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ORIGINAL ARTICLE

Microwave-assisted solvent-free extraction of essential oil from *Coleus aromaticus:* anti-phytopathogenic potential for fruit post-harvesting

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Abstract

This work evaluates the fungicidal effect of essential oil from *Coleus aromaticus* (*C. aromaticus*) by solvent-free microwave-assisted extraction with a yield of 0.54%. Fourier-transform infrared spectroscopy was utilised to identify the functional groups, which were O–H, C–O, C–H, and C=C. Gas chromatography–mass spectrometry analysis was performed to determine the primary essential oil components, namely, thymol (92.62%), thymoquinone (2.64%), creosol (1.77%), linalool (1.68%), *p*-Cymene-2,5-diol (0.73%), and *p*-Cymene (0.56%). The inhibitory effect of essential oil extracted from *C. aromaticus* against the isolated fungi, *Aspergillus niger* from mango, was investigated. The mycelial growth inhibition of the extracted essential oil by the poisoned food test and disc diffusion assay showed the reduction at 79.63 ± 1.7 and 70.45 ± 6.54%, respectively. In vivo experiment was conducted with artificially wounded and unwounded mangoes, applying the extracted essential oil to the wounded mangoes inoculated with *A. niger* that could decrease the disease incidence from 100 to 58.33 ± 14.43%. Meanwhile, the treatment of the extracted essential oil did not affect the quality of the mango and it also shows improvement in weight loss reduction of the mango in comparison to the chemical fungicide and untreated mango. Hence, essential oil from *C. aromaticus* by solvent-free microwave-assisted extraction could be used as an effective control for the fruit spoilage and potential source of fruit preservative.

Keywords Mango · Anti-fungal compounds · Natural essential oil · Green chemistry

Introduction

According to World Health Organization (2019), food intake has direct and indirect effects on human health, where 3.9 million deaths worldwide were caused by the deficient of vegetables and fruits consumption. Mango (*Mangifera indica* L.) is a highly beneficial fruit for health and predominantly has anticancer, antioxidant, anti-inflammatory potentials with high nutritional value of polyphenols and vitamin, all of which make a huge demand for mangoes (Lauricella et al. 2017). Mango is one of the major economically fruits with the estimated range of over 1000 million tons of production every year (Saúco 2004). Food and Agriculture Organization estimated that in the year 2011, around 15–50% of total amount of fruits and vegetables worldwide were lost during postharvest process. One of the attributes for causing the losses in mango production during postharvest is due to the pathogenic fungal infection. Fruits applied with chemical and synthetic fungicides can reduce fruit losses between 2 and 4%, while the fruits without treatment after postharvest can cause between 15 and 30% of losses in three weeks (Palou et al. 2016). The excessive application of chemical synthesised fungicide will cause adverse effects to the environment and living organisms. Highly concentrated chemical fungicide treatment is also capable of accumulating toxic material in the food product, which causes health problems and thus, have an undesirable impact on the export of mangoes.

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According to Food and Drug Administration (FDA), all alternative chemicals must be verified as generally regarded as safe (GRAS). These GRAS materials have no or low toxicological effects on living organisms and minimal impact to the environment (Palou et al. 2016). Several organic, inorganic, and plant essential oils have GRAS status, which are safe to apply for the postharvest process. One of the alternative methods to replace chemical fungicide is by the use of essential oil, as it is antimicrobial, has low mammalian toxicity, and low environmental impact (Sivakumar and Bautista-ba 2014). Essential oil is commonly used as natural medicine and natural pesticide. There are several methods that can be used to extract the essential oils. Solvent-free microwave-assisted extraction (SFME) is one of the latest technologies used for isolation and extraction of phyto-constituents from plant materials by electromagnetic energy (Filly et al. 2014). The direct interaction of electromagnetic energy will cause the vibration of plant molecules. Hence, the repulsion and attraction of molecules will cause frictional force which result in the production of heat energy. By this, it favours the release of compounds trapped inside the cells of plant material (Zill-E-Huma 2010). The advantages of SFME over conventional method are the shorter extraction time and higher yield. It is also more environmentally friendly compared with non-microwave-assisted extraction because there is no solvent needed for the extraction process (Lucchesi et al. 2004). The application of SFME method is more suitable for *Coleus aromaticus* due to its thicker tissues that can be penetrated by microwave to extract the essential oil.

