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Essential Oil with Mosquito-Repellent Potential from *Plectranthus amboinicus* Leaves against *Aedes aegypti via* Human Volunteer Study

(Minyak Pati dengan Potensi Penghalau Nyamuk daripada Daun *Plectranthus amboinicus* terhadap *Aedes aegypti* melalui Kajian Sukarelawan Manusia)

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ABSTRACT

Plectranthus amboinicus (PA) is a highly aromatic perennial shrub that belongs to the Lamiaceae family. It has thick, fleshy and succulent leaves and stem. The margins of the leaves contain numerous glandular hairs that produce essential oil. The essential oil of PA(PAEO) exhibits therapeutical, nutritional, antioxidant, antimicrobial, insecticidal, and insect repellent potentials. The objective of this study was to evaluate and compare the repellent properties of PAEO and DEET against *Aedes aegypti* mosquitoes, which are known carriers of dengue fever. The extraction of PAEO was carried out using hydrodistillation method while the chemical profile was analysed by gas chromatography-mass spectrometry (GC-MS) technique. The bioefficacy test was conducted according to the household insecticide products personal mosquito repellent evaluation method for biological efficacy (First revision) (MS1497:2007) provided by the Standard and Industrial Research Institute of Malaysia (SIRIM) with slight modification. 10% PAEO was used as test solution while 10% DEET was used as positive control. Fabric and patches treated with 10% PAEO were tested on human volunteers. Both the fabric and the patch, treated with 10% PAEO, exhibit excellent repellency (97 - 100%) against *Ae. aegypti* mosquitoes for a duration of up to 240 min (4 h) post-application. In contrast, 10% DEET, which initially showed a comparable repellency (99%) 30 min after application, experiences a gradual decline in repellency over time, reaching a 51% repellency rate at the end of the test (240 min post-application). A two-way ANOVA analysis indicates significant effect for type of treatment ($p = 0.016$) but with no significant difference between the exposure time. Tukey's test showed significant differences between 10% PAEO-treated patches and 10% DEET ($p = 0.016$), and between 10% PAEO-treated fabric and 10% DEET ($p = 0.026$). These findings suggest that PAEO is potentially an effective natural repellent against *Ae. aegypti.*

Keywords: *Aedes aegypti;* carvacrol; essential oil; natural mosquito repellent; *Plectranthus amboinicus*

ABSTRAK

Plectranthus amboinicus (PA) adalah sejenis syrub saka aromatik daripada famili Lamiaceae. Ia mempunyai batang dan daun yang tebal dan sukulen. Bahagian margin daun *P. amboinicus* mempunyai banyak rerambut kelenjar yang menghasilkan minyak pati. Minyak pati PA (PAEO) menunjukkan pelbagai potensi daripada segi terapeutik, pemakanan, antioksidan, antimikrob, insektisid dan penghalau serangga. Objektif kajian ini adalah untuk menilai dan membandingkan sifat penghalau nyamuk PAEO dan DEET terhadap *Aedes aegypti* yang merupakan vektor demam denggi. Pengekstrakan PAEO dijalankan menggunakan kaedah penyulingan hidro manakala profil kimia dianalisis dengan teknik kromatografi gas-spektrometri jisim (GC-MS). Ujian bioefikasi telah dijalankan berdasarkan kaedah *household insecticide products personal mosquito repellent evaluation method for biological efficacy (First revision)(MS1497:2007)* yang disediakan oleh Institut Penyelidikan Standard dan Perindustrian Malaysia (SIRIM) dengan sedikit pengubahsuaian. 10% PAEO telah digunakan sebagai larutan ujian manakala 10% DEET digunakan sebagai kawalan positif. Fabrik dan pelekat yang dirawat dengan 10% PAEO diuji ke atas sukarelawan manusia. Kedua-dua fabrik dan pelekat yang dirawat dengan 10% PAEO memberikan peratus perlindungan yang sangat baik (97 - 100%) terhadap *Ae. aegypti* sehingga 240 minit (4 jam) selepas aplikasi. Sebaliknya, 10% DEET menunjukkan peratus perlindungan yang standing dengan 10% PAEO pada awal ujian (30 minit selepas aplikasi) tetapi mengalami penurunan yang ketara dengan hanya 51% diakhir ujian (240 minit selepas aplikasi). Analisis ANOVA dua hala menunjukkan kesan jenis rawatan yang signifikan (p = 0.016) tetapi tiada perbezaan yang signifikan antara masa pendedahan. Ujian Tukey seterusnya mendedahkan perbezaan yang signifikan antara tampalan yang dirawat dengan 10% PAEO dan DEET 10% (p = 0.016) dan antara kain yang dirawat dengan 10% PAEO dan DEET 10% (p = 0.026). Keputusan ini menunjukkan potensi PAEO sebagai penghalau nyamuk semula jadi yang berkesan terhadap *Ae.aegypti*.

