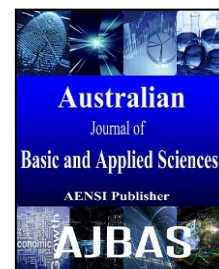




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### Extraction And Chemical Compositions of Ginger (*Zingiber Officinale* Roscoe) Essential Oils As Cockroaches Repellent

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#### ABSTRACT

Cockroaches are considered to be among nature's most adaptable creatures, which could pose a serious health issues in many countries. Their inclination for destruction and spreading pathogenic organism and disease has earned mans' loathing. The study aimed to determine the chemical compositions and potential of the essential oils from genius *Zingiber* of which *Z. officinale* (Family: Zingiberaceae) against German cockroaches. The essential oils were obtained by Supercritical Fluid Extraction (SFE) and Soxhlet extraction (SE) methods. Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyse the chemical compositions of the oils. Modified Ebeling Choice-Box test used in the repel test. Four concentrations; 10, 30, 50, and 100% (v/v) of the oils were prepared in water, used tween-80 to dissolve the oils; 1% tween-80 and naphthalene were negative and positive controls, respectively. The oils yields were 4.43 and 0.69 (w/w%) for SFE and SE, respectively. Thirty-five and sixty-six compounds were detected for SE and SFE, respectively. The most major components were:  $\alpha$ -zingiberene (16.98 & 13.74%),  $\alpha$ -farnesene (12.57 & 10.64%),  $\alpha$ -curcumene (8.75 & 8.03%),  $\beta$ -sesquihellandrene (8.02 & 8.23%), and citral (7.66 & 1.60%), respectively. The repellence depends on oil concentration and the IC<sub>50</sub> and IC<sub>90</sub> values were 16.0 and 28.0%, respectively, for SFES' oil. The study indicates the potential of this essential oil as repellent against the German cockroaches.

#### INTRODUCTION

Cockroaches are considered to be among nature's most adaptable creatures and have been living on the planet for at least 250 million years. They have strong survival skill, they are resistant to radiation than any other living things included animals and human being. However, their inclination for destruction and spreading pathogenic organism and disease has earned man's loathing (Buchanan, 2007). Furthermore, cockroaches are one of the common household pests which could pose a serious health issues in many countries. There are many types of the species of cockroach, including the American cockroach, German cockroach, Smooth cockroach, Australian cockroach, lobster cockroach and the Brown cockroach. The common species that found in homes, hostels, and restaurants are mostly the German cockroaches (*Blattella Germanica*) (Environmy\_adm, 2012).

There is ample evidence that proved substances produced by German cockroach (common species) producing allergic symptoms and others negative impact such as adulteration of food with excrement and defensive secretions, and transport of pathogenic organisms make this species one of the most troublesome annoyance pests of the world. Unfortunately, the number of cockroach does not appear to be reducing in a due time, as there is currently no efficient and proverbially applicable strategy that could actively eliminate or

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reduce the populations of this species (Thavara *et al.*, 2007). German cockroach was assumed to be associated with unsanitary conditions since ancient time. The fear of others knowing and observing the infestation may cause one to experience psychological problems and social anxiety. Normally, the German cockroaches will have a relatively short life (about 200 days). Females cockroaches can produce 5-10 egg cases during their life, and each egg case contains about 35-40 eggs. Its short generation time increases its chance of becoming resistant to the insecticides used to manage its population; therefore, chemical rotation and different products and strategies should be used to reduce the chance of resistance developing in the population (Phillips *et al.*, 2009). Environmentally compatible stored-product control agents are surely needed to replace synthetic pesticides that are harmful to environment and not effective due to the increasing difficulty of managing pesticide resistance (Donald *et al.*, 2009). Highly efficient cockroach repellents had gained a huge demand in the market recently.

