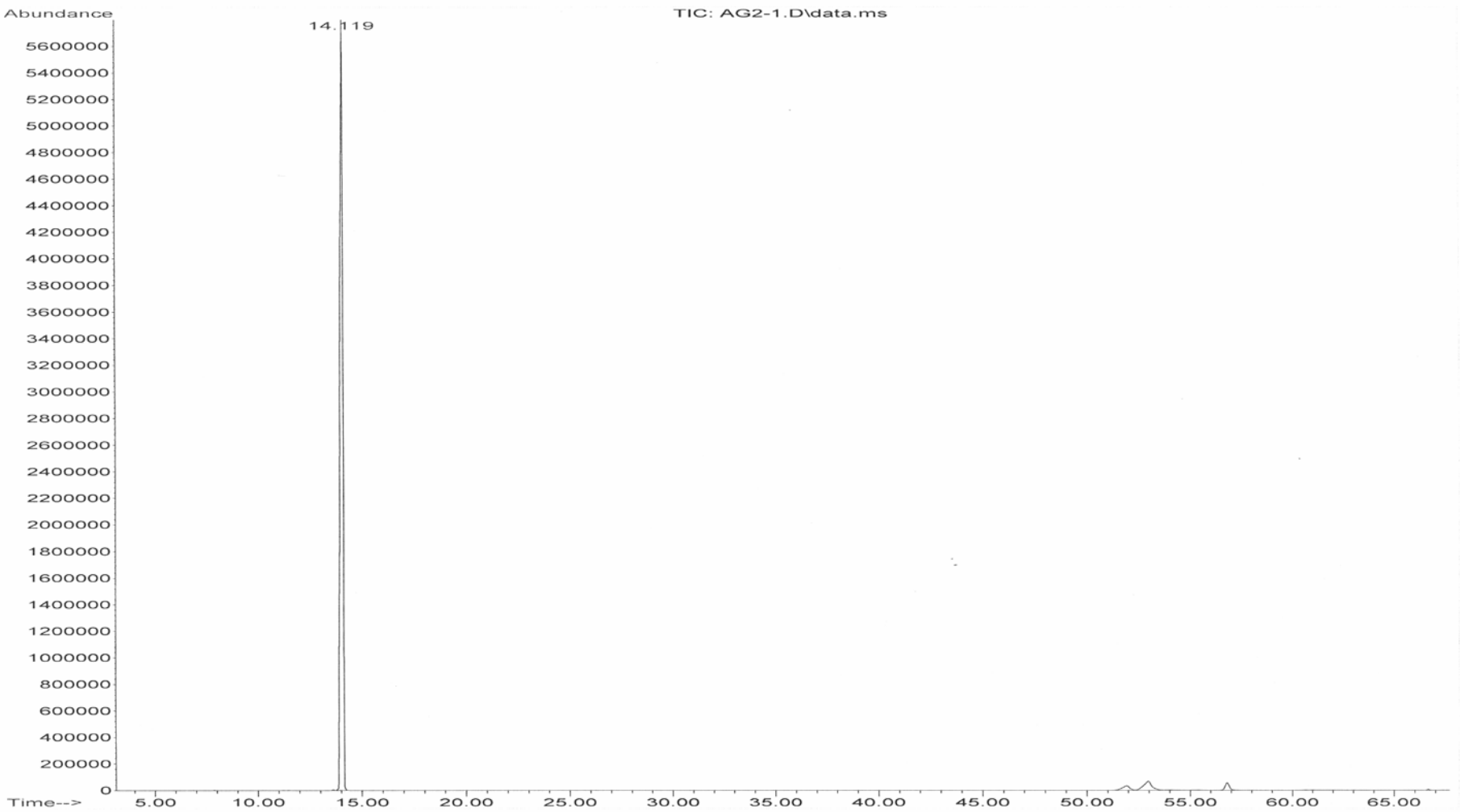