The essential oil extracted from *Coleus aromaticus* has multiple benefits, which include antibacterial property, antimicrobial activity, allopathic potential, and insecticidal properties. In addition, essential oils and nanoparticles extracted from *C. aromaticus* have a good antimicrobial activity, while the n-hexane, and dichloromethane extracts from *C. aromaticus* leaves exhibit activity against fungi, such as *Botrytis cinerea, Candida albicans*, and *Aspergillus niger* (Murthy et al. 2009). Therefore, it is important to replace the chemical synthetic fungicide with alternatives, which are biodegradable, has low toxicity, and is more effective through the application of essential oil. This study demonstrates the application of essential oil extracted from *C. aromaticus* by solvent-free microwave extraction for preventing fruit damage and as postharvest control for the evaluation of antifungal activity via in vitro and in vivo analyses.

Materials and methods

Plant material preparation

Fresh leaves of *Coleus aromaticus* were collected from the Institute of Sustainable Agrotechnology (INSAT), Perlis, Malaysia. The leaves were washed 2–3 times with tap water, followed by distilled water (Shiney et al. 2012). It was then directly used for solvent-free microwave extraction (SFME).

Extraction of essential oil from *Coleus aromaticus*

Extraction of essential oil was performed via SFME consists of MW apparatus (NEOS-GR, Milestone Srl, Italy) operating at 2.45 GHz (900 W maximum power). Firstly, 500 g of fresh *Coleus aromaticus* leaves was placed into Erlenmeyer flasks (500 ml) without the addition of solvents or water. The extraction was performed at atmospheric pressure with microwave power set at 500 W for 30 min and the temperature was monitored and controlled by infrared (IR) automatic temperature system sensor. The crude extract was collected continuously in a graduated cylinder until no more extract was obtained or overheating was detected. The extract was drained by gravity on a condenser outside the microwave irradiation cavity and was cooled down at room temperature and stored in a small bottle for further test. At the end of the extraction, the essential oil was received and dried with anhydrous sodium sulphate, then stored at 0 °C until its further use (Lucchesi et al. 2016). The extracted essential oil yield was estimated using the following equation:

$$R_{\mathrm{HE}}(\%) = \frac{m_{\mathrm{HE}}}{m_{\mathrm{S}}} \times 100\%$$

where m_{HE} essential oil mass (g), m_{s} mass of the starting material (g), and R_{HE} essential oil yield (%).

Analytical test of essential oil extracted from *Coleus aromatics*

Fourier-transform infrared spectroscopy (FTIR)

The Fourier-transform infrared spectroscopy (FTIR) was utilised to conduct the analytical testing on the essential oil by characterisation and identification of the functional groups or compounds reside (Pan et al. 2003). First, a drop of sample was kept between two plates with sodium chloride; a thin film was formed between the plates at the contact of sample. The sample was inserted in the FTIR for the analytical test, as described (Eberhardt et al. 2007). and scanned at 600–4000 cm^{-1}.

Gas chromatography–mass spectrometry (GC–MS)

The extracted essential oil from *Coleus aromaticus* was then further analysed at the National Institutes of Health using GC–MS to confirm the compounds present in the essential oil. Thermo Scientific Trace 1310 gas chromatography equipped with TSQ 8000 Evo triple quadrupole mass

spectrometer (Thermo Scientific, San Jose, CA, USA) was used and the stationary phase was with VF-5 ms fused silica capillary column (30 m × 0.25 mm; 0.25 µm film coating) obtained from Agilent (Santa Clara, CA, USA). Initially, the temperature was set at 40 °C (3 min), followed by a temperature increase to 250 °C (3 °C/min) with the final hold time for 3 min while helium was used as the carrier gas at a constant flow rate of 1 ml/min. The injector temperature was set at 250 °C with the injection volume of 10 µl in splitless mode. The MS was taken at 70 eV with an EI source at the mass range of *m/z* 35–650 (Mallikarjuna et al. 2011). The identification of MS fragmentation patterns was performed using the NIST Mass Spectral (MS) database.

Sample preparation from infected fruit

An infected mango (locally known as susu mango) was collected from Jabatan Pertanian Negeri Perlis, Malaysia. The infected mango was stored in a container and brought to the fermentation technology and microbiology laboratory in UniCITI Alam, Perlis Malaysia.

Isolation of pathogenic fungi from infected fruit

The isolation process of pathogenic fungi was carried out in a laminar flow after irradiation by ultraviolet (UV) light. The direct plating technique was used to isolate the pathogenic fungi from the mango fruit (Singh et al. 2002). The pathogenic fungus was isolated using Potato Dextrose Agar (PDA) medium. A part of the infected region of the fruit was cut by flame-sterilised forceps and transferred to the surface of PDA, where the plate was incubated for seven days at 25 °C (Thilagam et al. 2018).