Several chemicals were studied for repellent action against cockroaches, such as methyl neodecanamide, *N,N*-diethylphenylacetamide, propylneodecanamide, methyl neotridecanamide, alkyl neoalkanamides, eugenol and citral. Normally, insect repellents function by releasing a vapour barrier discouraging the insect or arthropod from coming into contact with the surface. Research shows that essential oils, steam distilled concentrates which containing volatile aromatic compounds from various part of plants such as rhizomes, flowers, roots, fruit and trees, have several properties against various haematophagous hexapods, thus some essential oil was being the basis of commercial and environmental friendly repellent formulations (Nerio *et al.*, 2010; Rajesh and Joshi, 2013).

Ginger (*Zingiber officinale* Roscoe.) has a long history of being used as a medicine and herbal since ancient time and had been used as an important cooking spice throughout the world. Phytochemical investigation of several types of ginger rhizomes has indicated the presence of bioactive compounds, such as gingerols, which are antibacterial agents and shogaols, phenylbutenoids, diarylheptanoids, flavanoids, diterpenoids, and sesquiterpenoids (Sivasothy *et al.*, 2011). Furthermore, there are many studies that proved their beneficial effects against the symptoms of diseases, acting as an anti-inflammatory, anti-tumour, anodyne, neuronal cell protective, anti-fungal and anti-bacterial agent (Mesomo *et al.*, 2012). Therefore, this research aimed to analyse the chemical compositions and investigate the potential of ginger essential oil in the cockroaches' repellent activity.

## MATERIALS AND METHODS

### **Plant Materials:**

The white ginger (*Zingiber Officinale* Roscoe.) obtained from the wet market at Jinjang Utara, Kuala Lumpur, Malaysia in 8<sup>th</sup> December 2012 and the collected ginger rhizomes were authenticated by botanist of the School of Environmental Sciences and Natural Resources, Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor, Malaysia.

### **Extraction of Ginger Rhizomes:**

The rhizomes were washed, cut into small pieces and allowed to dry in a less humid area. The dried gingers were grinded into coarse powder form and used for supercritical fluid extraction and soxhlet extraction of ginger essential oil. The extraction of ginger essential oil was conducted in laboratory scale of supercritical extraction unit pressure as described by Michele *et al.* (2013). Whereas, the essential oil of the ginger rhizomes (40 g dried and grinded ginger rhizomes) was extracted by Soxhlet (solvent semi-continuous extraction) method for 6 hours with n-hexane. The obtained oils were stored in hermetically closed dark bottles and kept at -4 °C for further studies. The percentage of oil (w/w%) from SFE and Soxhlet methods was calculated according to the following formula:

$$\text{The Oil Yield (\%)} = \frac{\text{Weight of Oil (g)}}{\text{Weight of sample (g)}} \times 100\%$$

### **Cockroaches Collection and Repellent Test :**

About 150 adult male and female of German cockroaches were brought from the Tong Hai Aquarium & Pet Shop at Penang on 3<sup>th</sup> July 2013. The cockroaches were kept in a cage and boxes and allowed to reproduce in several weeks until there were sufficient number to test and usually it is within two generation. Only the healthy nymphs and adults (male & female) cockroaches were then used in this repellence test. The cockroaches were reared in the laboratory by feeding on water and biscuits. The temperature was maintained at  $28 \pm 5$  °C. In the repellent test, four different concentrations; 10, 30, 50, and 100% (v/v) of ginger essential oil were prepared (used 1% tween-80 to dissolve the oil in water) and used for repellent against cockroaches. A 1% tween-80 and naphthalene were used as negative and positive controls, respectively.

The Ebeling *et al.* (1966) choice-box method was modified and used in this experiment. Then, the choice boxes (treated and untreated zone) were exposed to a photoperiod of 48 hours at 27 °C for 2 days. The cockroaches located at the treated and untreated zone were carefully observed and counted for every 1, 3, 6, 9, 12, 24, and 48 hours for 2 days of treatment. Each treatment with different concentration was conducted in duplicates. The percentage of repellency was calculated as follows:

$$\text{Repellency \%} = 100 \% - \left( \frac{T}{N} \times 100 \% \right)$$

Where the T stands for the number of cockroaches located at the treated zone and N stands for the total number (ten heads) of cockroaches been used in the repellency test. The mean percentage of the repellency was then calculated from the values obtained in two replicates. Data were evaluated through regression analysis. From the regression line; the IC50 and IC90 values were read representing the lethal concentration for 50% and 90% repellency (inhibition) of cockroaches.

