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The Action of Gelam (*Melaleuca cajuputi*) Stem Crude Extract as Natural Insecticide for *Camponotus* Sp.

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Abstract

Treatment of old historical wooden houses with synthetic preservative lined with chemical pesticide were known to cause hazards to human and the environment. Gelam tree crude extract was investigated in this study to explore its potential to repel wood damaging carpenter ants in order to reduce the use of hazardous pesticides and attack on the timber by wood boring insects. The crude extract from Gelam tree stem (*Melaleuca cajuputi*) was extracted by sequential extraction using hexane, dichloromethane and methanol. The repellent activity was tested at 20% w/v concentration using World Health Organization (WHO) recommended method on ant repellent testing for 3 hrs with 15 min's interval. All the extracts showed significant repellent activity on the tested *Camponotus* sp. ants. The toxicity activity on *Camponotus* sp. was determined by using 10% w/v concentration of crude extract mixed with honey. Toxicity activity of methanol extract (84.3%) showed the high toxicity percentage against *Camponotus* sp. The LT_{50} recorded for *Camponotus* sp. tested with hexane, dichloromethane and methanol crude extract were 19.11 hrs, 11.89 hrs and 9.43 hrs respectively. This study indicated that *M. cajuputi* stem has potential to be further studied and developed as natural insecticide against carpenter ants for the application on wooden buildings.

Keywords: Natural insecticide; *M. cajuputi*; *Camponotus* sp.; Repellence; Toxicity

Introduction

Many pre-British and colonial era houses have included wood in the building construction. The timbers used are rich food source for insects and fungi. Wood treatment of historical wood buildings is done by applying wood preservatives and pressure treated wood repairs which usually appeals to the historic preservation practitioners. Wood preservatives are applied by the impregnation of anticorrosive, mothproof or mildew-proof chemicals into wood products at atmospheric pressure. The service life of wood is extended after preservative treatment compared to the untreated wood in historical buildings [1].

Camponotus sp. or commonly known as Carpenter ants is one of the largest and most abundant species of ants that can be found in urban residential areas, which are usually in baits, gardens, cupboards, and kitchens [2]. *Camponotus* sp. has the ability to contaminate food and transmit many bacterial and viral diseases such as fever and cholera [3], while at the same time; the ants' bite or sting may result in severe anaphylactic allergic reactions and sometimes even death [4]. *Camponotus* sp. is one of the most significant structural pests in the world, causing serious damage by burrowing or nesting into the wood [5]. It is imperative to control the population of *Camponotus* sp. to reduce property damage especially historical wood buildings.

The efficient and effective control method of *Camponotus* sp. depends highly on the usage of synthetic insecticides [6]. Synthetic insecticide is defined as products pesticides formulated in vitro to kill insects [7]. Most popular synthetic insecticides that are commonly used to control insects are known as DDT (dichlorodiphenyltrichloroethane). Previous studies have reported the damage caused by the high usage of synthetic insecticide to the ozone layer and human health [6]. The misuse or overuse of synthetic insecticide has also led to the development of insecticide resistance in *Camponotus* sp. and destruction of non-target organisms causing imbalance ecological system [8]. Issues related to the high usage of synthetic insecticide in controlling urban pests can be solved by developing and using insecticides developed from natural products or naturally synthesised chemical compounds which have been identified as an alternative source to synthetic insecticides in order control the population of insects [9].

Natural insecticides are defined as a vital group of plants that can be found naturally in the wild, often slow growing protectant crops [10]. Protectants which can consider as an insecticide are safe and also beneficial to environment and humans when compared to synthetic insecticides, and has minimal residual effects [11]. Natural insecticides are mainly made of active components of the selected plant extracts and usually are very safe to be used in daily life. *M. cajuputi* tree can be found abundantly along east coast area of Peninsular Malaysia especially Kelantan in swampy land [12]. There have been numerous reports on the essential oil distillation [13-16] and evaluation of the essential oil as insecticides such as termicide [14], mosquitocide [17,18] and against *Tribolium castaneum* and *Sitophilus zeamais* [19]. Studies proved that the leaves of *M. cajuputi* retain antibacterial [20-22], anti-inflammatory and anaesthetic properties and have the potential to repel and kill insects [23].