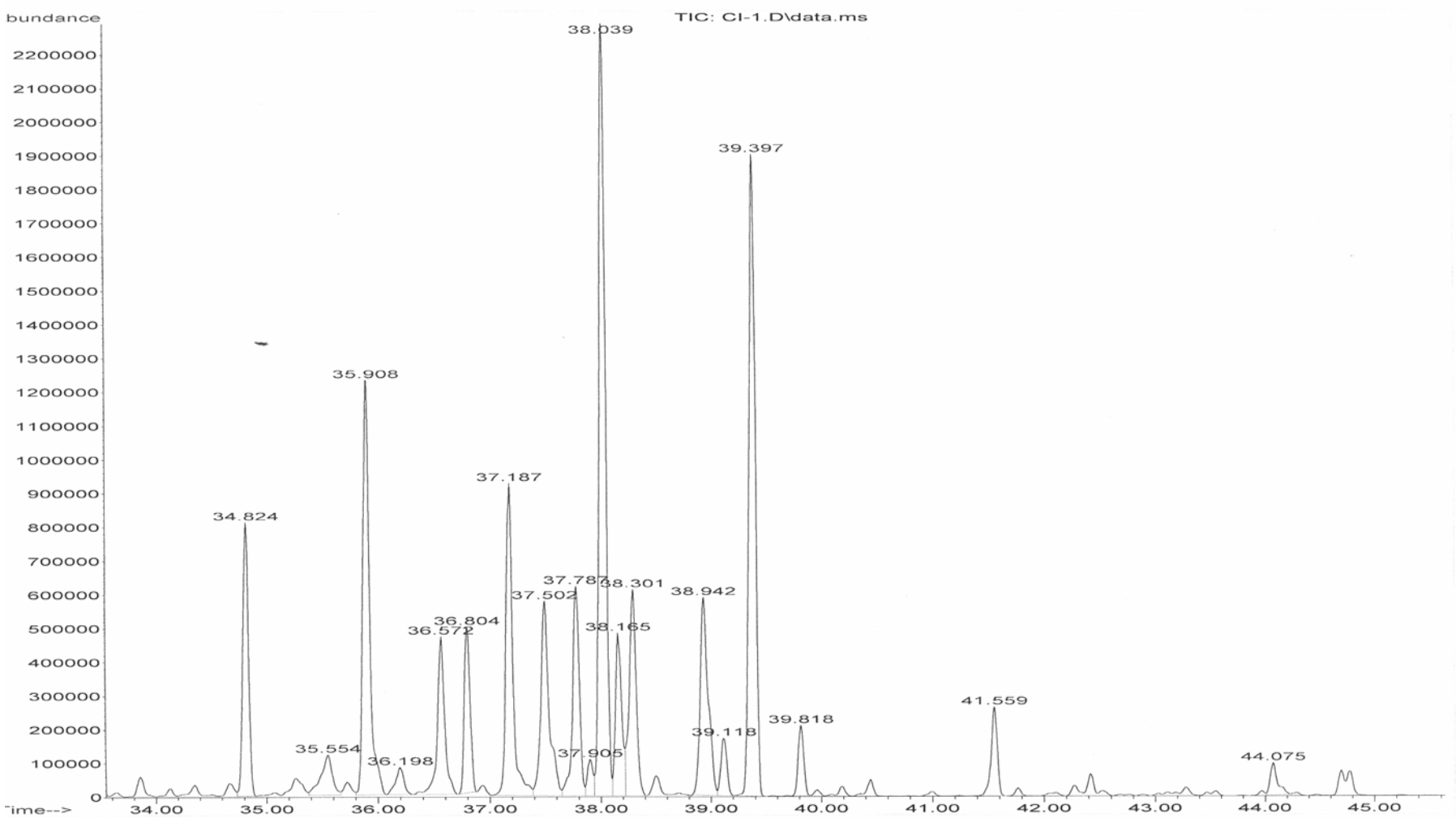


