

Comparative study on natural insect repellent properties of *Euphorbia hirta* and *Plectranthus amboinicus*

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ABSTRACT

Growing concern over the health and environmental risks of synthetic repellents like DEET and permethrin has spurred interest in natural, plant-based alternatives. Although many plants have long been used in Malaysia for traditional medicine and pest control, scientific evidence of their repellent potential remains limited. Therefore, this study aims to (1) evaluate the insect repellent efficiency of *Euphorbia hirta* (*E. hirta*) and *Plectranthus amboinicus* (*P. amboinicus*) against hematophagous insects, ants and (2) characterise the bioactive compounds responsible for their repellent properties. The active compounds of phenolic, terpenoids, thymol, and carvacrol from the plants were extracted using ethanol via Soxhlet extraction and concentrated using a rotary evaporator. Repellence tests were conducted at a 10% concentration, with formulations prepared based on terpenoid-to-phenolic ratios of 3:2, 1:1, and 2:3. Results revealed that *P. amboinicus* contained high levels of thymol (41.9%) and carvacrol (28.5%), while *E. hirta* showed notable amounts of quercetin (4.5%) and kaempferol (3.2%). *P. amboinicus* exhibited stronger repellence against ants at higher terpenoid-to-phenolic ratio of 3:2, whereas *E. hirta* demonstrated effective repellence even at lower ratio of 1:1, with minimal ant attraction. Overall, both plants showed promising insect repellent activity, with *P. amboinicus* being more effective at higher concentrations. These findings emphasise the attractiveness of plant-based repellents as sustainable pest control solutions, reducing reliance on synthetic chemicals and promoting eco-friendly practice.

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1. INTRODUCTION

The growing global concern over the adverse health and environmental impacts of synthetic insect repellents has driven significant interest in natural, plant-based alternatives. Synthetic repellents, including DEET (N,N-Diethyl-meta-toluamide) have been widely used as standard insect control agents due to their effectiveness. However, their prolonged use has been associated with skin irritation, neurotoxicity, and environmental persistence, which can contribute to ecological imbalance (Müller et al. 2009). The extensive use of these chemical repellents has therefore raised serious concerns about their toxicity, and long-term sustainability, emphasising the need to develop safer, natural substitutes. Furthermore, the increasing prevalence of insect-borne disease particularly in tropical and subtropical regions, has highlighted the urgent demand for sustainable, eco-friendly pest management solutions. As a result, plant-based repellents are gaining attention as promising alternatives.

Plants contain bioactive compounds that have been used for centuries in traditional medicine and pest control, contributing to their excellent insect-repellent, insecticidal, and antimicrobial properties (Wagner, 2020). Among the wide range of botanical species, *Euphorbia hirta* (*E. hirta*) and *Plectranthus amboinicus* (*P. amboinicus*) are particularly promising candidates for natural insect repellents. *Euphorbia hirta*, commonly known as asthma weed, is a tropical herb traditionally used for its diverse pharmacological activity, including anti-inflammatory, antimicrobial, and antioxidant effects (Mekam et al., 2019). Its insect-repellent activity is mainly attributed to non-volatile phenolic compounds such as flavonoids (quercetin and kaempferol) and alkaloids. These compounds can disrupt insect feeding and reproductive behaviours and have shown activity against *Aedes aegypti* and *Anopheles stephensi* (Elango & Rahuman, 2011; Kasman et al., 2025). Moreover, the long-lasting repellent effects of these compounds make it suitable for sustained pest control applications.

P. amboinicus is an aromatic herb which commonly known as Indian borage or Cuban oregano. Its bioactive components are usually extracted as essential oil, thymol or carvacrol. These volatile compounds exhibit strong insect-repellent properties by interfering with insect olfactory receptors, thereby masking host attractants such as carbon dioxide and body odours (Arena et al., 2025). Additionally, thymol, and carvacrol are well known for their antimicrobial, antifungal, and antioxidant properties, which have been traditionally used in pest management and medicine (Misra, & Pavithra, 2020). In contrast, the phenolic constituents found in *E. hirta* demonstrate greater chemical stability and prolonged repellence due to their slower degradation and oxidation rates compared to volatile oils.