Kata kunci: *Aedes aegypti;* karvakrol; minyak pati; penghalau nyamuk semula jadi; *Plectranthus amboinicus*

INTRODUCTION

Dengue is one of the most important and fast-spreading vector-borne disease in the tropical, subtropical and temperate regions. It is estimated that annually more than 400 million dengue cases and more than 20,000 deaths occur worldwide (Roy & Bhattacharjee 2021). The primary vector for dengue is *Aedes aegypti* mosquitoes. Dengue fever is caused by five dengue virus serotypes, known as DENV-1, DENV-2, DENV-3, DENV-4, and DENV-5. Even though majority of the infections are asymptomatic or manifested as mild fever, in some cases it is manifested as dengue haemorrhagic fever or dengue shock syndrome that can lead to death (Achee et al. 2015; Masrani et al. 2021; Zaini et. al. 2019). At present, there are no vaccines or effective medical treatments available for dengue (Awang Besar et al. 2019; Mohd Ngesom et al. 2021; Rahman et al. 2021). Personal protective measures such as the use of synthetic mosquito repellents play an important role in preventing this disease.

Mosquitoes are normally attracted and activated by a specific chemical or a blend of chemicals (kairomones) produced by a potential host (Lim et al. 2019; Wooding et al. 2020). Some of potential human-specific kairomones that found to attract mosquitoes include carbon dioxide, lactic acid, and 1-octen-3-ol (Nwanya et al. 2020; Wooding et al. 2020). Repellents that are normally applied on the skin or clothing create an unpleasant condition for mosquitos to land or feed, thus, interrupting the human-mosquito contact (Sneha, Nidhi & Aniket 2018). *N,N*-Diethyl-*m*-toluamide (DEET) is the most common active ingredient in majority of commercially available mosquito repellents. Other common synthetic repellents are permethrin and picaridin (Bhat & Aravind 2017). However, DEET has been reported toxic towards children and elderly and cause damage to plastics and synthetic fabric (Othman et al. 2019). DEET can also cause dermatitis, allergy, neurology and cardiovascular effect, seizures, slurred speech, hypotension, and bradycardia in children (Othman et al. 2019; Wu, Zhang & Yang 2019). Due to these adverse effects and the increased demand for safer and environmentally friendly products, intensive effort towards the development of natural products had been initiated (Abu Bakar et. al. 2023; Oyebamiji et al. 2023; Tisgratog et al. 2016).

Essential oils obtained from plants have been considered as one of the promising alternatives to synthetic repellents. Many studies had reported the potential of plant derivatives, such as aqueous extracts or essential oils, in controlling vector mosquitoes and proven to be more efficient than the synthetic chemicals (Asadollahi et al. 2019; Huang et al. 2019; Lim et al. 2019; Wathoni et al. 2018). Essential oils derived from plants contain a rich source of bioactive compounds that can be utilized in producing green repellent (Asadollahi et al. 2019). The essential oils of citronella, *Cymbopogon nardus* (L.) Rendle (citronella grass), and lemon eucalyptus are some of the proven commercially successful plant based repellents (Paluch, Bartholomay & Coats 2010). Another plant-derived repellent, *p*-menthane-3, 8-diol (PMD) has also been proven to be effective and safe, competing with DEET in disease prevention, and have received recognition by WHO (Maia & Moore 2011). Some essential oils are currently used as active ingredient in many commercial mosquito repellent products that can be applied directly on the skin, treated materials, sprays or repellent aromatic candles (Evergetis et al. 2018).

According to Pappathi et al. (2021), essential oil of *Plectranthus amboinicus* (PA) can be used as a possible mosquitosidal agent. *P. amboinicus* is a perennial aromatic succulent shrub that belongs to Lamiaceae family, is commonly found in tropical and warm region such as Africa, Asia and Australia (Huang et al. 2019; Prasad et al. 2020). PA which is also known by many names such as country borage, Mexican mint, Cuban oregano, Karpooravalli and Bangun-bangun is found abundantly in Malaysia. However, the potential of this plant has not been explored, especially as a mosquito repellent.

Numerous studies have explored the chemical composition and pharmacological properties of PA essential oil and reported the occurrence of various classes of phytocompounds, with terpenes, including monoterpenes and sesquiterpenes, being the most abundant components in its essential oils (Noriega 2020; Ting, Che Ku Jusoh & Hashim 2024). Specifically, oxygenated compounds with hydroxyl groups within these terpenes have demonstrated repellent activity against mosquitoes, as noted by Olivero-Verbel, Nerio and Stashenko (2009). The study by Evergetis et al. (2018) supports these findings, highlighting the potential mosquito repellent activity of Carvacrol, an oxygenated monoterpene.

This study was conducted to evaluate the potential of PAEO as a natural mosquito repellent in comparison to 10% DEET. PAEO was extracted from dried leaves using hydrodistillation method. The chemical composition was identified, and a bio efficacy study was performed using fabric and patches treated with 10% PAEO on human samples. Conducting the test with human volunteers is essential, as the host-seeking and landing response in mosquitoes is influenced by chemical cues such as carbon dioxide, lactic acid, convection heat, and body moisture produced by the host (Gross & Coats 2014; Wooding et al. 2020).

MATERIALS AND METHODS

PLANT MATERIAL

Leaves of *P. amboinicus* plants were collected from four locations within Hulu Langat district in Selangor; Taman Bukit (2.9815°N, 101.7928°E), SMK Jalan Empat Herbal Garden (2.9611°N, 101.7818°E), Taman West Country (2.9632°N, 101.7869°E) and Taman Kajang Prima (2.9793°N, 101.8137°E). All the samples were collected in the month of March, 2023. The plant was identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (ID097/2023) was deposited at UKM Herbarium.