Identification of isolated fungi

Identification of pathogenic fungi based on morphological characteristics, such as colour, structure of hyphae, and types of conidial characteristics, was carried out under the light microscope (Olympus CX23, Tokyo, Japan). Small amount of isolated fungus was transferred to a drop of lactophenol cotton blue (LCB) on a clean glass slide and teased apart by dissecting needles (Abdullah et al. 2016). A cover slip was placed on the slide by lowering it down to remove any air bubbles. The specimen was examined microscopically under low magnification (10×) and then under high magnification (40×) to obtain a clear observation of morphology. After the identification and verification of pathogenic fungi, were harvested from the growing edge of the pathogenic fungi using a sterile needle and placed on potato dextrose agar plate. The plates were incubated at 25 °C ± 2 °C (10 to 14 days) to produce conidia.

Effect of essential oil from *Coleus aromaticus* on antifungal activity

The effect of antifungal activity of *C. aromaticus* was examined using in vitro and in vivo tests. The effects of antifungal activity were compared by treatment with control (distilled water), essential oil from *C. aromaticus*, and commercial chemical fungicide (Globus 5.5).

In vitro antifungal activity test

Poisoned food technique

The in vitro antifungal activity of essential oil was determined via the poisoned food technique (Abdullah et al. 2016). Poisoned food technique was utilised to assess the effects of control, essential oil of *C. aromaticus* and 0.05% (v/v) Tween 80, commercial chemical fungicide (Globus 5.5), and 0.05% (v/v) Tween 80, where they were mixed in a sterilised PDA medium immediately at temperature of 50 °C. The mixture was swirled and immediately poured onto the sterilised petri dish and let to solidify. The plates were centrally inoculated with a 6-mm mycelial disc cut from the fungal culture. Then, the plates were incubated for seven days at 25 °C ± 2 °C. The diameter of fungal growth was measured and recorded after 7 days.

Disc diffusion method

The inhibition zone (diameter) formed by the essential oil extracted from *C. aromaticus* against pathogenic fungi was accessed by agar disc diffusion method using PDA in petri dish. A 6 mm diameter of sterilised filter paper discs with control (distilled water), essential oil from *C. aromaticus* and chemical fungicide (Globus 5.5) were kept independently at the centre of the agar plate with and inoculated with the test pathogenic fungi (Humber 1997). The plate was tightly sealed and incubated at 25 °C ± 2 °C (7 days). The mean diameter of the mycelia growth from both fungi was carried out in triplicates to determine the antifungal activities and expressed in terms of mycelia growth inhibition percentage (MGI; %), as shown in the following equation (Shao et al. 2015):

$$\mathrm{MGI} = \frac{(\mathrm{dc} - \mathrm{dt})}{\mathrm{dc}} \times 100\%$$

dc is representing the diameter of mycelia growth in control and dt is for the diameter of mycelia growth treated by the essential oil.

In vivo antifungal activity test

Effect of antifungal activity on artificially wounded and inoculated mangoes

By using the method stated earlier (Balouiri et al. 2016) for in vivo antifungal test, four wounds with 2-mm deep and 3-mm wide cut was created at equatorial side on each mango via a sterile cork-borer. Each wound was treated with total 30 µl of essential oil extracted from *C. aromaticus*, distilled water (control), and chemical fungicide (Globus 5.5). The control and treated mangoes were incubated at room temperature for 2 h. After two hours of incubation, 20 µl of pathogenic fungus was inoculated on each wound of mango (Karim et al. 2017). The control and treated sets of mangoes were placed in separate cardboard boxes with labelling and kept at 25 °C with the relative humidity of 95% for 10 days. The disease incidence was calculated by counting the number of rotten wounds for both treated and control fruits after 10 days of incubation via the following equation (Abd-Alla and Haggag 2013):

$$\text{Disease incidence} = (\text{number of rotten wounds})/(\text{number of total wounds}) \times 100\%.$$

Examination on mango quality

The fresh mangoes (susu mango) collected were free from injury and infections, and were used in the experimentation of this work. All mangoes were sprayed evenly with different treatments: control, essential oil, and chemical fungicide. Mangoes sprayed with sterile distilled water were used as the control, where the treated and control mangoes were air-dried at room temperature. They were placed on plastic tray in separate card boxes with a proper labelling and stored at 25 °C and 95% of relative humidity for 10 days (Balouiri et al. 2016). Each treatment has three replicates and each replicate is represented by one mango. After 10 days of storage, the treated and control mangoes were used for fruit quality examination. The fruit quality measurement involved colour changes, soluble solid content, firmness, and weight loss.

