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Research paper

Syzygium aromaticum flower essential oils as a source of antioxidant and mosquito repellent

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ABSTRACT

Context: *Aedes albopictus* is a vector of dangerous diseases such as dengue and Zika, requires effective repellents. ***Objectives:*** This study investigates the potential of *Syzygium aromaticum* essential oils as an antioxidant agent and their efficacy in repelling against *A. albopictus*. ***Methods:*** Hydro distillation and GC and GCMS, DPPH, FRAP. *In vivo* test used Azman Kamal rats.. ***Results:*** The essential oils of *S. aromaticum* comprised of eugenol (79.12%), β-caryophyllene (11.60%) and (*Z*)-Isoeugenol acetate (5.56%) as their major constituents. It also has a high antioxidant effect, with the DPPH test showed 80% inhibition and the FRAP test displayed a value of 300 µmol Fe^{2+} per mg of extract. In addition, the mosquito-repellent test showed that *S. aromaticum* EO has a 100% mosquito-repellent effect. ***Conclusion:*** *S. aromaticum* was found to be an effective antioxidant repellent against *A. albopictus* mosquitoes.

Keywords: *Aedes albopictus,* antioxidant, hydro distillation, mosquito repellant, *Syzygium aromaticum,*

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INTRODUCTION

Aedes albopictus, also known as the Asian tiger mosquito, is a potent vector for a spectrum of dangerous diseases, including dengue fever, the Zika virus and chikungunya (Silva *et al*. 2020). The serious health risks associated with these mosquito-borne infections underscore the urgent need for effective mosquito repellents (Onen *et al*., 2023). Mosquitoes, notorious for their annoying bites and potential transmission of diseases, become an unwelcome sight, especially in the warmer seasons (Onen *et al*., 2023). In the search for effective and safe insect repellents, natural solutions have attracted much attention (Mapossa *et al*., 2021). Essential oils from plant have proven to be a promising, environmentally friendly alternative for mosquito control (Asadollahi *et al*., 2019). Essential oils (EO), which are extracted from a variety of plants and herbs, have mosquito-repellent properties due to their ingredients such as citronellal, geraniol, eucalyptol and the now widely known sandalwood oil (Sharma *et al*., 2019). *Syzygium aromaticum* (*S. aromaticum*) originates from the Maluku Islands in Indonesia and is a versatile spice extracted from the dried flower buds of the *S. aromaticum* tree (Cortés-Rojas *et al*., 2014). It is characterized by a strong sweet and spicy aroma and an elongated shape. The EO of *S. aromaticum*, rich in eugenol and eugenyl acetate, contributes to its unique flavor, fragrance and repellent effect (Haro-González *et al*., 2021). Tannins and flavonoids, including kaempferol and rhamnetin, have antioxidant and anti-inflammatory effects (Mittal *et al*., 2014). The medicinal use of *S. aromaticum* is diverse, with eugenol acting as a natural analgesic (Nisar *et al*., 2021). Its antimicrobial properties are used in oral hygiene and infection control. In traditional medicine, *S. aromaticum* is valued for its carminative and anti-inflammatory properties for digestive complaints (Haro-González *et al*., 2021). This research aims to investigate the potential of certain plant EOs for effective defense against *A. albopictus*. Each of these oils has unique compositions and properties that play a crucial role in deterring these disease-carrying mosquitoes. Therefore, this study aims to shed light on nature's inherent ability to contribute to mosquito control through the use of the extracted EO, while potentially providing additional benefits for holistic well-being.

METHODS

Plant material and extraction of essential oil: The method for the preparation of *S. aromaticum* EO was developed according to Selles *et al*., (2020). The dried flowers of *S. aromaticum* were purchased from the local market. The flowers were air-dried and ground to a fine powder using a stainless steel blender. The EOs were extracted by hydro distillation. The EO was collected dried over anhydrous sodium sulphate (Na_2SO_4) and stored in a refrigerator at 4°C before GC/MS analysis and biological screening.

Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS): GC analysis of *S. aromaticum* EO was conducted using Shimadzu GC-2010 Plus capillary chromatograph which was equipped with a flame ionization detector (FID) and using split/split less mode injection technique, under the following conditions: carrier gas helium; similar temperature for injector and detector at 250°C. This chromatograph uses capillary column HP-5MS (30m x 0.25mm x 0.25µm). Operating

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conditions are; initial oven temperature, 60°C for 10 min, up to 230°C at 3°C/min, and then 230°C for 10 min. The GCMS analysis was performed using Agilent Technologies GCMS 7890A/5975C Series MSD system, equipped with HP-5MScapillary column (30 m × 0.25 mm I.D × 0.25 μm film thickness). The temperature program was set as above. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The ionization energy (IE) was 70 eV. The temperatures of the injection and transfer line were 250°C. The temperature of the ion source was set at 280 °C. The EO data were obtained by recording full scan mass spectra in the scan range of 45–550 A.M.U. The chemical constituents were identified by comparison of the calculated Kovat's Indices (KI) with literature values and matching their mass spectra with the database library (HPCH2205.L; Wiley7Nist05.L; NIST05a.L).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay: The DPPH scavenging assay was performed according to Tiveron *et al*. (2012) with slight modifications. A DPPH reagent was prepared by mixing 1.77 g of DPPH powder in 10 ml of methanol. Previously prepared EO (50 μL) was mixed with 100 μL DPPH reagent in a 96-well microplate. The microplate was incubated in the dark for 30 minutes. The absorbance of the solution was measured at a wavelength of 540 nm. The experiments were performed in triplicate. Ascorbic acid (5 mg/ml) was used as positive control.

Ferric Reducing Anti-oxidant Power (FRAP) assay: The FRAP assessment was performed according to the procedure described by Benzie and Strain (1996). The FRAP reagent contained 2.5 mM of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$ - $6H_2O$ and 25 mM of a 300 mM acetate buffer (pH = 3.6). A standard curve was generated with a concentration of 0.1–2 mmol / l $FeSO_4$ - $7H_2O$. The 30 μL EO was added to a 96-well plate. Then 200 μL FRAP reagent was added to the microplate and mixed with a microplate mixer for a few minutes. The reaction mixture was stirred at 37°C for 30 minutes and the absorbance was measured at 593 nm using a spectrophotometer (TECAN, Männedorf, Switzerland). The experiments were carried out in triplicate.

Mosquito rearing: Adult mosquitoes were bred from *A. albopictus* eggs from the FRIM buildings by collecting the eggs using ovitraps according to the method developed by Dieng *et al*., 2010. The eggs were reared in an optimal environment at a temperature of 27°C in controlled laboratory facilities to ensure successful hatching. The larvae were fed with 10% mouse liver. Meanwhile, the adult mosquitoes were fed with a mixture of 10% glucose and 5% vitamin B2 in 100 ml distilled water.

Azman Kamal (AZK) rats: This study was conducted with male AZK rats. The rat was bred through a selective inbreeding program at the Forest Research Institute Malaysia (FRIM) and registered in the Rat Genome Database (RGD) in 2021 (RGD, 2021). This rat is hairless, which makes it suitable for a mosquito repellent study. The average weight of the AZK rats was 145-250 g. They were kept in a normal environment (25°C, 12h/12h light/dark cycle) with a standard diet and water ad libitum. They were acclimatized for one week before the study. The study design and procedures followed the Institutional Animal Use and Care Committee (IACUC) FRIM guidelines. Prior ethical approval was obtained from the IACUC-FRIM (Approval number: IACUC-FRIM/01/3a-2022).