Figure 4: Chromatogram of positive active compound from *C. indicum* after TLC



4.17. Field application of synthetic active compound

Consumption of treated and untreated wood blocks by *C. gestroi* are given in Table 15 and 16. There was a significant difference in the wood consumption of *C. gestroi* for 500 ppm, 1000 ppm, 2000 ppm and 5000 ppm of synthetic 1,8-cineol and farnesene. All of the test concentrations significantly reduced feeding by *C. gestroi* as compared with control in the wood test. The antifeedant effect was more or less concentration dependent. Field application study on wood with 5000 ppm of 1,8-cineol gave maximum antifeedant effect (89.47%) on *C. gestroi* after 14 weeks.

On the other hand, there was no significant difference in the wood consumption by *C. gestroi* towards treated wood with 500 ppm farnesene. However there was a significant difference in the scores for farnesene treated wood at 1000 ppm, 2000 ppm and 5000 ppm. Farnesene provide effective protection and 5000 ppm of farnesene gave maximum antifeedant effect (67.77%) on *C. gestroi* after 14 weeks.

Table 15. Consumption and antifeedant activity of wood blocks treated with 1,8-cineol and farnesene against *C. gestroi* after 14 days placed in field condition.

Concentration	Mean consumption of rubber wood (g)					
	1,8-Cineol			Farnesene		
	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
500ppm	36.60 ± 4.51[a]	42.20 ± 3.19[e]	13.27	41.80 ± 3.19[a]	41.20 ± 3.18[a]	1.46
1000ppm	25.80 ± 3.42[b]	42.20 ± 3.19[e]	38.86	35.20 ± 2.86[b]	41.20 ± 3.18[a]	14.56
2000ppm	16.80 ± 2.86[c]	42.20 ± 3.19[e]	60.19	23.80 ± 2.77[c]	41.20 ± 3.18[a]	42.23
5000ppm	5.00 ± 1.58[d]	42.20 ± 3.19[e]	88.15	15.60 ± 2.70[d]	41.20 ± 3.18[a]	62.14

Means with the same letter are not significantly different between groups ($P>0.05$).
Means with different letter are significantly different between groups ($P\leq 0.05$).

Table 16. Consumption and antifeedant activity of wood blocks treated with 1,8-cineol and farnesene against *C. gestroi* after 16 weeks placed in field condition.

Concentration	Mean consumption of rubber wood (g)					
	1,8-Cineol			Farnesene		
	Treated	Control	Antifeedant activity (%)	Treated	Control	Antifeedant activity (%)
500ppm	259.20 ± 5.41[a]	295.40 ± 5.28[e]	12.25	318.50 ± 6.93[a]	309.00 ± 4.79[a]	-3.07
1000ppm	161.80 ± 4.52[b]	295.40 ± 5.28[e]	45.23	269.40 ± 4.68[b]	309.00 ± 4.79[a]	12.82
2000ppm	87.60 ± 3.76[c]	295.40 ± 5.28[e]	70.35	147.80 ± 3.79[c]	309.00 ± 4.79[a]	52.17
5000ppm	31.10 ± 2.68[d]	295.40 ± 5.28[e]	89.47	99.60 ± 2.67[d]	309.00 ± 4.79[a]	67.77

Means with the same letter are not significantly different between groups ($P>0.05$).
Means with different letter are significantly different between groups ($P\leq 0.05$).

Chapter 5. Discussion

In this study, the methanolic extract of *A. galanga* did not exhibit positive antifeeding effect for *Coptotermes gestroi, C. curvignathus* and *Macrotermes carbonarius* was not significantly different compared to the control. Whereas, the essential oil from *A. galanga* gave positive antifeeding effects and showed significant difference in the antifeedant effects. Hence, the essential oil was further used in antifeeding experiment in this study. Bioassay with different concentrations of essential oil of *A. galanga* showed that 2000 ppm and 5000 ppm exhibit similar antifeedant effect; however, it was not significantly different. This indicates that 2000 ppm can be considered as optimum concentration to provide maximum antifeedant effect. In previous studies, the ethanol rhizome extract of *Alpinia galanga* was toxic against the fruit fly, *Bactrocera dorsalis* at 5,987.05 ppm level (LC_{50}) after 24 hours (Sukhirun et al., 2009).

The methanolic extract of *C. indicum* did not provide positive result and was not significantly different compared to the control. Whereas, the essential oil from *C. indicum* showed significant difference in antifeedant effect *Coptotermes gestroi, C. curvignathus* and *Macrotermes carbonarius* compared to the control. Hence, the essential oil was further used in antifeeding experiment in this study. Bioassay with different concentrations of essential oil of *C. indicum* showed that 2000 ppm and 5000 ppm exhibit similar antifeedant effect; however, it was not significantly different. This indicates that 2000 ppm can be considered as optimum concentration to provide maximum antifeedant effect. However, according to Elango et al., (2012), the highest mortality of the Formosan subterranean termite, *Coptotermes formosanus* was found in the leaf hexane extract of *Aristolochia bracteolate* after 24 h (LD_{50} = 363 ppm).

Essential oils of *A. galanga* and *C. indicum* exhibited antifeedant effects on *C. gestroi, C. curvignathus* and *M. carbonarius*. Therefore, essential oils of *A. galanga* and *C. indicum* can be considered as potential pesticide to control *C. gestroi, C. curvignathus* and *M. carbonarius* due to its antifeedant action. In a previous study by Shunting et al. (2005), essential oils from air-dried and processed flowers of *C. indicum* possessed significant antimicrobial effect.