The leaves were then cleaned and air-dried for up to 21 days in a well-ventilated room. The temperature in the room was between 28 °C and 32 °C. The dried leaves were collected and weighed. Once the mass of the leaves remained stable, the leaves were ground using a 750 W Preethi Galaxy electric grinder, sieved to get a uniformed particle size of ≤1 mm^2 and stored in an air-tight container below 4 °C until extraction.

EXTRACTION OF ESSENTIAL OIL

The extraction of essential oil was carried out using a modified method based on British Pharmacopeia (2022). 225 g of dried leaves sample was subjected to hydrodistillation using a Clevenger apparatus with a 1 g:10 mL solid to solvent ratio for 6 h. The chiller was set at 5 °C. The distillate containing oil and water was collected and transferred to a separating funnel. The yellowish essential oil layer that formed at the top was separated and dried using anhydrous sodium sulphate. Dichloromethane was added to the remaining aqueous layer to separate and collect any remaining essential oil in it. The essential oil was dried using 2 g anhydrous sodium sulphate. The anhydrous sodium sulphate was later filtered out and the purified essential oil was stored in a 5 mL air-tight vial at 4 °C. The yield of the essential oil was calculated and expressed as percentage (v/w), as described in Equation (1) (Colucci 2018; Deng et al. 2023; Hidayatulfathi et al. 2019):

$$\text{Yield (\%)} = \frac{\text{volume of essential oil obtained}}{\text{initial weight of the dried leaves}} \times 100 \quad (1)$$

CHEMICAL IDENTIFICATION

The volatile compounds extracted by hydrodistillation were analysed using Agilent 6890N Network GC System and 5975C inert MSD with Triple-Axis Detector. Helium was used as a carrier gas at 1.0 mL/min. A 30 m Agilent silica capillary column with an internal diameter of 0.25 mm and film thickness of 0.25 µm was used. The injector temperature was 230 °C with an injection size of 0.1 µL. The oven temperature was first maintained at 40 °C for 2 min. Then, a two-step temperature increase was performed; to 175 °C at a rate of 5 °C/min, and to 250 °C at 10 °C/min. The ion source temperature was set at 220 °C while the transfer line temperature was set at 280 °C. The phytocompounds were identified based on their retention time (RT) and by matching their mass spectra (> 80% match) of NIST (National Institute of Standards and Technology) library data and literature data (Hassani et al. 2012; Manjamalai, Alexander & Berlin Grace 2012; Monzote et al. 2020).

REARING OF MOSQUITOES

An established colony of adult *Ae. aegypti* mosquitoes were reared and maintained in screened cages at the Insectarium of Test facility of Medical Entomology GLP Laboratory at Institute for Medical Research, Jalan Pahang, Kuala Lumpur, Malaysia. The mosquitoes were fed with 10% sucrose solution while the temperature and humidity were maintained at 27±2 °C and 75±10%, respectively. Female mosquitoes aged between 5 and 7 days (MS1497:2007) (Hidayatulfathi et al. 2017; Misni, Sulaiman & Othman 2008) were isolated from the group and employed for the experiment. Prior to the test, these mosquitoes were deprived of food overnight.

PREPARATION FOR REPELLENT TEST

The test solutions (10% DEET and 10% PAEO) were prepared by diluting the essential oil in absolute ethanol solution and prepared fresh before every test. The repellent activity of 10% PAEO was tested in the form of treated fabric and patch. 10% DEET was used as positive control (MS1497:2007) (Luker et al. 2023; Misni, Sulaiman & Othman 2008). A thin and white cotton fabric was chosen to prepare the treated fabric based on the ability of the material to allow kairomones and body heat to be released through it to activate host-seeking behaviour in mosquitoes.

TABLE 1. Chemical constituents and yield of essential oil from *Plectranthus amboinicus* based on part of plant used, method of extraction and location of sample collection