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Mosquitoes repellency test: A mosquito repellent test was performed according to Peng *et al*., 2022. The test was carried out with AZK rats from the FRIM animal house. Before the experiment, the mosquitoes were not fed for 24 hours. On the day of the experiment, the AZK rats were injected with 0.1% ketamine and rendered unconscious. In a repellent kit, 3 mosquitoes were isolated and exposed to the rats for 5 minutes. In the negative control group, the skin of the rat was not rubbed with any sample. In the group treated with EO, one drop of 100 µl of the sample was applied to the rat's body.

Statistical analysis: one-way analysis of variance (ANOVA), examined through Bonferroni’s test at significance level p = 0.05.

RESULTS

The chemical analysis of *S. aromaticum* EO revealed the presence of 28 (99.72%) volatile constituents (Figure 1). According to Table 1, the result showed that the EO of *S. aromaticum* contains eugenol (79.12%), β-caryophyllene (11.60%) and (Z)-Isoeugenol acetate (5.56%) as major constituents. It also contains moderate amounts of α-Humulene (1.25%), 2-Heptyl acetate (1.04%), Methyl salicylate (0.16%), Caryophyllene oxide (0.16%). The EO of *S. aromaticum* also contains small amounts of compounds ranging from 0.01% to 0.09% which are (*E*)-β-ocimene (0.01%), *trans*-Cadina-1(6)-4-diene (0.01%), β-Selinene (0.01%), α-muurolene (0.01%), (*E*)-anethole (0.02%), (*E,E*)-α-farnesene (0.02%), Germacrene B (0.02%), α-cadinol (0.02%), Benzyl acetate (0.03%), Ethyl benzoate (0.03%), 2-Undecanone (0.03%), α-selinene (0.03%), δ-Cadinene (0.03%), Caryolan-8-ol (0.03%), Linalool (0.04%), γ-Cadinene (0.05%), aromadendrene (0.06%), 2-nonanone (0.07%), bicycogermacrene (0.07%), (*E*)-iso-eugenol acetate (0.07%), α-cadinene (0.08%), and chavicol (0.09%).

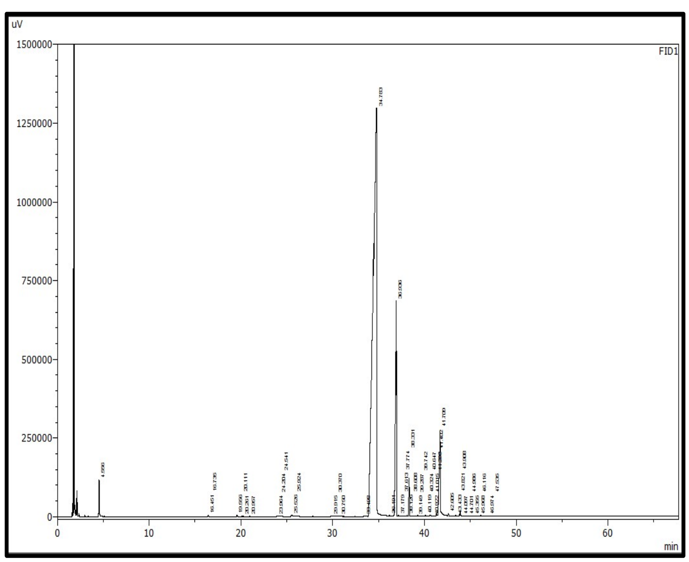

Figure 1: GC chromatogram of *S. aromaticum* EO

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Ascorbic acid showed the highest DPPH inhibition percentage, which was 82% (Table 1). However, BHT showed significantly lower DPPH inhibition compared to ascorbic acid, which was 75%. Moreover, the result showed that the *S. aromaticum* EO 100% has higher antioxidant activity, which can inhibit DPPH by 80%, which is close to ascorbic acid value. *S. aromaticum* EO 50% showed lower values and no significant difference with BHT. *S. aromaticum* 50% showed lower values compared to *S. aromaticum* EO 100%.

Table 1: Volatile constituents of *S. aromaticum* EO.