Previous studies using methanolic extract of ginger, *Zingiber officinale* has shown to possess insecticidal activity (Singh et al., 2005; Oparaeke et al., 2005). Past researchers found that essential oils from various plants are effective against several insect species with varying potencies (Ho et al., 1996; Huang et al., 1999; Tunc¸ et al., 2000; Zhu et al., 2001; Kostyukovsky et al., 2002; Garcia et al., 2005); acting as toxins, growth inhibitors, development disruptors, deterrents or repellents. Essential oil components of *Flourensia* spp. have been reported as having insect antifeedant (Faini et al., 1997), phytotoxic (Mata et al., 2003), antifungal, antialgal and antitermite properties (Tellez et al., 2001). In another study, the orange oil extract (OOE), was known to be toxic to insects in laboratory experiments, in which 68% termites were killed (Raina et al., 2007).

Essential oils of *A. galanga* and *C. indicum* were used for separation using column chromatography due to their positive antifeeding activity. Fraction 4 of *A. galanga* essential oil from column chromatography and fraction 5 of *C. indicum* essential oil from column chromatography showed positive antifeeding effect against all three species of termites investigated. The positive fractions were further separated by using TLC before GCMS analysis.

Analysis of the column chromatography fractions using Gas Chromatography-Mass Spectrometry showed that the active compound that gave positive antifeeding effect

in *A. galanga* was 1, 8-cineol. Whereas, the active compound that gave positive antifeeding effect in *C. indicum* was farnesene.

This study successfully identified 1, 8-cineol from *A. galanga* and farnesene from *C. indicum* as the antifeedant compound which elicited antifeeding behavior in the termites, *C. gestroi*, *C. curvignathus* and *M. carbonarius.* 1, 8-cineol is not a new active compound. It was identified as the marker compound for the genus *Alpinia.* In previous studies 1, 8-cineol was used as an insecticide and insect repellent (Klocke et al., 1987; Sfara et al., 2009), whereas, farnesene was never used (as a single entity) as an insecticide before. Farnesene was used in combination with other chemical as an insecticide. A study by Cui et al., (2012), reported that the commercial pesticide, imidacloprid with farnesene further reduced the numbers of apterous aphids, *Lipaphis erysimi* (Kaltenbach) and *Myzus persicae* (Sulzer) on Chinese cabbage.

This study found that 100 ppm concentration of synthetic compound 1,8-cineol did not provide positive antifeedant activity towards *C. gestroi*, whereas 200 ppm, 500 ppm and 1000 ppm 1,8-cineol showed positive antifeedant activity against *C. gestroi*. This study showed that 1000 ppm synthetic 1, 8-cineol gave maximum antifeedant activity whereas 200 ppm 1, 8-cineol concentration is considered as minimum concentration that can cause antifeedant activity on *C. gestroi*. However, for synthetic farnesene, 100 ppm and 200 ppm did not provide positive antifeedant activity towards *C. gestroi* whereas 500 ppm and 1000 ppm showed positive antifeedant activity from *C. gestroi*. Hence this study shows that 1000 ppm gave maximum antifeedant activity whereas 500 ppm concentration is considered as minimum concentration that can cause antifeedant activity on *C. gestroi*. Similarly, 1000 ppm synthetic 1,8-cineol gave maximum antifeedant activity whereas 500 ppm 1,8-cineol is considered as minimum concentration that can cause antifeedant activity on *C. curvignathus*. This study also shows that 1000 ppm synthetic farnesene gave maximum

antifeedant activity whereas 500 ppm concentration is considered as minimum concentration that can cause antifeedant activity on *C. curvignathus*. 1000 ppm synthetic 1, 8-cineol gave maximum antifeedant activity and 500 ppm synthetic 1, 8-cineol is considered as minimum concentration that can cause antifeedant activity on *M. carbonarius*. In the case of synthetic farnesene, 1000 ppm farnesene gave maximum antifeedant activity and 500 ppm is considered as minimum concentration that can cause antifeedant activity on *M. carbonarius.*

On the basis of ED_{50} values, *C. gestroi* were significantly more susceptible towards the essential oil from *C. indicum* (4677 mg/kg) compared to *A. galanga* (6607 mg/kg). Similarly, *C. curvignathus* and *M. carbonarius* were significantly more susceptible towards the essential oil of *C. indicum* (3467 mg/kg and 1905 mg/kg) compared to that of *A. galanga* (4365 mg/kg and 2512 mg/kg). The susceptibility of *C. gestroi* towards the essential oil from *C. indicum* might be due to the synergistic effect of other compounds with farnesene which cause the antifeedant activity. In contrast, *C. gestroi, C. curvignathus* and *M. carbonarius* were significantly more susceptible towards the commercial termiticide, chlorpyrifos compared to the synthetic compound, 1, 8-cineol and farnesene. *Macrotermes carbonarius* were more susceptible to the essential oils (*A. galanga and C. indicum*), synthetic compounds (1, 8-cineol and Farnesene) and commercial termiticide (chlorpyrifos) compared to *C. gestroi and C. curvignathus*. This is because of *M. carbonarius* were never exposed to any pesticide in their life span since their habitat are in the forest. Whereas, *C. gestroi* and *C. curvignathus* which are found in trees and dead wood, have been treated with pesticides and chemicals.