M. cajuputi has been used for many purposes in human daily life, such as flavouring in cooking; the good smell of the leaves is used for fragrance and refreshing agent in the cosmetics, perfumes, detergents and soap. Bakar et al. [17,18] reported the effectiveness of essential oil extracted from *M. cajuputi* leaves collected from Negeri Sembilan, Malaysia as natural insecticide against dengue vectors in different experimental conditions. However, very limited studies are done to evaluate the effectiveness of extract from *M. cajuputi* stem, branch and bark as potential pesticides. Studies done previously focused on the use of the *M. cajuputi* stem extract for its allelopathy effect as reported by Pattarawadee et al. [24] and as larvacide of *Aedes albopictus* [25]. There is detailed study or reports on the isolation of crude extract from *M. cajuputi* stem and its potential as insecticide against urban pests. Therefore, this study was conducted to identify the potential of *M. cajuputi* stem extract as natural insecticide for its repellent and toxic activity against *Camponotus* sp.

Materials and Methods

The methodology in this study is inclusive of *M. cajuputi* tree stem sampling, collection of *Camponotus* sp. crude extract preparation using stem powder, bioassay technique and statistical analysis. The method used for this study is adopted and modified from the steps used by WHO (World Health Organisation) to control the disease causing vector, Aedes mosquito [26].

Collection of *M. cajuputi* stem

M. cajuputi stem samples were collected from secondary forest of Bang Khao, Pattani, Thailand at the coordinate of 6˚49ˈ24.04˝ N, 101˚9 ˈ44.4˝ E using knife, axe and chisel. The dust and sand from stem sample was removed to prevent non-tree contamination. The collected stem sample were air dried under shade at room temperature (27°C) for five days until the stems were completely dried and in crispy form [27]. The dried stem samples were cut into small pieces to be ground into fine powder by using electric grinder. The finely ground stem samples were sealed in polyethylene bags and stored in chiller at 4°C [28].

Collection of *Camponotus* sp.

Carpenter ants (*Camponotus* sp.) were collected by using honey as a trap to find its colony. Honey were mixed with three drops of water in an aluminium foil that was shaped into the size of a 500 mL mineral water bottle cap and kept in a garden around Prince of Songkla University, Pattani, Thailand. The area was observed to host *Camponotus* sp. The trail left by *Camponotus* sp. eventually led to its colony. The colony consisting of *Camponotus* sp. was then collected into a plastic bag using a small and soft painting brush to prevent injuries to the ants. The *Camponotus* sp. was kept alive in the plastic bag which was poked with tiny holes for aeration and supplied with honey as a source of food and water before being used for bioassay test. The collected live *Camponotus* sp. in transparent plastic bag was maintained at room temperature of 26°C to 30°C and 70% RH.

Crude extract preparation using *M. cajuputi* stem powder

In order to obtain the crude extract, the *M. cajuputi* stem was extracted using three different extraction solvents: n-hexane, dichloromethane and methanol [29]. Crude extract of *M. cajuputi* stem was prepared by using a slightly modified method described by Shankar et al. [30] ie. Sequential extraction technique.

Isolation of the crude extract from *M. cajuputi* stem powder was started with the use the most non-polar extraction solvent, n-hexane (Sigma Aldrich, St. Louis, USA), followed by dichloromethane (Sigma Aldrich, St. Louis, USA) and methanol (Sigma Aldrich, St. Louis, USA). The ground stem samples were collected in a sieve cloth, tightly tied and then were placed into a 2 L glass jar. Extraction solvent was measured to one litre and poured into the glass jar till the sieve cloth was totally soaked and covered with the solvent. The sample was allowed to soak for 24 hrs. After 24 hrs, the sieve cloth was removed from the glass jar and the sieve cloth was squeezed completely to ensure all the solution to drop into the glass jar.