No	Chemical name	RI	RT	%
1	2-Heptyl acetate	1038	4.556	1.04
2	(*E*)-β-ocimene	1050	16.735	0.01
3	2-Nonanone	1091	19.556	0.07
4	Linalool	1098	20.111	0.04
5	Benzyl acetate	1163	23.964	0.03
6	Ethyl benzoate	1170	24.204	0.03
7	Methyl salicylate	1190	25.526	0.16
8	Chavicol	1253	29.915	0.09
9	(*E*)-Anethole	1283	30.370	0.02
10	2-Undecanone	1291	30.750	0.03
11	Eugenol	1356	34.783	79.12
12	β-Caryophyllene	1418	36.936	11.60
13	Aromadendrene	1439	37.179	0.06
14	α-Humulene	1454	38.126	1.25
15	*trans*-Cadina-1(6)-4-diene	1475	38.608	0.01
16	β-Selinene	1489	39.742	0.01
17	α-Selinene	1498	40.119	0.03
18	Bicycogermacrene	1500	40.647	0.07
19	α-Muurolene	1501	40.824	0.01
20	(*E,E*)-α-Farnesene	1508	40.922	0.02
21	γ-Cadinene	1513	40.949	0.05
22	δ-Cadinene	1524	41.402	0.03
23	α-Cadinene	1537	41.595	0.08
24	Germacrene B	1556	41.655	0.02
25	(*Z*)-Isoeugenol acetate	1563	41.709	5.56
26	Caryolan-8-ol	1571	43.433	0.03
24	Caryophyllene oxide	1581	43.908	0.16
27	(*E*)-Iso-eugenol acetate	1611	44.097	0.07
28	α-Cadinol	1653	44.701	0.02

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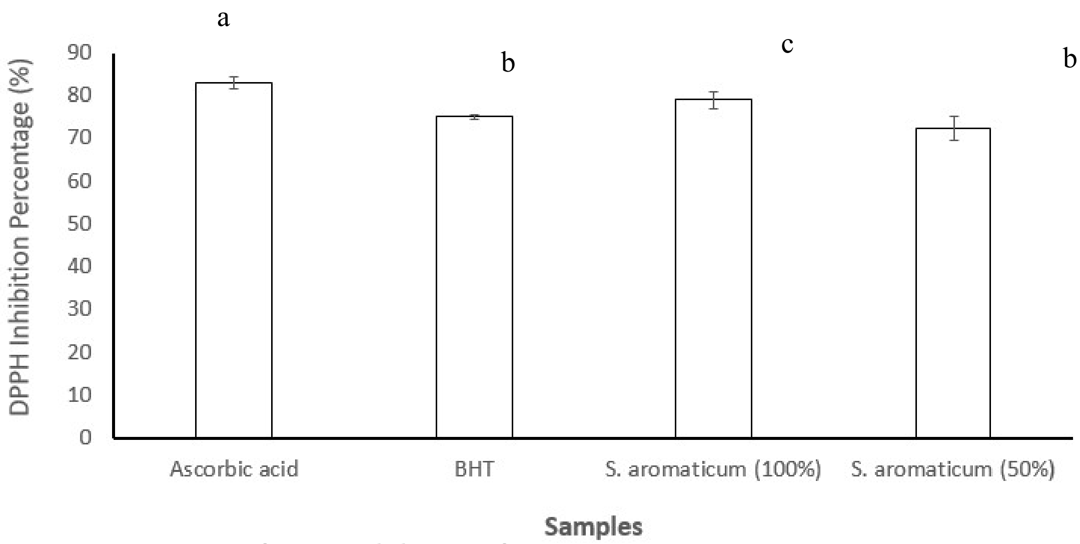

Figure 3: Percentage of DPPH inhibition of *S. aromaticum* EO.