In the field application study on wood, it was found that 500 ppm, 1000 ppm, 2000 ppm and 5000 ppm of 1, 8-cineol showed positive antifeedant response from *C. gestroi*. This study shows that 5000 ppm of 1, 8-cineol gave maximum antifeeding response and

considered as the concentration that caused maximum antifeedant effect on *C. gestroi*. However, for the synthetic farnesene, 500 ppm did not give positive antifeedant response towards *C. gestroi* whereas 1000 ppm, 2000 ppm and 5000 ppm showed positive antifeedant response from the *C. gestroi*. This study also shows that 5000 ppm gave maximum response and it's considered as the concentration that can cause maximum antifeedant effect on *C. gestroi*. The synthetic compound 1, 8-cineol caused significant protection to wood weight loss against *C. gestroi.* Similarly, the synthetic compound farnesene provided effective protection to the wood weight loss respectively. Therefore, these treatments are effective against biodeterioration of wood by *C. gestroi.* This suggests that though chemical are an effective control against termites but they are harmful to non target organism. Therefore, the search for alternative control measures is essential. These botanicals can be used in combination with chemical pesticide to lower their harmful effect.

The results of this study suggest that 1, 8-cineol and farnesene have the potential to be developed (in a broader scale) as effective pesticide in integrated pest management programs in controlling termites (pest), *C. gestroi* and *C. curvignathus.* Eucalyptus oil and eucalyptol (1, 8-cineol) were effective against *Aspergillus niger, Penicillium chrysogenum,* and *Penicillium* sp. but at higher concentrations of 600 ul/ml and 500 ul/ml, respectively (Matan *et al.*, 2009).

The extracts of *A. galanga* and *C. indicum* were found to be effective against termites. Although these botanicals are effective at a higher ED_{50} then the commercial termiticide but in this study however the extracts of *A. galanga* and *C. indicum* are nontoxic and safe for the environment, biodegradable and renewable source. The plant extract could be exploited to develop new wood preservatives to protect wooden structures, agricultural crops, plants and trees, as these are less harmful to the environment and humans. Long term field studies are required to develop these botanicals as commercial termiticides

Nevertheless this future study in developing this extract for commercial use is of utmost importance globally for human health and environment.

Chapter 6. Conclusion

The antifeedant activity studies of methanolic extracts and essential oils from *Alpinia galanga* rhizomes and *Chrysanthemum indicum* leaves on *Coptotermes gestroi, Coptotermus curvignathus* and *Macrotermus carbonarius* were conducted in the laboratory using dual choice bioassays. The effectiveness of the essential oils and the active compounds identified, were tested in the field for 16 weeks. The key findings from the above studies are listed below:

1. Essential oils of *A. galanga* and *C. indicum* gave positive antifeeding effect in the antifeedant bioassays on paper disc.
2. The methanolic extracts of *A. galanga* and *C. indicum* showed negative antifeeding effect in the feeding bioassay on paper disc.
3. Essential oils of *A. galanga* and *C. indicum s*howed good antifeedant properties against *C. gestroi, C. curvignathus* and *M. carbonarius.*
4. Antifeedant activities of *A. galanga* and *C. indicum* essential oils may be due to the presence of the compounds 1, 8-cineol and farnesene, respectively.
5. The comparison of the antifeedant activity of *A. galanga* rhizome oil and *C. indicum* leaf oil with analytical grade 1, 8-cineol, farnesene and commercial termiticide, chlorpyrifos showed that chlorpyrifos gave better antifeedant activity than the analytical grade chemicals followed by the essential oils.
6. Results from the ED_{50} values showed that, *M. carbonarius* were more susceptible than *C. gestroi and C. curvignathus* to the antifeedant effect of the essential oils (*A. galanga and C. indicum),* synthetic compounds (1, 8-cineol and Farnesene) and

commercial termiticide (chlorpyrifos).

7. The field application study using treated wood showed that the synthetic compound 1, 8-cineol gave better antifeedant activity compared to farnesene. This is the first study which proves that 1, 8-cineol can be potentially used in wood preservation against termites.

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