The solution obtained were filtered using Whatman filter paper No. 1 one spoon of anhydrous sodium sulphate (Sigma Aldrich, St. Louis, USA) was added into the solution before being filtered. Finally, all filtrates were evaporated using a rotary evaporator that was connected to a vacuum and water cooler. The concentrated extracts were stored in the chiller at 4ºC until required for bioassays test.

Bioassay techniques of extractives

Repellence activity of crude extract from M. cajuputi against Camponotus sp.: The *Camponotus* sp. repellence potential by *M. cajuputi* stem extracts was tested using mosquito repellent test protocol published by WHO with slight modification and also by following the steps and experimental setup as described by Mensah et al. [31,32]. Repellent activities of the *M. cajuputi* stem extracts prepared using three different solvents (n-hexane, dichloromethane and methanol) were evaluated by dissolving 20% w⁄v of the extracts in acetone. A volume of one mL of 20% w⁄v diluted crude extract solution was used to soak the edges of a nine cm round Whatman filter paper No.1 and was placed in the centre of a ring. Commercially bought honey (2 g) was used as bait and was placed in the centre of the Whatman filter paper No.1 a Whatman filter paper No.1 soaked at the edges with one mL of acetone was used as negative control.

A total of 30 live *Camponotus* sp. were used for this repellence experiment. The ants were starved for 24 hrs before repellency test were conducted. The live ants were then introduced onto each ring (150 mm × 25 mm) but outside the 9 cm Whatman filter paper. Three rings with crude extract soaked filter papers were prepared for each of the three different crude extract as a triplicate for this experiment. The experiment was monitored for 3 hrs with intervals of 15 min to calculate the repellency rate of each of the *M. cajuputi* stem extracts. The final repellency rate for each crude extract was calculated after 180 min of exposure time using modified WHO landing inhibition formula as used by Thavara et al. [33]. Number of *Camponotus* sp. Found on the treated zone and control zone in the experimental ring setup was recorded.

Toxicity of crude extract from M. cajuputi against Camponotus sp.: The toxicity rate of three prepared *M. cajuputi* stem crude extract to *Camponotus* sp. was identified by using force feed method. A model set up with two transparent plastic boxes that made up the foraging area and habitat for *Camponotus* sp. were prepared. In the habitat area, the transparent plastic box was filled with damp soil and dry leaves to mimic *Camponotus* sp. habitat in wild. In the foraging area, a cotton ball soaked with water was placed at a corner and an aluminium foil containing a mixture of honey and 10% *M. cajuputi* stem crude extract isolated using n-Hexane was placed at another corner of the plastic box. The same setup was used for dichloromethane and methanol crude extract. The plastic box setup for foraging area was connected to

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the habitat box by using a transparent pipe for the *Camponotus* sp. to move from the habitat box to foraging area box.

The collected live *Camponotus* sp. were transferred into the habitat box (30 live ants per box) and provided with honey and water to allow the ants to adapt to the box condition for 24 hrs. After 24 hrs, *Camponotus* sp. was starved for 24 hrs. After starving for 24 hrs, the ants were provided with water and honey mixed with the *M. cajuputi* stem n-hexane crude extract. The experiments were done in triplicates. The same method was repeated using dichloromethane and methanol crude extract. The number of dead ants was recorded for 24 hrs with the interval of 3 hrs.

Statistical analysis

The toxicity test were analysed using Probit analysis [34] to identify the lethal time taken to kill 50% and 90% of the tested *Camponotus* sp. population.

Results

Yield of crude extract from *M. cajuputi* stem powder

The percentage of crude extract yield from *M. cajuputi* stem samples found to be different for the three solvents used i.e. n-hexane, dichloromethane and methanol. Figure 1 shows that dichloromethane extract has the highest yield percentage of crude extract (0.51% w/w) followed by methanol extract 0.2% (w/w) and lastly hexane extract (0.08% w/w). This result is supported by similar study conducted by Pattarawadee et al. [24] with *M. cajuputi* crude leaf and stem extracts using hexane, methanol and dichloromethane as extraction solvent which showed comparable result to this study. However, the percentage yield of leaf extract is higher compared to stem extract and this could be due to the high content and accumulation of active compound resulting from secondary metabolism in the leaf which is higher compared to the stem [35].