S. aromaticum EO has a lower FRAP value compared to ascorbic acid (Figure 4). Ascorbic acid showed high FRAP value which was 1400 μmol Fe2+ mg. However, BHT showed a lower value, which was 500 μmol Fe2+ mg. *S. aromaticum* EO at 100% showed a significantly lower FRAP value of 300 μmol Fe2+ mg compared to ascorbic acid and BHT. *S. aromaticum* EO at 50% showed no significant difference compared to *S. aromaticum* EO at 100%.

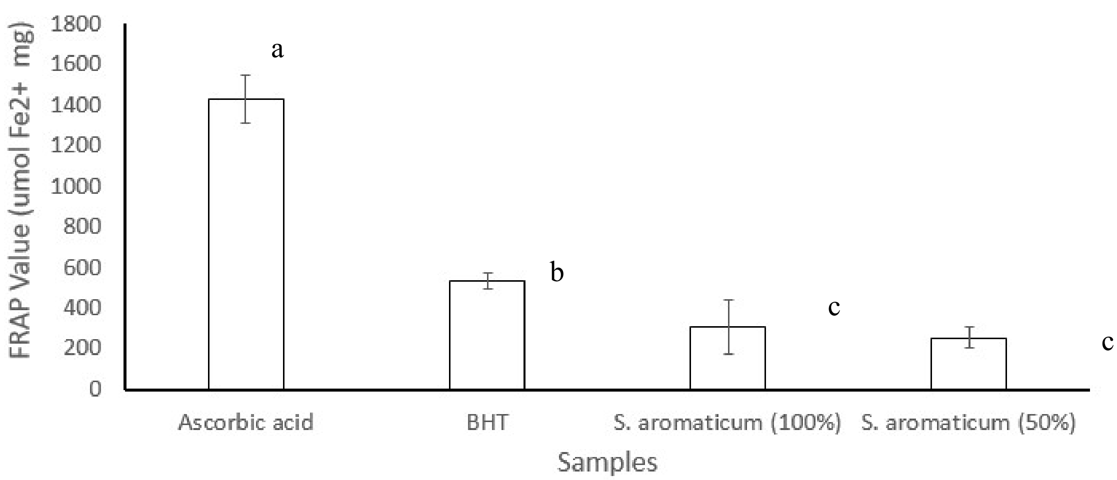

Figure 4: FRAP value of *S. aromaticum* EO.

S. aromaticum can prevent mosquito bites (Table 2). Compared to the control group, the mosquitoes in the control group sucked AZK rat blood after 1 minute 08 sec of exposure.

Table 2: Effect of EO on adult mosquitoes.

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Essential Oil Samples	**Number of Mosquitoes (n)**	**Time taken (min)**	**Effect onMosquito**
Control	3	5	Sucking blood after 1 min 08 sec after exposure
S. aromaticum	3	5	No biting and sucking blood