Sample	Extraction	Distillation time	Location of sample	Yield	Chemical constituent	Reference
Dried leaves	Hydrodistillation	5 h	Africa	1.25 % (v/w)	carvacrol (23.0 %), camphor (22.2 %), δ-3-carene (15.0 %), γ-terpinene (8.4 %), O-cymene (7.7 %)	Hassani et al. (2012)
Fresh leaves	Steam distillation	NI	Malaysia	0.19% (v/w)	carvacrol (85.14%), thymoquinone (1.65%), terpinen-4-ol (0.70%), octenol (0.62%) and thymol (0.23%)	Norazsida, Pakeer & Taher (2017)
Fresh flowers	Hydrodistillation	5 h	India	0.1% (v/w)	carvacrol 50.98%, β- caryophyllene (21.54%), trans α-bergamotene (17.73%)	Joshi (2011)
Fresh aerial parts	Hydrodistillation	5 h	India	0.3% (v/w)	carvacrol (77.14%), β- caryophyllene (5.74%), caryophyllene oxide (3.72%), α-humulene (1.95%)	Joshi (2011)
Fresh leaves	Hydrodistillation	3 h	India	0.5% (v/w)	carvacrol (14.21%), thymol (18.09%), z –caryophyllene (18.06%), trans caryophyllene (5.30%), *p*-cymene (10.83%)	Manjamalai, Alexander & Berlin Grace (2012)
Fresh leaves	Hydrodistillation	NI	Malaysia	0.18% (v/w)	3-carene (20.78%), carvacrol (19.29%), α-terpinene (6.04%), o-cymene (5.06%), δ-terpinene (8.94%), camphor (17.96%)	Erny Sabrina et al. (2014)
Dried whole plant	Hydrodistillation	3 h	Yemen	1.43%	thymol (36.90%), linalool (13.24%), z-isoeugenol (4.22%), δ-cadinene (4.66%)	Hussein, Ahmed, & Algabali (2017)
Fresh leaves	Hydrodistillation	3 h	Brazil	0.12% (v/w)	carvacrol (88.61%), eugenol (1.59%), z-caryophyllene (3.39%), oct-1-en-3-ol (1.79%)	Pinheiro et al. (2015)
Aerial parts	Hydrodistillation	NI	Cuba	0.70-0.75%	carvacrol (71%), γ-terpinene (4.3%), *p*-cymene (4.3%), β- caryophyllene (4.2%%), trans α-bergamotene (2.7%), caryophyllene oxide 1.6%)	Monzote et al. (2020)
Fresh shoots	Hydrodistillation	3 h	Brazil	0.17-0.61%	carvacrol (38.48 – 51.07%), trans caryophyllene (19.80 – 26.65%), α-bergamotene (14.20 – 18.38%), α-humulene (1.95%)	Merlin et al. (2020)

*NI – not informed

Apart from that, white colour was chosen to avoid colour bias as dark colours are known to attract mosquitoes more than light colours. Furthermore, the ability of the fabric to readily absorb the test solution and dry rapidly is also taken into consideration. The fabric was cut into 5 × 10 cm rectangles. 500 µL of 10% PAEO was applied evenly on each piece of fabric using a micropipette. The fabric was then left at room temperature for 60 min to allow the solvent to evaporate fully before conducting the test.

The patch (Figure 1) was carefully designed to enable the essential oil to be confined within the absorbent pad and released slowly through a breathable fabric when applied. A water and oil-proof film was used to prevent the essential oil from seeping out into the adhesive layer and possibly coming into contact with the skin. The low allergy adhesive layer can be attached directly to the skin or the clothing.

REPELLENCY TEST

The repellent activity of PAEO against *Ae. aegypti* was evaluated at the test facility of the Medical Entomology GLP Laboratory at the Institute for Medical Research (IMR), Jalan Pahang, Kuala Lumpur, Malaysia. The test was conducted according to the household insecticide products-personal mosquito repellent evaluation method for biological efficacy (MS1497:2007) provided by the Standard and Industrial Research Institute of Malaysia (SIRIM). Three human volunteers; two females and one male, including the author, participated in the test. All the volunteers were briefed on the procedure and asked to report any undesirable effects, such as skin irritation or burning sensations, immediately. The volunteers signed a consent form prior to the test.

The forearms and hands of all the volunteers were washed with unscented neutral soap, thoroughly rinsed, and allowed to dry before experimentation. A protective glove with a rectangular opening area of 24 cm^2 (3 cm × 8 cm) was used in the experiment. The opening in the glove is designed to limit the landing of mosquitoes to the exposed area only. The glove was used on both arms. The left arm was not treated and acts as negative control while