#### **Gas Chromatography- Mass Spectrometry (GC-MS):**

The essential oil extracted from ginger (*Z. officinale*) were analysed by an Agilent Technologies 7890A Gas Chromatography- Mass spectrometry (GC-MS: Model 3171A) with HP-5 elastic quartz capillary column (30 m x 250 µm x 0.25 µm) film thickness and desorbed at 350 °C for 10 min. Helium was used to inject samples (1 µL) in splitless mode. The flow rate of the carrier gas (Helium) was 1.23 mL min<sup>-1</sup>. The initial oven temperature was 60 °C and the pressure was 10.726 psi. Mass detector was conducted with electron multiplier voltage (EMV) mode at 1200 EM voltage and scanning rate of 1 scan s<sup>-1</sup>. The components of ginger essential oil were identified by comparing the mass spectra with those recorded in the National Institute of Standards and Technology (NIST) library (Ding *et al.*, 2012).

#### **Statistical Analysis:**

One-way ANOVA test was applied in this study to compare the means of the percentage yield of ginger essential oil using SE and SFE at the 5% significance level. The statistical analyses were performed using Microsoft Excel 2010 software, and the differences were considered significant when  $p \leq 0.05$ . Besides this, the repellency data were expressed as mean ± SEM for the analysis of the variance of the duplicate values of repellency percentage against cockroaches (Appel and Tanley, 2000).

## **RESULTS AND DISCUSSION**

#### **Yield of Ginger Essential Oil Based on Different Extraction Methods:**

Both extraction methods were performed at the optimized temperature and pressure in order to obtain the higher percentage yields of ginger essential oil. The extraction yields were calculated under the fixed extraction period which is 120 min for SFE and 360 min for SE. From the ANOVA test, the mean of percentage yield among methods was significantly different as  $P$  value is less than 0.05.

Different methods of extraction have different extraction yield and efficiencies. A primary rule in the industries is to lower down the economic cost from the production of natural products which contained higher concentration of natural bioactive compounds, while it can be achieved by excellent extraction yield (Mandana *et al.*, 2012). Therefore, the conventional extraction (Soxhlet) and Supercritical Fluid Extraction methods were used in this experiment for the comparison in terms of quality and quantity. Both extractions are done by using non-polar solvent which is CO<sub>2</sub> for SFE and n-hexane for SE. Hence the mean yields of the oils were 4.43 ± 0.29 wt.% for SE and 0.69 ± 0.04 wt.% for SFE.

Although supercritical fluid extraction allows to reduce solvent consumption and shorten the extraction times, but the extraction yield of traditional technique which is the Soxhlet extraction is equivalent to or even higher than those obtained with advanced techniques. From the experiment, the extracted oil from the Soxhlet method was shown to be dark brown in colour, which means that there might be more other organic compounds and undesired impurities present in the extracted oil, while from the Supercritical Fluid extraction method, the obtained oil showed a pale yellow in colour which means the oil contained only desired components and less organic solvent. As a result, the Soxhlet method has shown the highest yield of ginger essential oil when compared to the Supercritical Fluid extraction method, but the oil from SFE is cleaner than Soxhlet method. Furthermore, SFE does not require filtration steps and can avoid thermal degradation. Meanwhile, SFE showed more advantages than the conventional methods.