Statistical analysis

All data are shown as a mean ± standard deviation. Analysis of variance (one-way ANOVA; Microsoft Excel 2010) was conducted, utilising a probability level less than 5% ($p < 0.05$). A value of $p < 0.05$ was represented to be statistically significant.

Results and discussion

Analytical test of essential oil

The fresh leaves of *C. aromaticus* were utilised to obtain the essential oil by solvent-free microwave extraction (Gaston et al. 2015) where the yield was 0.54%. The extraction procedure involved the basic concept shown in Fig. 1.

Fourier-transform infrared spectroscopy (FTIR) measurement

Fourier-transform infrared spectroscopy (FTIR) measurement was done to observe the presence of functional group in the essential oil, as shown in Fig. 2. From the study Govindaraju and Arulselvi (2018), the extracted essential oil from *C. aromaticus* showed wavenumbers from 806 to 3446 cm^{-1}, which corresponded to the O–H, C–H, C=C, C–O, and =CH stretching groups. However, this study found that the band at 3268.74 cm^{-1} located at wavenumbers 3000–2700 cm^{-1} corresponds to the O–H functional group, whereas the peak at 1100.32 cm^{-1} corresponds to C–O functional group (Aging and Munajad 2018). Next, peaks at 1636.08 cm^{-1} and 1100.32 cm^{-1} correspond to C=C and C–H group, respectively (Guillén and Cabo 1999). Conclusively, the functional groups present in the extracted essential oil from *C. aromaticus* are O–H, C–O, C–H, and C=C groups.

GC–MS analysis

The chemical composition and retention time of essential oils from *C. aromaticus* are presented in Fig. 3. In this study, a total of six compounds were identified from the essential oils of *C. aromaticus*. The major constituents were thymol (92.62%), thymoquinone (2.64%), creosol (1.77%), linalool (1.68%), followed by *p*-Cymene-2,5-diol (0.73%) and *p*-Cymene (0.56%) as minor constituents. Earlier reports from Singh et al. (2002) documented the essential oil constituents as thymol (94.3%), 1,8-cineole (1.8%), carvacrol (1.25%), *p*-Cymene (0.3%), terpinen-4-ol (0.2%), and spathulenol (0.2%). However, Murthy et al. (2009) reported that the major constituents were carvacrol (70%), followed by β-caryophyllene (6.2%), γ-terpinene (5.3%), *p*-Cymene (5.6%), and other compositions. The chemical compounds and variability of the