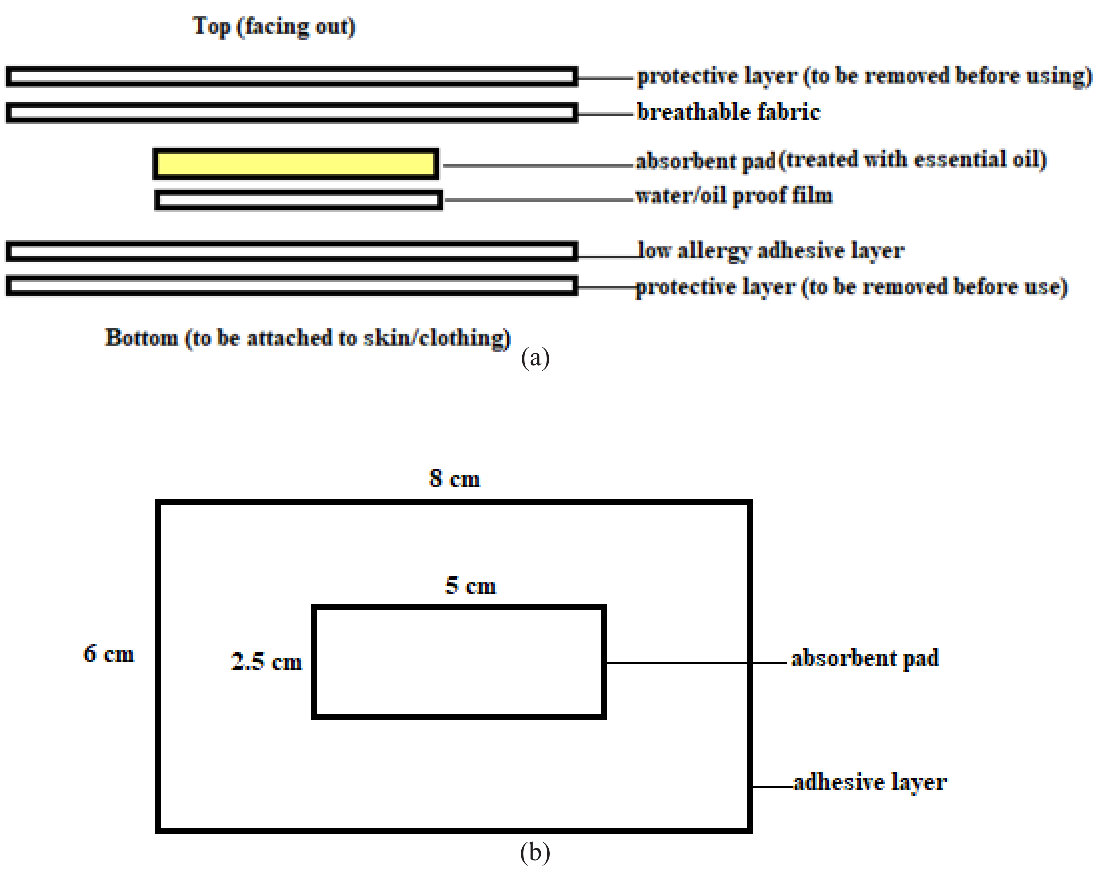


FIGURE 1. Side view (a) and top view (b) of the patch

the right arm was treated with 10% DEET solution, 10% PAEO-treated fabric or 10% PAEO-treated patch. For each treatment, the tests were conducted in triplicates using a fresh set of mosquitoes. The tests were conducted between 8.00 am and 1.30 pm, which is within the peak time for *Ae. aegypti* activity (Lim et al. 2019).

A laboratory cage (60 cm × 60 cm × 60 cm) with two equal compartments (30 cm× 60 cm × 60 cm), separated by a transparent polyethylene Perspex partition board was used for the test. 25 blood-starved (starved overnight), adult female *Ae. aegypti* mosquitoes were released into each compartment and left to acclimatize in the cages for 30 min. The control and treated arms were then introduced simultaneously into the cage through a circular opening, secured by netting. The number of mosquitoes landing in the confined area within 5 min was counted and recorded. This procedure was repeated every 30 min for 4 h post-application (30, 60, 90, 120, 180, 210, 240 min). Each test was repeated thrice to get a mean value. The effectiveness of the test solutions was determined by calculating the percentage of protection using as described in equation (2) (Colucci 2018; Deng et al. 2023; Hidayatulfathi et al. 2019):

$$R\ (\%) = \frac{C-T}{C} \times 100\% \qquad (2)$$

where C refers to the number of mosquitoes landing on the control arm; and T refers to the number of mosquitoes landing on the treated arm.

STATISTICAL ANALYSIS

Two-way ANOVA and Tukey's test were conducted using IBM SPSS Statistics software to examine the effect of type of treatment and exposure time on the protection. Level for significance was set at $p = 0.05$. A Tukey's test was conducted for further mean comparisons between treatments.

RESULTS AND DISCUSSION

THE YIELD OF ESSENTIAL OIL

A total of 1.9 mL of essential oil was extracted from 225 g of ground dried *PA* leaves after a 6 h hydrodistillation process. The yield was 0.8% (v/w), which can be considered as a good yield when compared to majority of reports stated in Table 1. However, it is slightly lower compared to the yield obtained by Hassani et al. (2012). This might be due to the difference in environmental factors such as geographical region, climate, development stage of the plant, plant parts used, and method of sample preparation (Arumugam, Kumara Swamy & Sinniah 2016; Aziz et al. 2018). The oil was pale-yellow in colour and had a pungent smell.

THE CHEMICAL IDENTIFICATION

Chemical identification of PAEO by GC-MS analysis showed the presence of 83 phytocompounds. However, only 6 compounds were found in a higher abundance than 1% (Table 2) while 54 other compounds were below 0.1%. The dominant phytocompound detected was carvacrol (77.26%) which is in line with the majority findings stated in Table 1. Norazsida, Pakeer and Taher (2017) reported the presence of 85.14% of carvacrol PAEO, while Erny Sabrina et al. (2014) reported a lower content of carvacrol of 19.29%. As mentioned earlier, the difference in the yield and the occurrence of major components in PA may be attributed to the difference in environmental factors (Arumugam, Kumara Swamy & Sinniah 2016; Aziz et al. 2018). Other compounds such as caryophyllene (6.63%), trans-α-bergamotene (4.49%), α-humulene (1.80%), caryophyllene oxide (1.18%) and phytol (1.18%) were also found in PAEO (Figure 2).

THE REPELLENCE TEST

A two-way ANOVA was conducted to study the influence of exposure time and type of treatment on percentage protection indicates significant main effect for type of treatment, $p = 0.011$. However, there was no significant effect noted between the exposure time. Additionally, a Tukey HSD post hoc test was employed to assess the significance of differences between pairs of group means for types of treatments. The result indicates a significant difference between 10% PAEO-treated patch and 10% DEET ($p = 0.016$) and 10% PAEO-treated fabric and 10% DEET ($p = 0.026$). However, there is no significant difference noted between 10% PAEO-treated patch and 10% PAEO-treated fabric. Figure 3 shows the mean percentage protection provided by 10% DEET, 10% PAEO-treated fabric and 10% PAEO-treated patch at 240 min.