#### **Analysis of Ginger Essential Oil by Gas Chromatography- Mass Spectroscopy:**

The chemical compositions of the ginger oil from two different extraction methods were identified by using GC-MS. There are 73 compounds being identified and tabulated in Table 1, representing about 96.74% and

92.44% in essential oil extracted from both SFE and SE methods, respectively. Sixty-six compounds were found in SFEs' oil, while only thirty-five compounds found in SEs' oil. Chromatographic analysis shows that the chemical profiles of the ginger essential oil are similar and contain monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes. The monoterpene hydrocarbons are believed to be the contributor to the aroma ginger. However the monoterpene constituents are less abundant in the essential oil of dried ginger than in fresh ginger.

The compounds identified in ginger essential oils are almost in agreement with previous studies. Suresh *et al.* (2012) reported the two main components in the ginger essential oil are geranial and zingiberene, while Padalia *et al.* (2011) reported citral is the main components of the rhizomes essential oil of *Z. officinale* which extracted by SFE extraction method. In this study, the GC-MS analysis indicated the most abundant constituents from both SFE and SEs' oils were:  $\alpha$ -zingiberene (16.98% & 13.74%),  $\alpha$ -farnesene (12.57% & 10.64%),  $\alpha$ -curcumene (8.75% & 8.03%),  $\beta$ -sesquihellandrene (8.02% & 8.23%), citral (7.66% & 1.60%),  $\beta$ -citronellol (5.66% & 2.55%) and geraniol (0.18% & 3.25 %) respectively. These components represent about 70% of the total constituents in the oils. Comparing the results mentioned with those in the literature, it indicated that some different in the major constituents of the ginger essential oil, this might be due to the fact that the geographic origin of the ginger and also the effect of the different extraction methods.

The result indicated the oil extracted from both methods contained citral. However, SFEs' oil contained higher concentration of citral (7.66%) than in SEs' oil (1.66%). In literature, citral has been reported as one of the most significant member of acyclic monoterpenoid in the ginger essential oils, which has possessed a strong repellent against cockroaches. Meanwhile the result shows that geraniol was also presents in SFEs' oil (0.18%). Previous study indicated the monoterpenoid compounds, citral and geraniol were toxic to the cockroaches by contact or injection. Hence the essential oil extracted from SFE has the high potential in repellent activity against cockroaches.  $\beta$ -citronellol was present in both SFE and SEs' oil with the concentration of 5.66% and 2.55%, respectively. The concentration of beta-citronellol in SFEs' oil is much higher than in SEs' oil. As mention in literature, beta-citronellol had possessed a repellent activity against the mosquito. Hence citronellol in the SFE's oil has high potential in cockroaches' repellent activity as well. Citronellic acid is present in SFEs' oil with 0.70% and 0.88% in soxhlets' oil. As reported by Jang *et al.* (2005), citronellic acid has the repellent properties to repel the mosquito (Jang *et al.*, 2005). Meanwhile numerous studies have been conducted for the insecticidal activities against German cockroaches by using the citronellic acid.

$\alpha$ -Pinene has the potential in repellent activity against *Periplaneta Americana*. Therefore it has the potential to repel *Blattella germanica* as both species are come from the same order (*Blattodea*) and family (*Blattidae*). Once again it proved that SFEs' oil is suitable to be used as the test sample against cockroaches as  $\alpha$ -pinene do not present in soxhlets' oil. Previous study noticed that  $\alpha$ -terpineol is active as Terpinen-4-ol and  $\alpha$ -Pinene which are similar in insecticidal activities.  $\alpha$ -terpineol was found in both SFE and SEs' oils. However, the concentration of  $\alpha$ -terpineol in SFE's oil (1.02%) is higher than in SEs' oil (0.71%).

Beside this, linalool was detected in SFEs' oil with 0.64% but it is absent in the soxhlets' oil. The essential oil which contained high level of linalool was toxic to the small aphids as mention in literature. Previous study used the linalool to test for the insecticidal activities against adult German cockroaches and found that it is slightly toxic (Jang *et al.*, 2005). Although SE method has higher yield of oil than SFE, but SFEs' oil contained more chemical components than SEs' oil. SFEs' oil showed more chemical constituents than SE. This might be due to fact that the extraction temperature for SFE is lower than SE. In higher temperature of extraction process, the volatile components such like terpenes, mostly were decomposed or destructed, thus the chemical components of SEs' oil is less than in SFEs' oil. From the chromatographic analysis of chemical components, both oils contained oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes but monoterpenes hydrocarbons does not present in SE' oil. Therefore, this result clearly showed SFEs' oil has the highest concentration of the active components which are proved to have efficiency repellent against cockroaches. Thus, the SFEs' oil was chosen as the test sample for the repellent test against the German cockroaches.