Previous study recorded few comparison studies evaluate the relative effectiveness of *E. hirta* or *P. amboinicus* against ants; instead, majority of research focusses on each species separately. Despite that both species exhibit antioxidant properties, their specific bioactive pathways and molecular targets are poorly characterised. Therefore, suitable extraction method should be introduced as solvent polarity and extraction temperature significantly influence the yield and concentration of key bioactive compounds such as flavonoids and terpenoids, ultimately affecting reproducibility and potency of extract. Variability in extraction processes and lack of standardised formulation methods present significant challenges to the large-scale and pharmaceutical utilisation of these plant extract. This gap in knowledge limits the broader adoption of these plants as viable alternatives to synthetic repellents, particularly in regions where mosquito-borne diseases such as malaria, dengue, and Zika pose significant public health challenges. Therefore, this study aims to address these gaps by characterising the bioactive compounds

presents in *E. hirta* and *P. amboinicus* responsible for insect-repellent activity, and by evaluating and comparing their repellent efficacy at varying trafenoid-to-phenolic ratios (3:2; 1:1; and 2:3). The effectiveness of each formulation will be assessed based on the repellence over time.

2. MATERIAL AND METHOD

2.1 Material

Both plants used in this study, *E. Hirta* and *P. Amboinicus* were collected from nurseries. The ethanol 95% (Vchem, United State) was used for extraction. All dilution and cleaning were performed using distilled water. Summary of research is presented in Fig. 1.

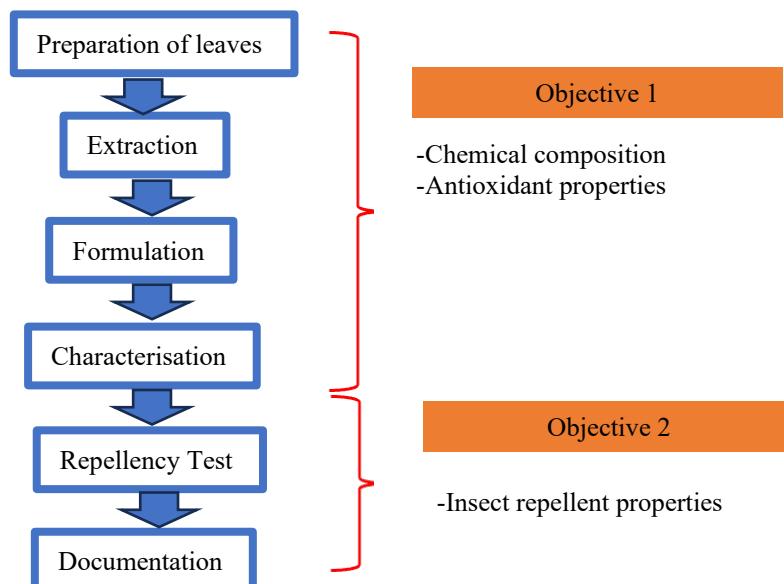


Fig. 1: Research framework of repellency test for Ant using *E. hirta* and *P. amboinicus*

Source: Author's own data

2.2 Preparation of Extraction

E. hirta and *P. amboinicus* leaves were collected and separated from each plant. The leaves were placed on trays and oven-dried at 65 °C for 4.5 h. The dried leaves were then ground into fine particles (<2 mm) using a mechanical grinder to increase surface area and enhance contact with the solvent during extraction. The extraction process was conducted using Soxhlet Extractor (Beh-Labor Behrotest, Germany). A total of 150 mL 95% ethanol was added to four round bottom flasks. Approximately 15 g of dried leaf powder from each plant was placed into separate thimbles and inserted into Soxhlet apparatus. The sample-to-solvent ratio was maintained between 1:5 to 1:10 to optimise the extraction efficiency. The temperature of each round bottom flask was maintained at 65 °C, and extraction was carried out for 5 h. After extraction, the crude extracts were collected in conical flasks and labelled. They were then transferred

into a rotary evaporator (Heidolph, Germany) and concentrated under reduced pressure at 40 °C to remove the ethanol. This process allowed the recovery of concentrated plant extracts by gentle evaporation of the solvent without degrading the thermally sensitive bioactive compounds.