Figure 4 shows the percentage protection offered by each individual treatment (DEET 10%, PAEO 10% treated fabric, and PAEO 10% treated patch) at different intervals post-application. There was no significant difference in treatment efficacy across different exposure times. Figure 5 illustrates the comparison of percentage protection provided by all three treatments at each time intervals from 30 to 240 min post-application. At 30 min post-application, all three treatments exhibit an equivalent percentage protection ranging from 97% to 99%. Beyond this point, the percentage protection provided by 10% DEET decreases over time, reaching 51% at 240 min post-application. However, the treated patch consistently maintained a high percentage protection up to 100% throughout the test. A complete protection (100%) was achieved at 150 and remained up to 240 min post-application. Meanwhile, treated fabric achieved complete protection at 210 and was maintained up to 240 min post-application. Based on Figure 5, it is evident that significant differences among the three treatments emerge from 150 to 240 min post-application.

The result clearly underscores the potential of PAEO as a natural mosquito repellent. It's worth noting that even though Lalthazuali and Mathew (2017) reported that 20% PAEO provided 100% repellence against *Ae. aegypti* for up to 6 h when compared to 20% DEET, their study did not involve human volunteers as test subjects and utilized a very high concentration of both PAEO and DEET.

The findings presented herewith suggested that the heightened repellent effect of PAEO compared to DEET can be attributed to the presence of carvacrol as the main compound. Carvacrol is acknowledged for its efficacy as a pest control agent. It can cause rapid neurotoxic effects in insects by blocking chloride uptake into neurons through the chloride channel. Moreover, it has been observed to influence the sensory receptors of insects, thereby contributing to its repellent activity (Bagul & Rajput 2018). The distinct, pungent odour associated with carvacrol (Ashaari et al. 2021) may play a crucial role in repelling mosquitoes by potentially blocking the odorant and gustatory receptor proteins located on their antennae, preventing them from finding their host as mentioned by Rodriguez et al. (2015).

CONCLUSION

The identification of chemical constituents from the essential oil shows the presence of carvacrol as the major component with an abundance of 77.26%. Mosquito bioassay conducted using 10% PAEO tested in the form of treated fabric and treated patch demonstrated a better repellent activity when compared to 10% DEET. PAEO-treated patch shows the highest repellent activity against *Ae. aegypti* compared to 10% PAEO-treated fabric and 10% DEET. The result proves that PAEO has the potential to contribute to the development of a new green insect repellent that can help reduce human-mosquito contacts and most importantly mosquito-borne diseases such as dengue. This finding is new in the field of green repellents