**Table 1:** GC-MS Analysis of Chemical Constituents of Ginger (*Z. officinale* Roscoe.) Essential Oils

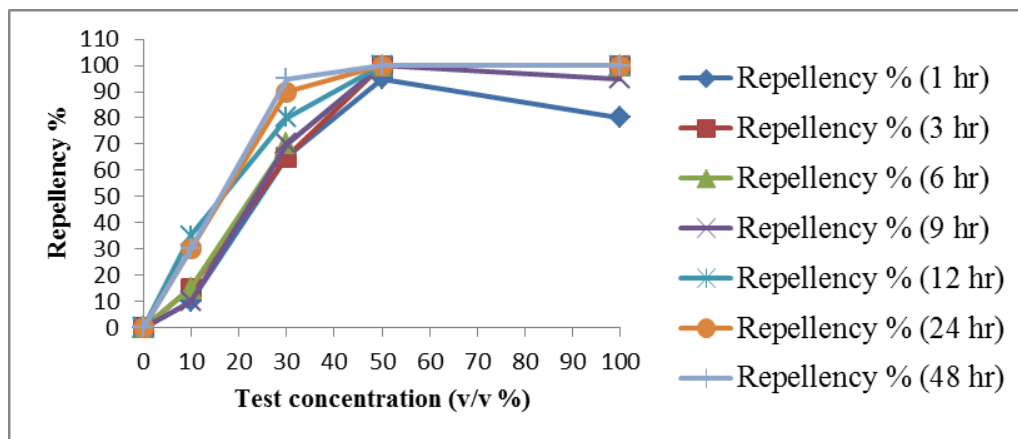
Chemical Component	Retention Time	Relative Area (%)		Formula
		SFE	Soxhlet	
<i>Monoterpenes</i>				
$\alpha$ -Pinene	7.012	0.24	-	C <sub>10</sub> H <sub>16</sub>
Camphene	7.413	0.73	-	C <sub>10</sub> H <sub>16</sub>
$\beta$ -Myrcene	8.854	0.54	-	C <sub>10</sub> H <sub>16</sub>
2-Carene	12.597	0.23	-	C <sub>10</sub> H <sub>16</sub>
2,6-Octadiene,2,6-dimethyl-	23.875	0.11	-	C <sub>10</sub> H <sub>18</sub>
Cyclopropane, tetramethylpropylidene	32.789	0.16	-	C <sub>10</sub> H <sub>18</sub>
o-Cymene	39.124	0.25	-	C <sub>10</sub> H <sub>14</sub>
<i>Oxygenated Monoterpenes</i>				
Eucalyptol	10.171	3.18	1.13	C <sub>10</sub> H <sub>18</sub> O