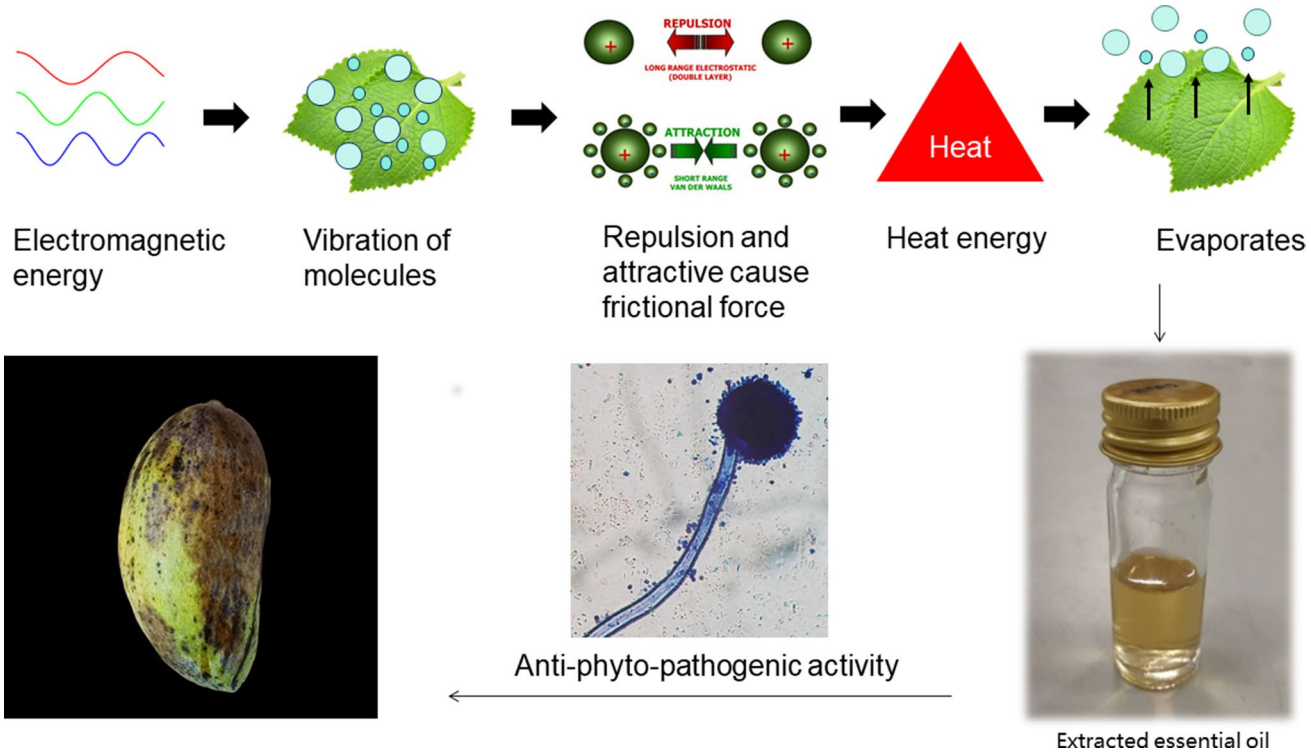


Fig. 1 Schematic representation for the extraction of essential oil from *Coleus aromaticus*. An application for anti-phytopathogenic activity is displayed. Microscopic image of the pathogen (*Aspergillus niger*) is shown

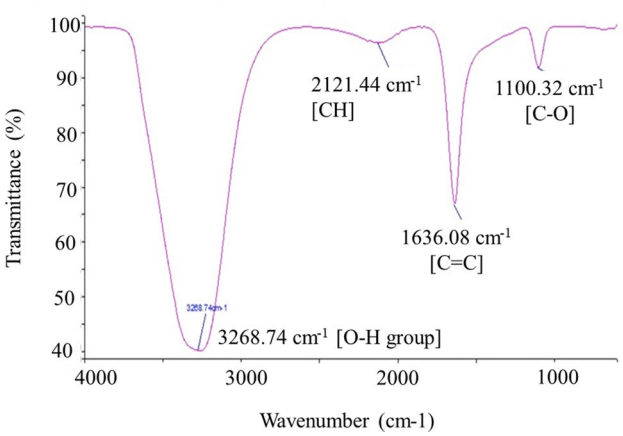


Fig. 2 Fourier-transform infrared spectroscopy analysis on essential oil extracted from *Coleus aromaticus*. Scanning range from 600 to 4000 cm^{-1} is shown. The presence of chemical groups at the specific wavenumber is shown

essential oil might vary with growth factors and genetic diversity or the method of extraction used (Mallavarapu et al. 1999). Table 1 illustrates the comparison of existing functional groups determined via FTIR, with the structure of compounds (thymol, thymoquinone, creosol, linalool and *p*-Cymene-2,5-diol, and *p*-Cymene) identified from GC–MS analysis.

Isolation and identification of isolated fungi

From the fungus isolated from the infected mango, the colony was initially white in colour and black-to-dark brown colour after conidial production was noticed 36 h after growth. The septate, hyaline mycelia with black colour conidia and the spore-bearing structures are indications for *Aspergillus niger* (Bennett 2007). For morphological identification, the fungi were stained with lactophenol cotton blue and observed under the light microscope. The obtained image shows the roughened, round conidia, which is similar to the morphology to that of *A. niger* (Krishnapillai and Wijeratnam 2013), earlier studies also showed similar morphology (Singh and Mumbai 2014; Svanström, 2013). From the above-mentioned study (Krishnapillai and Wijeratnam 2013), *A. niger* was known to cause the black mould rot of mangoes, one of the postharvest diseases of mangoes.

In vitro antifungal activity test

The in vitro antifungal activity was conducted using poisoned food technique and agar disc diffusion. Tests were conducted by comparing the effect of essential oil extracted from *C. aromaticus*, commercial chemical fungicide (Globus 5.5), and distilled water acted as the control.