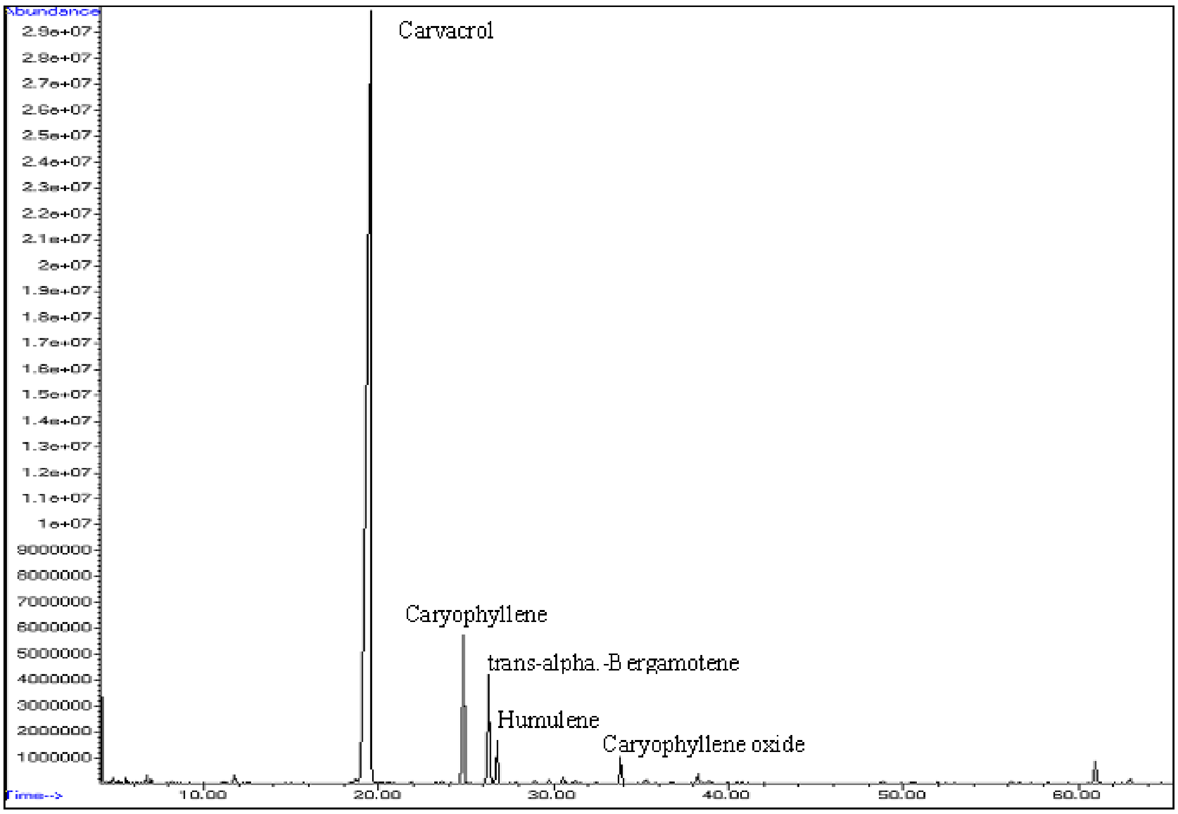
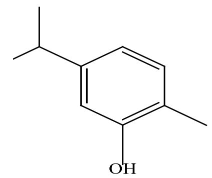
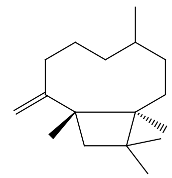
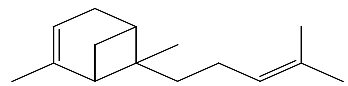
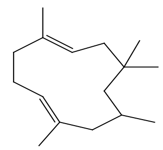
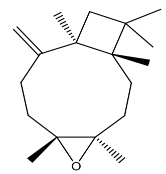
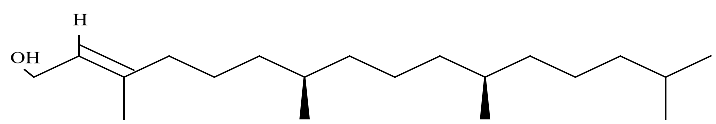
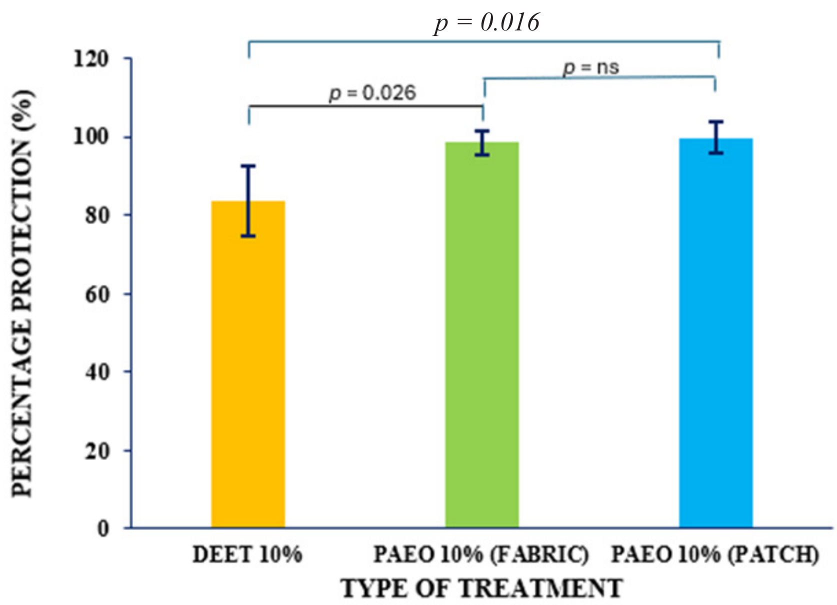


FIGURE 2. GC-MS chromatogram of essential oil from dried *Plectranthus amboinicus* leaves