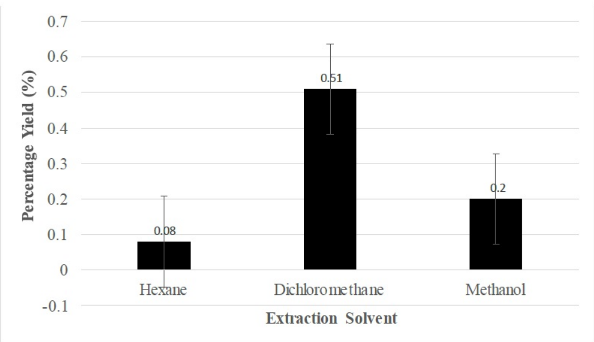

Figure 1: Percentage yield of crude extract using different extraction solvent (hexane, dichloromethane and methanol).

Repellence percentage of crude extract from *M. cajuputi* stem against *Camponotus* sp.

The repellence test using the three crude extracts obtained from *M. cajuputi* stem on *Camponotus* sp. showed hexane extract has the highest repellent effect against *Camponotus* sp. (97.3%), followed by dichloromethane (83.4%) and methanol (42.8%) (Figure 2). This finding is in similarity with study done by Khanam et al. [36,37] who reported that crude extract from plant Zingiber cassumunar using non-polar solvent i.e. hexane and petroleum ether are both strong repellent against *T. castaneum* (red flour beetle) and *T. confusum* (confused flour beetle).

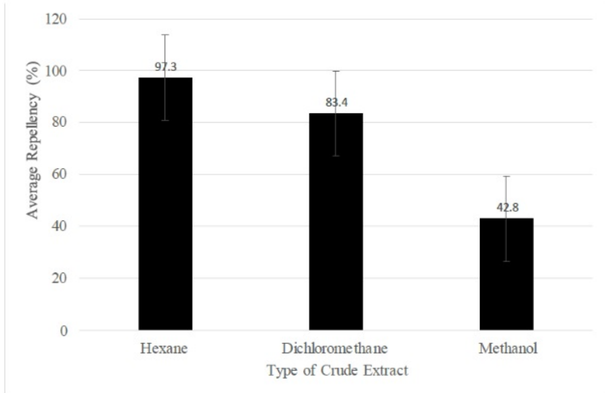

Figure 2: Average repellence percentage of different crude extract (hexane, dichloromethane and methanol) against *Camponotus* sp. (carpenter ants).

Toxicity percentage of crude extract from *M. cajuputi* stem against *Camponotus* sp.

The toxicity percentage of three prepared *M. cajuputi* stem crude extract for *Camponotus* sp. were found to be different for all the solvents used. The toxicity percentage for methanol extract has the highest toxicity effect to *Camponotus* sp. (84.3%) followed by dichloromethane extract (77.7%) and hexane extract (62.3%) as shown in (Figure 3). The control used showed 0% of toxicity effect to *Camponotus* sp.

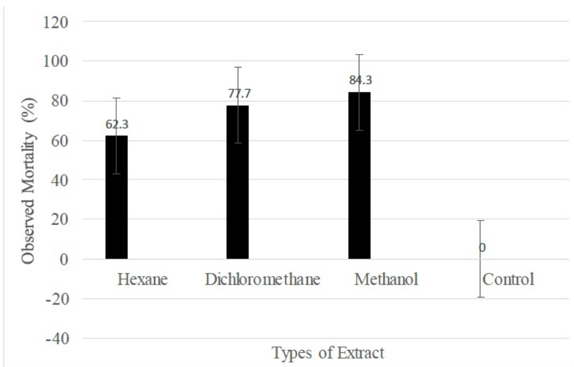

Figure 3: Mortality percentage of *Camponotus* sp. tested with different crude extracts.