Chemical Component	Retention Time	Relative Area (%)		Formula
		SFE	Soxhlet	
Linalool	12.929	0.64	-	C <sub>10</sub> H <sub>18</sub> O
Camphor	14.182	0.10	-	C <sub>10</sub> H <sub>16</sub> O
Fenchol	14.697	0.10	-	C <sub>10</sub> H <sub>18</sub> O
Citronellal	14.926	0.43	0.48	C <sub>10</sub> H <sub>18</sub> O
Endo Borneol	15.515	1.03	0.77	C <sub>10</sub> H <sub>18</sub> O
L-Terpinen-4-ol	16.116	0.23	-	C <sub>10</sub> H <sub>18</sub> O
$\alpha$ -Terpineol	16.602	1.02	0.71	C <sub>10</sub> H <sub>18</sub> O
Myrtenol	16.940	0.12	-	C <sub>10</sub> H <sub>16</sub> O
Decanal	17.380	0.72	1.43	C <sub>10</sub> H <sub>20</sub> O
$\beta$ -Citronellol	18.691	5.66	2.55	C <sub>10</sub> H <sub>20</sub> O
Geraniol	19.074	0.18	3.25	C <sub>10</sub> H <sub>18</sub> O
Cis-p-Mental-2,8-diene-1-ol	19.286	0.13	-	C <sub>10</sub> H <sub>16</sub> O
Citral	20.133	7.66	1.60	C <sub>10</sub> H <sub>16</sub> O
Fragranol	20.779	0.08	-	C <sub>10</sub> H <sub>18</sub> O
Cyclohexanol,2-(2-hydroxy-2-propyl)-5-methyl	22.284	0.09	-	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>
2,5-Dimethyl-2-vinyl-4-hexen-1-ol	24.218	-	1.28	C <sub>10</sub> H <sub>18</sub> O
Lavandulol	24.601	0.43	-	C <sub>10</sub> H <sub>18</sub> O
cis-Geraniol	25.002	0.67	-	C <sub>10</sub> H <sub>18</sub> O
$\alpha$ -Copaene	25.282	0.78	0.36	C <sub>10</sub> H <sub>18</sub> O
Trans-Isoeugenol	27.119	0.12	-	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
1,7-Cctadien-3-one,2-methyl-6-methylene	41.029	-	2.68	C <sub>10</sub> H <sub>14</sub> O
<i>Sesquiterpenes</i>				
$\alpha$ -Longipinene	24.842	0.30	-	C <sub>15</sub> H <sub>24</sub>
$\beta$ -Elemene	25.866	1.27	1.58	C <sub>15</sub> H <sub>24</sub>
$\beta$ -Ylangene	26.902	0.36	0.26	C <sub>15</sub> H <sub>24</sub>
$\beta$ -Cubebene	27.319	0.21	-	C <sub>15</sub> H <sub>24</sub>
$\gamma$ -Elemene	27.520	0.85	0.53	C <sub>15</sub> H <sub>24</sub>
$\alpha$ -Bergamotene	27.737	0.90	-	C <sub>15</sub> H <sub>24</sub>
$\gamma$ -Muurolene	27.886	0.16	-	C <sub>15</sub> H <sub>24</sub>
$\beta$ -Patchoulene	28.178	0.33	0.31	C <sub>15</sub> H <sub>24</sub>
Alloaromadendrene	28.517	1.41	0.48	C <sub>15</sub> H <sub>24</sub>
$\alpha$ -Curcumene	29.482	8.75	8.03	C <sub>15</sub> H <sub>22</sub>
$\beta$ -Selinene	29.814	1.37	0.94	C <sub>15</sub> H <sub>22</sub>
$\alpha$ -Zingiberene	30.312	16.98	13.74	C <sub>15</sub> H <sub>24</sub>
Bicyclo (4.4.0)dec-1-ene,2-isopropyl-5-methyl-9-	30.718	-	0.82	C <sub>15</sub> H <sub>24</sub>
$\alpha$ -Farnesene	30.855	12.67	11.01	C <sub>15</sub> H <sub>24</sub>
$\beta$ -Sesquihellandrene	31.067	8.02	8.41	C <sub>15</sub> H <sub>24</sub>
Dihydrocurcumene	32.646	0.07	--	C <sub>15</sub> H <sub>24</sub>
Guaia-3,9-diene	35.799	0.18	-	C <sub>15</sub> H <sub>24</sub>
$\gamma$ -Selinene	35.925	0.43	-	C <sub>15</sub> H <sub>24</sub>
$\alpha$ -Funebrene	37.836	0.91	1.97	C <sub>15</sub> H <sub>24</sub>
<i>Oxygenated sesquiterpenes</i>				
Spir(4,5)dec-6-en-8-one,1,7-dimethyl-4-(1-methyl)-	24.819	-	0.54	C <sub>15</sub> H <sub>24</sub> O
Elemol	31.828	0.86	0.73	C <sub>15</sub> H <sub>26</sub> O
$\beta$ -Bisabolol	32.086	0.24	0.20	C <sub>15</sub> H <sub>26</sub> O
2-Cyclohexene-1-carboxaldehyde	33.293	-	1.95	C <sub>15</sub> H <sub>24</sub> O
Nirolidol	32.463	0.74	0.59	C <sub>15</sub> H <sub>26</sub> O
7-epi-cis-sesquisabinene hydrate	33.367	0.83	-	C <sub>15</sub> H <sub>26</sub> O
Epishiobunol	33.642	0.10	-	C <sub>15</sub> H <sub>26</sub> O
T-Muurolol	35.393	0.25	-	C <sub>15</sub> H <sub>26</sub> O
$\beta$ -Selinol	35.668	0.65	0.50	C <sub>15</sub> H <sub>26</sub> O
$\gamma$ -Eudesmol	35.817	-	0.30	C <sub>15</sub> H <sub>26</sub> O
Sparhulenol	36.886	0.44	-	C <sub>15</sub> H <sub>24</sub> O
Trans-Farnesol	39.730	0.05	-	C <sub>15</sub> H <sub>26</sub> O
Trans-Farnesal	40.503	0.19	-	C <sub>15</sub> H <sub>24</sub> O
Bergamotol-Z- $\alpha$ -trans-	40.875	0.44	-	C <sub>15</sub> H <sub>24</sub> O
Isoaromadendrene epoxide	41.865	0.16	-	C <sub>15</sub> H <sub>24</sub> O
<i>Sesterterpenes</i>				
Trans-Geranylgeraniol	38.889	0.16	-	C <sub>20</sub> H <sub>34</sub> O
<i>Others</i>				
Zingiberone	34.563	7.96	18.21	C <sub>11</sub> H <sub>14</sub> O <sub>3</sub>
2-Undecanone	21.254	0.35	0.26	C <sub>11</sub> H <sub>22</sub> O
Total		96.74%	92.44%	