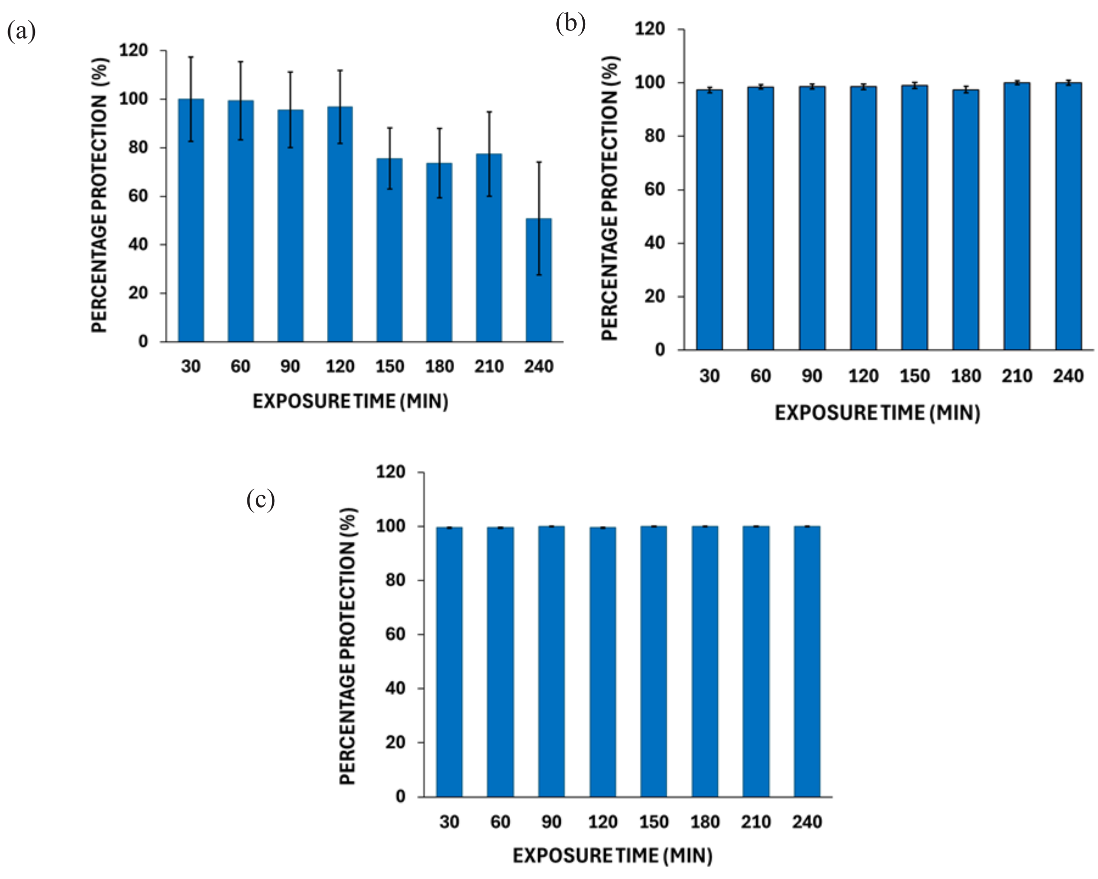
TABLE 2. The six most abundant chemical compounds found in the essential oil of *Plectranthus amboinicus* leaves identified through GCMS

Compound	Ret.time (min)	Peak area (%)	Chemical formula	Library match (%)	Chemical structure	Chemical classification
Carvacrol	19.504	77.26	$C_{10}H_{14}O$	87		Monoterpene
Caryophyllene	24.863	6.63	$C_{15}H_{24}$	99		Sesquiterpene
trans-α-Bergamotene	26.297	4.94	$C_{15}H_{24}$	91		Sesquiterpene
Humulene	26.758	1.80	$C_{15}H_{24}$	98		Sesquiterpene
Caryophyllene oxide	33.841	1.18	$C_{15}H_{24}O$	95		Sesquiterpene
Phytol	60.958	1.18	$C_{20}H_{40}O$	83		Diterpene



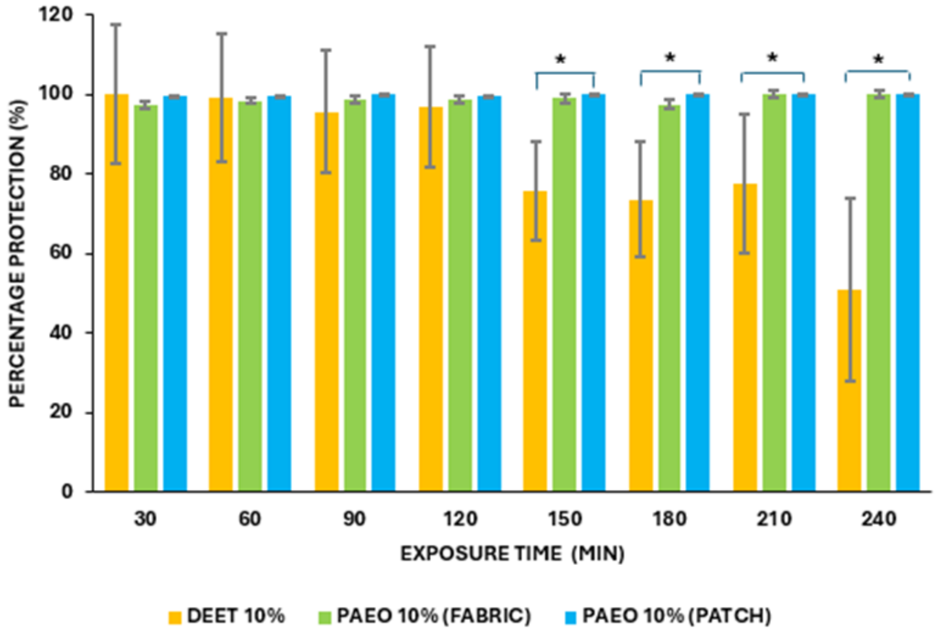
Data shown are the mean value ± SD for the three types of treatments. Values that are significantly different at $p < 0.05$ are marked at the top of the bars, while (ns) refers to no significance. Error bars represent SD

FIGURE 3. Comparison of repellent efficacy of individual treatments at 240 min post application



Data shown are the mean value ± SD for the three types of treatments. (ns) refers to no significant difference. Error bars represent SD

FIGURE 4. Mean percentage protection over exposure time for 10% DEET (a), 10% PAEO-treated fabric (b) and 10% PAEO-treated patch (c)



(*) indicates significant difference between treatments

FIGURE 5. Comparison of repellent efficacy (percentage protection) between treatments at each time interval (exposure time)

as there have not been any similar study or result published up to date on the use of PAEO as green mosquito repellent using human volunteers as test subject.

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