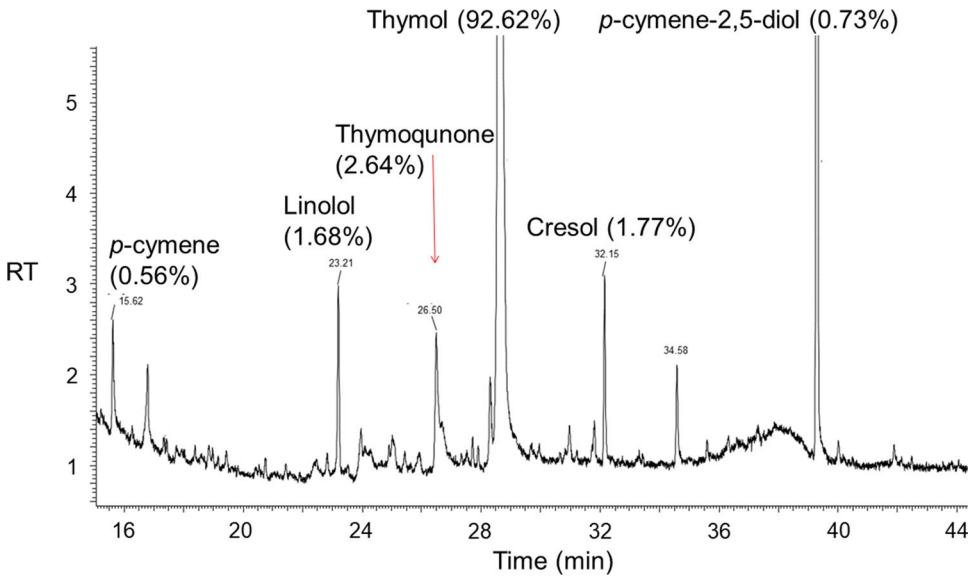


Fig. 3 Gas chromatography–mass spectrometry of essential oil extracted from *Coleus aromaticus*. The identified major compounds are indicated. Calculated in per cent

Poisoned food technique antifungal test

The essential oil extracted from *C. aromaticus* has elicited a notable antifungal activity against the isolated *A. niger* from the mango. Results from the poisoned food technique antifungal can be seen from Fig. 4a–c; Ta−2, whereby the mycelia growth on the essential oil extracted from *C. aromaticus* was 18.3 ± 0.15 mm and the inhibition of mycelia growth was 79.63% ± 1.70%. However, the mycelia growth for chemical fungicide (Globus 5.5) was 12.7% ± 0.17 mm, where the inhibition was 85.92% ± 1.70%, higher than the extracted essential oil from *C. aromaticus*. Meanwhile, the mycelia growth on control (distilled water) was 48.0 ± 0.26 mm and with 46.67% ± 2.94% of inhibition. In order to allow for uniform mixing of the extracted essential oil and commercial chemical fungicide, an emulsifier, Tween 80, was used to ensure even distribution on the surface.

Disc diffusion antifungal test

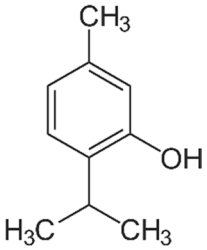
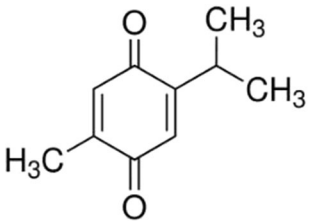
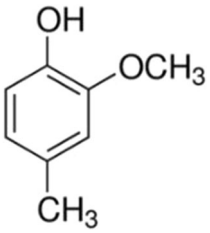
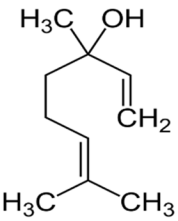
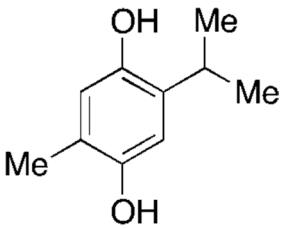
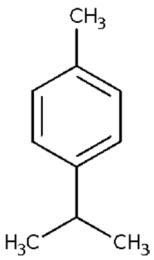
Next, for the disc diffusion antifungal test, there was no inhibition found on the control (distilled water), while for the treatment by the essential oil of *C. aromaticus*, the mycelia growth was 14.3 ± 0.05 mm and the mycelia growth inhibition were 70.45% ± 6.54%. However, the chemical fungicide (Globus 5.5) showed a higher inhibition on the mycelial growth of 81.55% ± 3.08%, whereas the mycelial growth was 15.7 ± 0.06 mm (Fig. 4d–f; Table 3).