### Repellent Activities of Ginger Essential Oils against German Cockroaches:

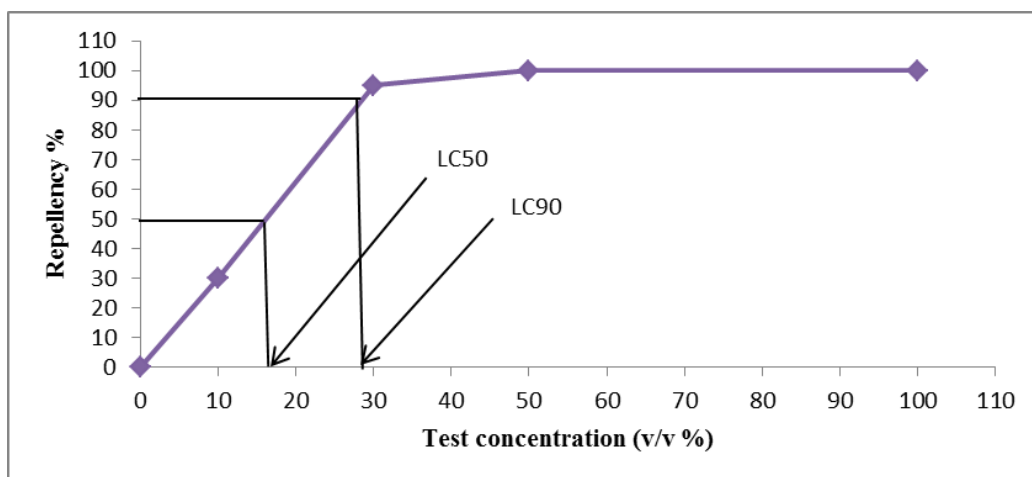
Figure 1 shows the essential oils of *Z. officinale* provided complete repellency (100%) against German cockroaches with highest concentration. All concentrations of the ginger essential oils showed repellent activity against German cockroaches after 48 hours of exposures. The result shows the percentage of repellent against