2.3 Insect repellent formulations

The preparation of the insect repellent solutions was carried out using a solvent-to-extract ratio of 90:10 (v/v). This preparation was based on the preparation method suggested by Elango and Rahuman (2011) at 5–10% (v/v) for repellence test against *Aedes aegypti* and *Anopheles stephensi*. To prepare 10 mL of each repellent solution, the required amount of crude extracts from *P. amboinicus* and *E. hirta* were formulated according to Table 1. This preparation is based on the equal-step-variation to provide consistent influence of terpenoid-to-phenolic composition on each plant. The extract in ethanol was first dissolved in distilled water and then mixed thoroughly using a magnetic stirrer to ensure homogeneity. The prepared solutions were transferred into labelled airtight containers and stored at 4 °C until further use for repellence test. The pH of insect-repellent formulation was measured to evaluate their stability and suitability. The pH was collected from a calibrated pH meter (Mettler Toledo). Each sample was tested in triplicate, and the results were expressed as the mean ± standard deviation.

Table 1: Ratio concentration of *P. amboinicus* and *E. hirta* per volume

No.	<i>Plectranthus amboinicus</i>	<i>Euphorbia hirta</i>
Sample 1	3	3
Sample 2	1	1
Sample 3	2	2

Source: Author's own data

2.4 Characterisation study

Fourier Transform Infrared Spectroscopy (FTIR) (Bruker Vertex 70) in attenuated total reflectance (ATR) was employed to identify and characterise functional groups and molecular structures in of *P. amboinicus* and *E. hirta*. FTIR analysis is particularly valuable in phytochemical studies for identifying the presence of functional groups such as hydroxyl (–OH), carbonyl (C=O), and alkene (–C=C–) groups, which are common in bioactive compounds like flavonoids, terpenoids, and phenolics. The liquid extract sample were directly placed onto the ATR crystal and scanned within the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} to obtain the characteristic absorption spectra.

UV-vis spectroscopy (Shimadzu UV 1800 PC) is an essential analytical technique for evaluating antioxidant activity. The antioxidant potential of the plant extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, with absorbance measured across wavelength from 400 to 600 nm. Previous studies have shown that the characteristic absorption peak for DPPH occurs at 517 nm, corresponding to its antioxidant activity (Murtaza et. A., 2023). This wavelength was therefore used to quantify the antioxidant potential of the plant extracts. For the assay, accurate stock solutions of plant extracts and the DPPH reagent were prepared. About 1 mg of the DPPH was dissolved in 50 mL of 99% ethanol to achieve the desired concentration, and the solution was stored in the dark for 30 minutes to prevent DPPH degradation. Separately, 8 mg of ascorbic acid was dissolved in 8 mL of 99% ethanol to

serve as antioxidant reference. The test mixtures were then prepared as follows: 20 mL of mixed solution containing equal volumes of *E. hirta* extract and DPPH solution, and 10 mL solution consisting of 5 mL of *P. amboinicus* extract and 5 mL of *E. hirta* extract in ethanol. The mixtures were left to stand for 30 minutes at room temperature to allow the reaction to occur before absorbance measurements were recorded.

2.5 Repellency test

The repellency bioassay followed a modified surface-choice test, similar to the method described by Qin et. al. (2017), where treated and untreated food source was simultaneously exposed to worker ants, and attraction was measured over time. Approximately four candies were placed on white papers to observe ant attraction to each candy as shown in Fig. 2(f).