The results of susceptibility tests against *Camponotus* sp. when fed with crude extract from three solvents are different. Lethal time 50 (LT_{50}) and lethal time 90 (LT_{90}) for *Camponotus* sp. were analysed using Probit. LT_{50} is defined as lethal time taken for the crude extract to kill 50% of the *Camponotus* sp. population, whereas LT_{90} denotes lethal time taken for the extract to kill 90% of the *Camponotus* sp. population. The Probit value for LT_{50} is 19.11, 11.89, and 9.43 and LT_{90} is 58.42, 43.53 and 32.93 for *Camponotus* sp. tested with hexane, dichloromethane and methanol crude extract (Figures 4-6). The time taken to kill *Camponotus* sp. with hexane extract was longer than other crude extracts as the number of ants which ate the crude extract mixture were very few. *Camponotus* sp. in this study was observed to be repelled by the smell of hexane crude extract hence less ants were recorded to consume the bait. Therefore, hexane extract shows very low toxicity effect. The time taken for dichloromethane extract to kill *Camponotus* sp. is less compared to hexane extract. This proves that mortality rate increases as the polarity of the compound increases. Besides that, the smell of dichloromethane is very mild compared to the smell of hexane extract, which might not repel the *Camponotus* sp. from eating the crude extract of *M. cajuputi* stem. From the above tests, smell of the compound with the solvent influences the toxicity rate of the compound. The time taken for methanol extract to kill the carpenter ants were the shortest compared to hexane and dichloromethane extract. This strongly proves that the toxicity rate of *M. cajuputi* stem against *Camponotus* sp. increases with the increasing polarity of the solvents in the order of methanol>dichloromethane>hexane. This polarity order is completely the reverse for susceptibility test with *M. cajuputi* stem extract against *Camponotus* sp.

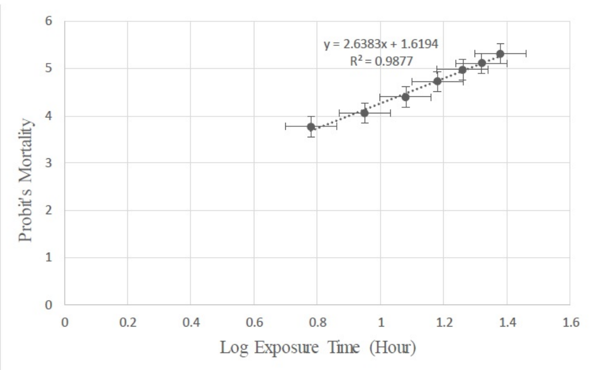

Figure 4: Probit's mortality of *Camponotus* sp. against hexane extract.

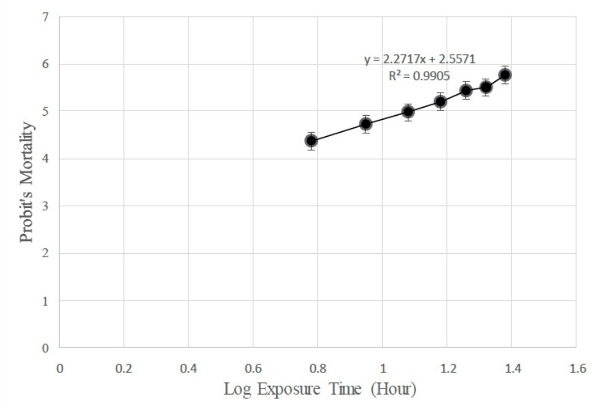

Figure 5: Probit's mortality of *Camponotus* sp. against dichloromethane extract.

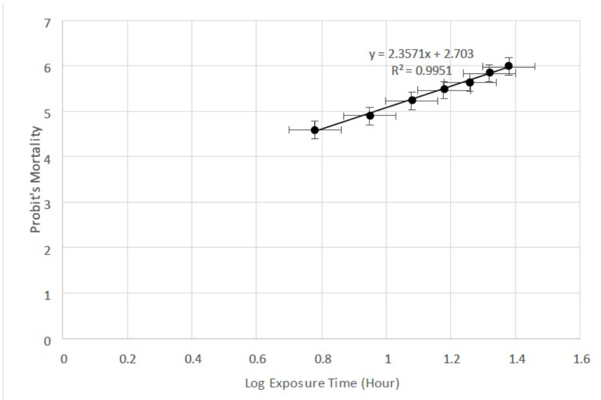

Figure 6: Probit's mortality of *Camponotus* sp. against methanol extract.