cockroaches was increased when the test concentration is increased from 10 to 100 (v/v%). This indicated the repellency of ginger essential oil against cockroaches was concentration dependents. At 10 of concentration, repellency was 30% after 48 hour of treatment. Concentration of 30 caused 95% repellency after 48 hour treatment; while the both concentrations (50 and 100) were repels 100% after 48 hours of exposure. The four different oil concentrations showed repellency in varying degrees against German cockroaches. The pure ginger essential oil (100%) showed excellent repellency (100%) and found to be effective as naphthalene in the repellency against German cockroaches.



**Fig. 1:** The percentage of repellency versus test concentration within 48 hours

Figure 2 shows the  $IC_{50}$  and  $IC_{90}$  values (percentage concentration) of the ginger essential oil against German cockroaches, indicated after 48 hour treatments. The  $IC_{50}$  and  $IC_{90}$  are the measurement of the concentration of the inhibitor which required inhibiting a given biological function by half and 90%, respectively. In contact repellency test, German cockroaches were exposed to four different test concentrations 10, 30, 50 and 100 (v/v%) of SFEs' ginger (*Z. officinale*) oil. The result revealed the  $IC_{50}$  and  $IC_{90}$  values of the oil after 48 hours treatments, has the highest repellency rate, which were 16.0 v/v% and 28.0 v/v%, respectively. Thus it would practical for use as cockroaches repellent as it requires very low dose (28.0 v/v%) to be effective for more than 90% repellency. However, the essential oil is volatile and the effectiveness was reduced when applied for long period. Therefore, a new product, using natural sources like ginger rhizome (*Z. officinale* Roscoe.) essential oil, could be formulated effectively and substitute the current synthetic repellent. Ginger oil is readily degradable and less hazardous to human and animals health by having significantly lower toxicity level.



**Fig. 2:**  $IC_{50}$  and  $IC_{90}$  values of ginger essential oil against German cockroaches after 48 hour of treatment

### Conclusion:

The essential oil content of the oils obtained by soxhlet extraction (SE) and supercritical fluid extraction (SFE) were  $4.43 \pm 0.29$  and  $0.69 \pm 0.04\%$  (w/w%), respectively. Almost sixty-six compounds were identified in SFEs' oil, while only thirty-five compounds in SEs' oil. The major constituents for SFE and SEs' ginger (*Z.*

*officinale* Roscoe) essential oil were  $\alpha$ -zingiberene (16.98% & 13.74%),  $\alpha$ -farnesene (12.57% & 10.64%),  $\alpha$ -curcumene (8.75% & 8.03%),  $\beta$ -sesquihellandrene (8.02% & 8.23%), citral (7.66% & 1.60%), and  $\beta$ -citronellol (5.66% & 2.55%), respectively. Meanwhile, results showed the alpha-pinene which has been proved to possess an insecticidal activity against cockroaches, were presents in SFEs' oil with 0.24% in concentration, but absent in soxhlets' oil. Whereas, citral, which also been proved toxic to the cockroaches species and it was present in SFE's oil with higher concentration (7.66%). It is important to note that oil with more components may be more efficiency because of the synergetic work. Hence the SFEs' essential oil was chosen as the samples in the repellence test against German cockroaches. Furthermore, SFE is more convenient and advanced method for the extraction of high-quality ginger essential oil compared to SE method and the extraction time is shorter and less solvent are required for the extraction. Furthermore, SFE method yielded cleaner essential oil and contained more volatile components than SE method. In bioassay test, the repellence of the oil against cockroaches increased with increasing of oil concentrations. The IC<sub>50</sub> and IC<sub>90</sub> values were 16.0 v/v% and 28.0 v/v%, respectively. Hence this oil is a promising repellent and may play a vital role in cockroaches' repellent agent.

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