DISCUSSION

In this study, the yield of EO obtained through hydro distillation was 5.5 %. The *S. aromaticum* gave a unique odor due to their appreciable volatile content. Eugenol was detected as the major compound with 72.19%, followed by β-caryophyllene (11.60%) and (Z)-isoeugenol acetate (5.56%). The composition of the EO can change due to many factors, such as the geographical area of cultivation, agronomic techniques, harvesting method, phenological stage and storage conditions, which cause the formation or degradation of certain components (Haro-González *et al*., 2021). As shown in Figure 2, the GC chromatogram of the EO of *S. aromaticum* shows the highest peak at an Rt value of 34.783, indicating the existence of the compound eugenol. Almost all varieties of *S. aromaticum* contain eugenol (Landowska *et al*., 2021), which may serve as an antioxidant and mosquito repellent. Eugenol is a phenolic aromatic compound. Eugenol is a clear yellow to pale yellow liquid with an oily consistency and a pungent aroma (Liñán-Atero *et al*., 2024). It is slightly soluble in water and highly soluble in organic solvents (Ulanowska *et al*., 2021). In addition, there are several peaks: isoeugenol, caryophyllene and humulene groups, which are among the highest compounds in *S. aromaticum* EO (Amelia *et al*., 2017). In addition, the antioxidant activity of *S. aromaticum* EO was determined by DPPH. The DPPH assay is commonly used to evaluate the efficacy of various antioxidant compounds as free radical scavengers (Baliyan *et al*., 2022). Figure 3 shows that the EO of *S. aromaticum* inhibits 80 % of DPPH. This indicates that the EO can reduce free radical attacks and decrease the oxidation process. This result is consistent with a previous study by Gülçin *et al*. (2012), which showed that the EO of *S. aromaticum* is an effective antioxidant in various in vitro assays, including DPPH. The strong DPPH inhibitory effect of *S. aromaticum* EO may be due to the presence of eugenol, which is known to have antioxidant activity (Ulanowska and Olas, 2021). Isoeugenol has also been reported to have an antioxidant effect in previous reports (Zhang *et al*., 2017). In addition, caryophyllene and humulene have been attributed to an antioxidant effect (Yin *et al*., 2019). All of these major constituents confer antioxidant efficacy to scavenge DPPH.Besides that, the FRAP value in Figure 4 shows that *S. aromaticum* has a significantly lower FRAP value compared to ascorbic acid. The FRAP assay values represent the corresponding concentration of electron-donating antioxidants in the reduction of ferric iron (Fe^{3+}) to ferrous ion (Fe^{2+}) (Halvorsen *et al*., 2002). In previous studies, ascorbic acid was used as a control to test whether an ingredient has a high FRAP value (Johari and Khan, 2022). In general, ascorbic acid has a high FRAP value between 10 and 14 μMol Fe^{2+} per mg of extract. In previous studies, most reports

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found a FRAP value of about 4 to 6 µMol Fe^{2+} per mg of extract . Thus, the results of this study are consistent with previous reports showing that the FRAP value of *S. aromaticum* is lower than that of ascorbic acid. Moreover, mosquito repellents using EOs are considered safer and more accessible than synthetic chemical repellents and may have a lower likelihood of resistance development in mosquitoes (Sutthanont *et al*., 2022). There is enormous potential for the commercialization of EOs as a finished product for mosquito repellent (Sneha, 2022). It is believed that the bioactivity of any plant EO is due to its main constituents (Haris, 2022). *S. aromaticum* is one of the plants that can be used as an alternative larvicide to kill mosquito vectors as it contains many chemical compounds including eugenol compounds, eugenol acetate, methyl eugenol, B-caryophyllene, methyl eugenol, saponins, flavonoids and larvicidal tannins (Budiman *et al*., 2022). AZK rats were used as subjects because they have no fur and their skin is easily accessible. This specification facilitates the conduct of this study and the collection of data. The results of the study presented in Table 2 show that *S. aromaticum* can effectively prevent mosquito bites. It is assumed that the repellent effect is due to the fact that *S. aromaticum* EO contains compounds that effectively repel mosquitoes. Compared to the untreated control group, the mosquitoes in the control group sucked the blood of the AZK mice after 1 minute and 08 seconds of exposure. The limitation of this study was that it used only a small number of AZK rats as representatives for humans. A large number of subjects are required to test the ability of EOs to repel mosquitoes. In addition, the mosquito repellency test requires a longer exposure time, as the short test time of 5 minutes may not correspond to real-life conditions. To obtain a true picture of the activity of EO-based mosquito repellents, which are more effective and long-lasting, it may also be necessary to investigate the mechanism of how EO repels mosquitoes.

CONCLUSION

Analysis of the chemical composition of the *S. aromaticum* EO revealed eugenol as the major constituent. The *S. aromaticum* EO has antioxidant potential. The potential of *S. aromaticum* as an effective repellent against *A. albopictus* mosquitoes has been demonstrated. In our tests, this natural remedy achieved a surprising repellency rate of 100%, proving its promising effectiveness in mosquito-repellent activity.

DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

DECLARATION OF HONOUR

We declare in our honor that our results are not fake and made up.

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