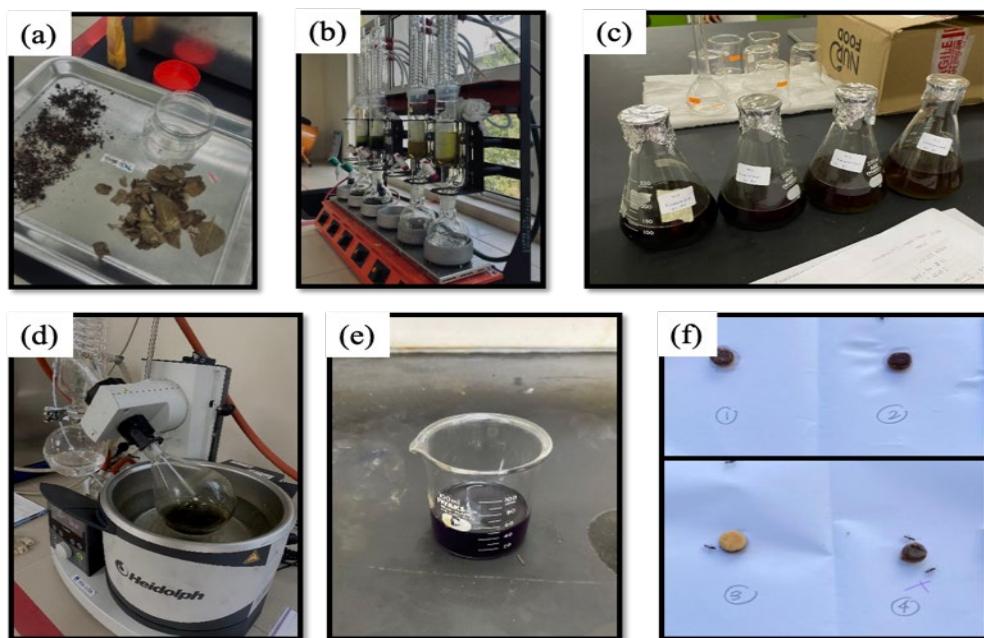


Fig. 2: Preparation of repellency test for ants using *E. hirta* and *P. amboinicus* (a) Preparation of leaves (b) extraction process using Soxhlet extraction (c) four different samples of extraction of leaves (d) removal of ethanol solvent using rotatory evaporator (e) final extraction of leaves (f) repellency test at 5 minutes

Source: Author's own data

To evaluate the ant-repellent activity, three candies were sprayed with 1.5 mL of the natural insect-repellent solution prepared as described in Table 1, while one candy served as control (without repellent). Repellency was assessed based on the number of *Tapinoma melanocephalum* ants attracted to the treated and untreated candies. The test was performed in triplicate ($n=3$), and mean and standard deviation were calculated to represent data consistency. Observations were recorded at 5, 10, 20, 30, and 60 minutes after exposure at room temperature. The overall procedure, from sample preparation to repellency testing, is illustrated in Fig. 2. The repellent assessment followed the general procedure adapted from WHO guidelines for insect repellent efficacy testing, with minor modifications.

3. RESULT AND DISCUSSION

3.1 FTIR analysis

Fig. 3 shows the FTIR spectra of (a) *P. amboinicus* and (b) *E. hirta* revealed distinct peaks corresponding to key functional groups associated with bioactive compounds responsible for insect repellent activity in Fig. 4. The FTIR spectrum of *P. amboinicus* in Fig. 3 confirms the presence of bioactive constituents' characteristics of its essential oil, such as thymol and carvacrol. The broad absorption band at 3300 cm^{-1} corresponds to O–H stretching vibrations, which have been reported to contribute to antioxidant and insect-repellent activity (Preethi & Manickam, 2017). The peaks near 3035 cm^{-1} , associated with C–H stretching, indicate the presence of aliphatic and aromatic structures. A strong absorption band between 1500 and 1700 cm^{-1} relates to C=O stretching, indicate the presence of trace aldehydes and ketones that enhance the bioactivity of essential oil components (Muhammad et. al., 2024). The peak at 1067 cm^{-1} corresponds to C–O stretching and aromatic ring vibrations, consistent with hydroxyl and methyl substituted aromatic rings known to improve insect repellent efficacy (Raut & Karuppayil, 2014). The existence of these characteristics peaks supports previous findings that phenolic monoterpenes such as carvacrol and thymol play a key role in insecticidal and repellent activity (Leesombun et al., 2022). In contrast, the broad absorption band observed at 3300 cm^{-1} corresponds to O–H stretching vibrations, indicating the presence of hydroxyl ($-\text{OH}$) groups commonly associated with alcohols or phenolic compounds. These compounds are known for their antioxidant and antimicrobial properties, which contribute to insect-repellent activity. The peak at 2900 cm^{-1} , representing C–H stretching, is attributed to non-aromatic hydrocarbons or fatty acids. A strong peak around 1700 cm^{-1} suggests C=O stretching, corresponding to carbonyl groups from aldehydes, ketones, or carboxylic acids. These compounds are often volatile and have been associated with flavonoids and phenolic compounds known for their insect-repellent potential. A sharp peak at region $1300\text{--}1000\text{ cm}^{-1}$ corresponds to C–O stretching vibration involving aromatic ring structures. This region is particularly important for identifying bioactive compounds unique to *E. hirta*.