The results of in vitro poisoned food technique and disc diffusion test hinted that essential oil extracted from *C. aromaticus* was effective, with the inhibition rate of above 50%. The presence of thymol could contribute to the inhibitory effect on fungi due to the presence of phenolic compound (De Almeida Alves et al. 2000). Thymol has the capability of aligning the fatty acid chains by dissolving into the cytoplasm membrane and increase the passive permeability (Lambert et al. 2001). This will distort the membrane fluidity and integrity, which changes the structure of fatty acids, proteins, phospholipids, and polysaccharides, thereby causing the leakage of cytoplasmic contents (Xing et al. 2014). Furthermore, previous study on antifungal efficacy of essential oil from *C. aromaticus* was studied (Lucchesi et al. 2004) against various fungi, namely, *Aspergillus flavus*, *A. ochraceus*, *A. niger*, *A. oryzae*, and other fungi species, which showed more than 50% of inhibition rate through the use of essential oil.

In vivo antifungal activity

The in vivo antifungal activity test was conducted by utilising artificially wounded and unwound mango to compare the effects of essential oil extracted from *C. aromaticus*, commercial chemical fungicide (Globus 5.5), and distilled water that served as control. From Fig. 5a–d, mangoes treated with essential oil extracted from *C. aromaticus* and chemical fungicide (Globus 5.5) have significant ability to reduce the incidence caused by *A. niger* under room temperature, compared with that of control (distilled water) ($p < 0.05$). From Fig. 5a, mango treated with distilled water shows 100% of disease incidence, while mango treated with extracted essential oil had significantly minimised the incidence of fungi on artificially inoculated mango to 58.33% ± 14.43%, whereas 16.66% ± 14.43% of disease incidence was noticed in the presence of commercial chemical fungicide. The applications of essential oil and chemical fungicide show the inhibitory effect on the mangoes, where it minimised the formation of mycelium and sporulation, compared

Table 1 Structure and functional groups of target compounds

Compound	Functional group	Structure
Thymol	OH group CH group C=C group	
Thymoquinone	CH group C = C group C–O group	
Creosol	OH group CH group C=C group	
Linalool	OH group CH group C=C group	
p- cymene-2, 5-diol	OH group CH group C=C group	
p- cymene	CH group C=C group	

with that of the control. All treated mangoes and control were infected by the isolated fungi. Figure 5a shows the fully-grown mangoes and a black mould rot on the wound after 10 days; Fig. 5b portrays a partially infected mango, showing only a black mould rot on some wounds; and Fig. 5c exhibits the development of soft water-soaked lesion around the wound. The results of in vitro were similar to that of the in-vivo test (Fig. 5a–d). However, the result of essential oil by in vitro test showed a higher antifungal effect compared with that from the in vivo