Discussion

Hexane is a non-polar solvent that will extract non-polar compounds from the stem sample. The yield percentage of non-polar compound in this study was found to be very low as non-polar compounds in plants are usually in the form of essential oil which is mostly found accumulated in the leaves compared to stem. The result obtained in this study was also in conformity with the findings by Koul et al. [38] from compound profiling of *M. cajuputi* leaves which showed high percentage of essential oil i.e. Non-polar compound was present. The crude extract yield percentage for all the three solvents used in this study are consistently lesser than 1% which indicates that high quantity of stem sample will be required in future for preparation of natural insecticide product from this plant material which is not economical.

The repellent effect of hexane crude extract could be due to the presence of non-polar compound isolated from *M. cajuputi* such as essential oil which could contribute to the high repellence effect. The strong smell of the essential oil acts as repellent to the *Camponotus* sp. from going into the treated area and this biological effect of essential oil were highlighted by Bakkali et al. [39]. On the other hand, methanol and dichloromethane crude extracts showed weak repellent activity that only distract *Camponotus* sp. from going into the treated area. The repellency rate decreases as the polarity of the compound in the crude extract increases in the order of solvent used: hexane>dichloromethane>methanol. A similar results was reported by Bigi et al. [40] in a study on activity of ricinine isolated from *Ricinus communis* against the leaf cutting ants (*Atta sexdens var. rubropilosa Forel*).

Blum [41] Reported that non-polar compounds are often more toxic to insects compared to the polar derivatives. This report contradicts the results obtained for this study which could be explained by the presence of strong smell due the non-polar compounds in the hexane crude extract. The strong smell repels *Camponotus* sp. from ingesting the filter paper with bait and hence the low percentage of toxicity was recorded. On the other hand, dichloromethane and methanol extract did not emit strong smell that could prevent the ants from eating the honey bait and thus attracts more individuals nearing the bait and results in higher toxicity effect against carpenter ants. This study also indicates that the repelling activity of non-polar compounds in hexane crude extract coupled with the toxicity effect of the extract could be used to make an early conclusion that the hexane crude extract of *M. cajuputi* stem has the potential to be developed as insecticide.

Result obtained in this study which indicates toxicity rate of the crude extract directly proportionate with the increasing of solvent polarity is in line with the study by Terezan et al. [42] on the activities of extracts and compounds from Spiranthera odoratissima St. Hil (*Rutaceae*) against ants. This study proves that methanol extract of *M. cajuputi* stem is more toxic to ants compared to dichloromethane and hexane extract. More studies are needed to investigate the compound profile and fraction concentration in each crude extract of *M. cajuputi* which will give a better understanding in the plant's repellent activity and mortality of *Camponotus* sp.

M. cajuputi stem has potential to be used as natural insecticide to repel and cause high mortality in 24 hrs of exposure to *Camponotus* sp. High repellence rate of *M. cajuputi* stem to *Camponotus* sp. was shown by using hexane extract with 97.3%. High toxicity rate of *M. cajuputi* stem to *Camponotus* sp. was shown by methanol extract with the percentage of 84.3%. Probit's analysis showed that the minimum time needed to kill 50% of the carpenter ants' population using methanol extract will require 9.43 hrs and 32.93 hrs to kill 90% of the population. *M. cajuputi* stem is more effective as repellent to carpenter ants compared to toxic and causing mortality to the ants. This preliminary study on *M. cajuputi* stem extract is a first report which showed that other than this plants's essential oil (Cajuputi oil) from leaves, the extract from stem has the potential to be developed as natural insecticide especially *M. cajuputi* which often a pest to wooden historical buildings.

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