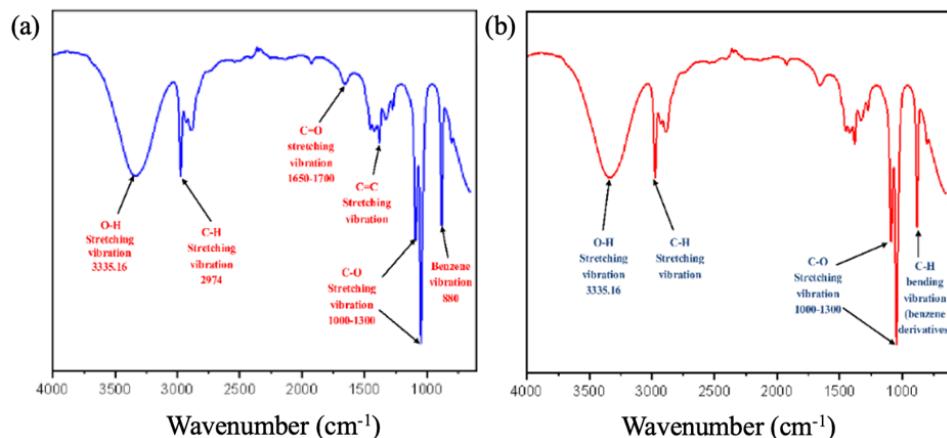


Fig. 3: Characterisation of FTIR analysis for (a) *P. amboinicus* and (b) *E. hirta*

Source: Author's own data

<https://doi.org/10.24191/mjct.v8i2.6849>

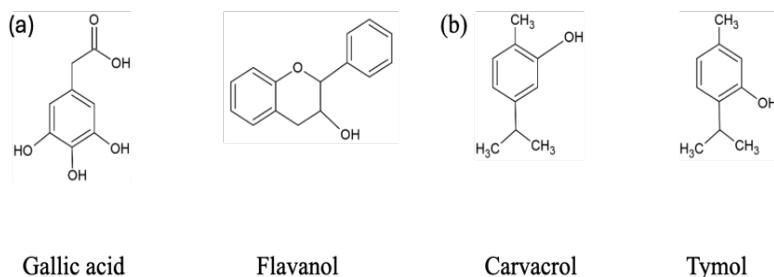


Fig. 4: Functional groups responsible for antioxidant properties of *E. hirta* (a) flavonoid and phenolic acid of *P. amboinicus* and (b) thymol and carvacrol for insect repellent properties in plants leaves

Source: Author's own data

Overall, the FTIR results confirm the presence of $-\text{OH}$, $\text{C}=\text{O}$ and $\text{C}-\text{O}$ groups in both plant extracts, validating the existence of phenolic and terpenoid compounds such as flavonoids, thymol, and carvacrol. These functional groups are known contributors to antioxidant and insect repellent properties by disrupting insect repellent mechanisms and providing oxidative stability. Although previous research by Othaman et al. (2024) examined the phytochemical composition of *E. hirta* and *P. amboinicus* for their pharmacological and antimicrobial properties, but their work didn't include FTIR characterisation. In contrast, our work confirms the presence of these functional groups establish direct link between functional groups and their potential insect repellent bioactivity.

3.2 Antioxidant properties

The antioxidant properties of the *E. hirta* and *P. amboinicus*, as analysed by UV-vis spectrophotometry, are presented in Fig. 5. The spectra were presented by the blue and orange lines, display four distinct peaks demonstrated that both plants share a common set of antioxidant compounds. These peaks correspond to the absorption bands of bioactive components such as flavonoids, phenolic acids, and tannins, which are known contributors to antioxidant activity. Notable, *E. hirta* showed higher absorbance peaks compared to *P. amboinicus*, suggesting a higher concentration or greater potency of antioxidant compounds. This finding implies that *E. hirta* possesses stronger radical-scavenging capabilities and may be more effective in neutralising free radicals.

Fig. 5. clearly shows that the *E. hirta* broad absorption bands around 270-350 nm attributed to flavonoids and phenolic acids. These compounds have strong electron-donating ability, explaining the high DPPH scavenging activity reported for *E. hirta* extracts (Basma et al., 2011). In contrast characteristic band for *P. amboinicus* was observed at 665 nm related to chlorophyl derivatives and essential oil pigments. However, their antioxidant effect via DPPH is lower than *E. hirta* which observed at 657 nm due to the volatility of terpenoids create less efficient radical scavengers compared to stable phenolics (Nerio et al., 2010). This suggests that both plants contain chlorophyll derivatives or similar compounds that are crucial for scavenging free radicals and may be responsible for their antioxidant and insect repelling properties (Tak & Isman, 2017).

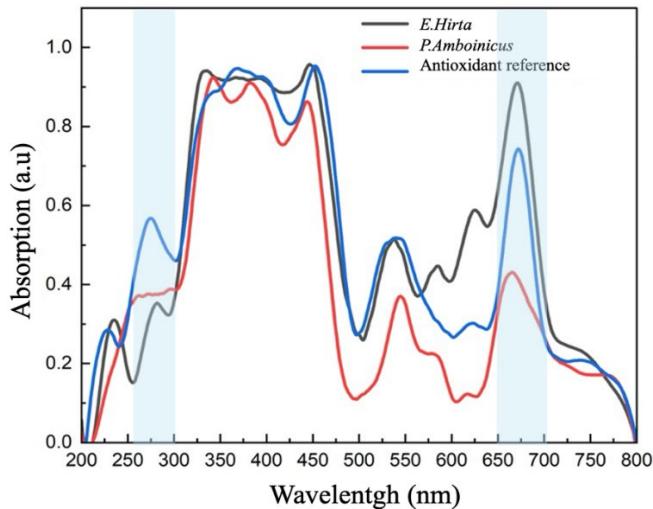


Fig. 5: UV absorption peaks of antioxidant properties of *E. hirta* and *P. amboinicus*.

Source: Author's own data

A pronounced difference was observed at 608.5 nm, where *E. hirta* recorded a higher absorbance compared to *P. amboinicus*. Similarly, a more distinct variation occurred at 535.5 nm, where this peak region is typically associated with phenolic compounds, which are non-volatile and well known for their insect-repellent and antimicrobial properties (Raut & Karuppayil, 2014). The stronger absorbance of *E. hirta* in this region supports the hypothesis that it contains a higher concentration of these bioactive substances, contributing to its greater effectiveness as a natural repellent. Finally, *E. hirta* displayed a notable peak at 506 nm (3.138), whereas *P. amboinicus* exhibited a weaker response at 505 nm (1.675). These results collectively indicated that *E. hirta* possesses more intense absorption characteristics across the visible range, reflecting its higher content of antioxidant and bioactive compounds compared to *P. amboinicus*. As previously reported, a higher abundance of carotenoids or related pigments can enhance a plant's functional role in pest control by affecting insect sensory and behavioural pathways (Heath et al., 2013). These pigments not only contribute to insect terrence but also provide ultraviolet (UV) protection and oxidative stress. Consequently, *E. hirta* appears to be more stable option for long term applications, as its carotenoid content may contribute to both chemical and environmental stability. Flavonoids can disrupt insect feeding and reproductive behaviours while offering additional protection against UV degradation and oxidative stress (Basma et al., 2011).

Overall, the comparative analysis indicates that stable, non-volatile compounds such as flavonoids and phenolic acids are responsible for the sustained repellent activity and environmental persistence observed in *E. hirta*. In contrast, *P. amboinicus*, which exhibits a pronounced absorbance peak of volatile essential oils that provide rapid but short-lived repellence. This finding is consistent with previous research showing that volatile compounds in *P. amboinicus* contribute to immediate repellency but lack the longevity associated with the more stable, non-volatile phenolics found in *E. hirta* (Jeramillo-Colorado et al. 2020). Unlike previous work focusing solely on GC-MS or phytochemical screening without spectroscopic correlation.

3.3 Repellency test

The repellency test against ants was conducted using three samples as 1, 2, and 3 corresponding to increasing concentration of *E. hirta* and *P. amboinicus* (Fig. 6). Sample 1 served as the control, and findings indicate that sample 2 exhibited the lowest number of ant attraction compared to sample 3. The reduced attraction of ants at higher concentrations of *P. amboinicus* extract is attributed to its stronger repellent effect, primarily due to the higher presence of bioactive compounds such as thymol (41.9%) and carvacrol (28.5%) (Nerio et al., 2010). Previous study has reported that these compounds disrupt the sensory system of ants through volatile odours, which overwhelm the ants' ability to detect food source and pheromone trails. At elevated concentrations, the intensity of these chemical signals overstimulates and interferes with the ants' sensory receptors, resulting in avoidance behaviour. Furthermore, both thymol and carvacrol have been identified as neurotoxic agents to insects, enhancing their repellent action (Raut & Karuppayil, 2014). The combined effect of neurotoxicity and strong odour stimuli triggers a natural avoidance response as part of the ants' instinctive survival mechanism. This dose-dependent repellency effect suggest that it is higher concentrations of *P. amboinicus* extract produce stronger repulsive behaviour (Muhammad et al., 2024). Hence, the essential oil of *P. amboinicus* demonstrate potential as a natural and eco-friendly alternative to synthetic repellents for ant control, supporting its application in Integrated Pest Management for home and agricultural usage.

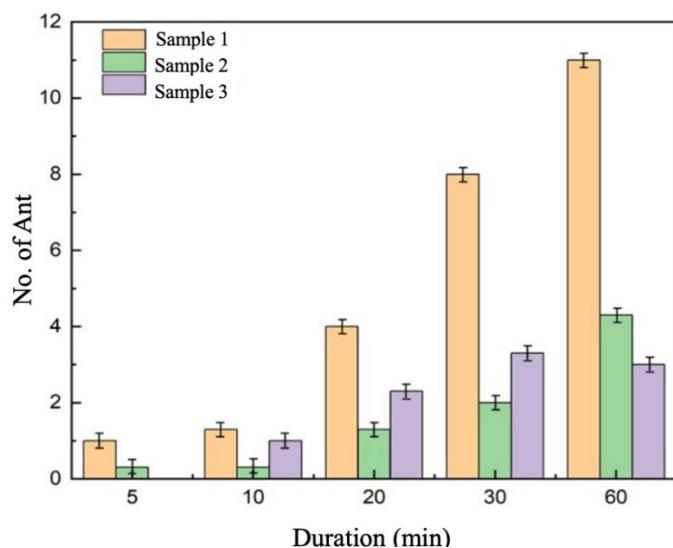


Fig. 6: Repellence assessment of attraction ants to candy at different time

Source: Authors' own data

The pH test is essential in the formulation of natural insect repellents because it ensures the product's safety, effectiveness, and compatibility with human skin. Table 2 indicates that sample 2 exhibits the highest alkaline result among the samples. Recent research is very limited in validating the effectiveness of insect repellents as it predominantly focused on their active ingredients rather than the regulating of pH levels. However, an alkaline insect-repellent solution can influence insects' sensitivity. Consequently,

numerous insects exhibit sensitivity to alteration in pH, particularly within their respiratory systems. An alkaline formulation ($\text{pH} > 7$) might disrupt the protective waxy coating, leading to moisture loss in insects and ultimately resulting in their demise.

Table 2: The final pH of insect-repellent solution

Samples	pH
1	8.13 ± 0.01
2	8.16 ± 0.00
3	8.15 ± 0.01

Source: Authors' own data

4. CONCLUSION

The insect repellent properties of *E. hirta* and *P. amboinicus* against ants were effectively assessed in this work. The factors contributing to their insect propellant properties were identified through their main active compounds such as flavonoids, phenolic compounds, thymol, and carvacrol. *E. hirta* was shown to be effective even at lower levels, but *P. amboinicus* demonstrated higher repellency at increased concentrations. Their bioactivity and antioxidant properties were validated by analytical methods such as FTIR and UV-vis spectroscopy. It is recommended to introduced advanced testing methods, Y-tube olfactometry to increase the accuracy and repeatability of repellency assessments. Lastly, further research into the molecular mechanisms and synergistic effects of these plants will optimise their repellent capacity, paving the way for sustainable, chemical free pest management solutions.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHORS' CONTRIBUTIONS

Hanis Nur Nabilah binti Hazri: Conceptualisation, methodology, formal analysis, investigation and writing-original draft; **Arbanah Muhammad:** Conceptualisation, supervision, methodology, and review validation, and editing formal analysis; **Nur Hazirah Rozali Annuar:** Conceptualisation, methodology, formal analysis; **Siti Rahika Abdul Halim:** Conceptualisation, formal analysis, and validation.

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