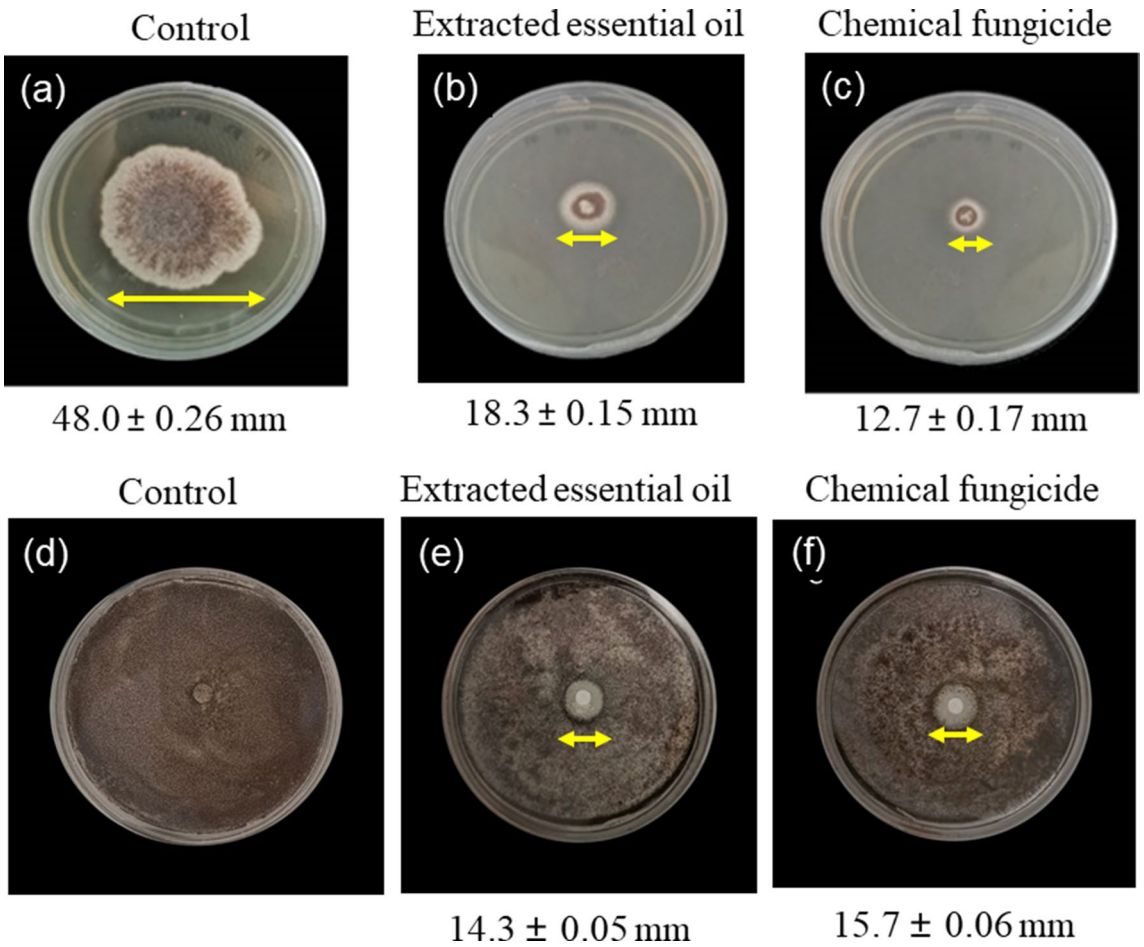


Fig. 4 Poisoned food technique. Effect of treatment on colony diameter of *Aspergillus niger* growth after 7 days incubation. **a** Treated with sterile distilled water (control); **b** Treated with essential oil extracted from *Coleus aromaticus* + Tween 80; **c** Treated with chemical fungicide (Globus 5.5 + Tween 80); Disc diffusion test is with effect of treatment on colony diameter of *Aspergillus niger* growth after 7 days incubation. **d** Treated with sterile distilled water (control); **e** Treated with essential oil extracted from *Coleus aromaticus* + Tween 80; **f** Treated with chemical fungicide (Globus 5.5 + Tween 80)

Table 2 Consolidated data by poisoned food technique

Treatment	Mycelia growth (mm)	Mycelia growth inhibition (%)
Control (distilled water)	48.0 ± 0.26	46.67 ± 2.94
Essential oil *C. aromaticus* + Tween-80	18.3 ± 0.15	79.63 ± 1.70
Chemical fungicide + Tween-80	12.7 ± 0.17	85.92 ± 1.70
ANOVA analysis (*P*-value)	**1.493E-06**	**1.22708E-06**

$P < 0.05$

test, in which this might be related to the structure of the fruits. Under in vivo condition, the extracted essential oil does not show the strong inhibitory effect compared to the in vitro condition. Earlier study (Feng et al. 2013) stated that the growing environment of fruits is more complex compared with the culture media, whereby environment is able to prevent the green fungicide effect on the survival of microbes. The exocarp and mesocarp of the mangoes will reduce the chance of essential oil penetration and block the antifungal activity. Therefore, higher dose of essential oil is required to increase the antifungal activity when applied on the fruits.

Table 3 Consolidated data by disc diffusion test

Treatment	Mycelia growth (mm)	Mycelia growth inhibition (%)
Control (distilled water)	–	–
Essential oil *C. aromaticus*	14.3 ± 0.05	70.45 ± 6.54
Chemical fungicide	15.7 ± 0.06	81.55 ± 3.08
ANOVA analysis (*P*-value)	**2.53308E-08**	**6.87628E-07**

$P < 0.05$

Effect of essential oil on mango quality

From Table 4, the treatment of extracted essential oil from *C. aromaticus* and commercial chemical fungicide does not show significant differences in colour index and soluble solids content (SSC), where $p > 0.05$. Previous study (Feng et al. 2013) stated that there is no significant difference in colour, SSC, and firmness of cherry tomatoes when treated with 500 ppm of cassia oil and mixed with calcium chloride after 30 days of storage at 20 °C. From this study, some variations in colour index value were noticed, where it might have been affected by the colour pigment distribution on the mangoes during its (mango) degradation. Generally, fruit firmness is decreased with storage time. However, the fruit treated with essential oil improves firmness of the fruit in compare to the untreated fruit after storage. The result is in agreement with other findings (Hosseini et al 2020). Apart from that, it also improves significantly reduction of weight loss. The percentage of weight loss is lower compare to other methods. Similar results were observed by Karunanayake et al. (2020), where they found the weight loss was significantly higher in the control fruits which had no essential oil treatment in compare to the fruits receiving essential oil treatment. The hydrophobic nature of essential oil helps in

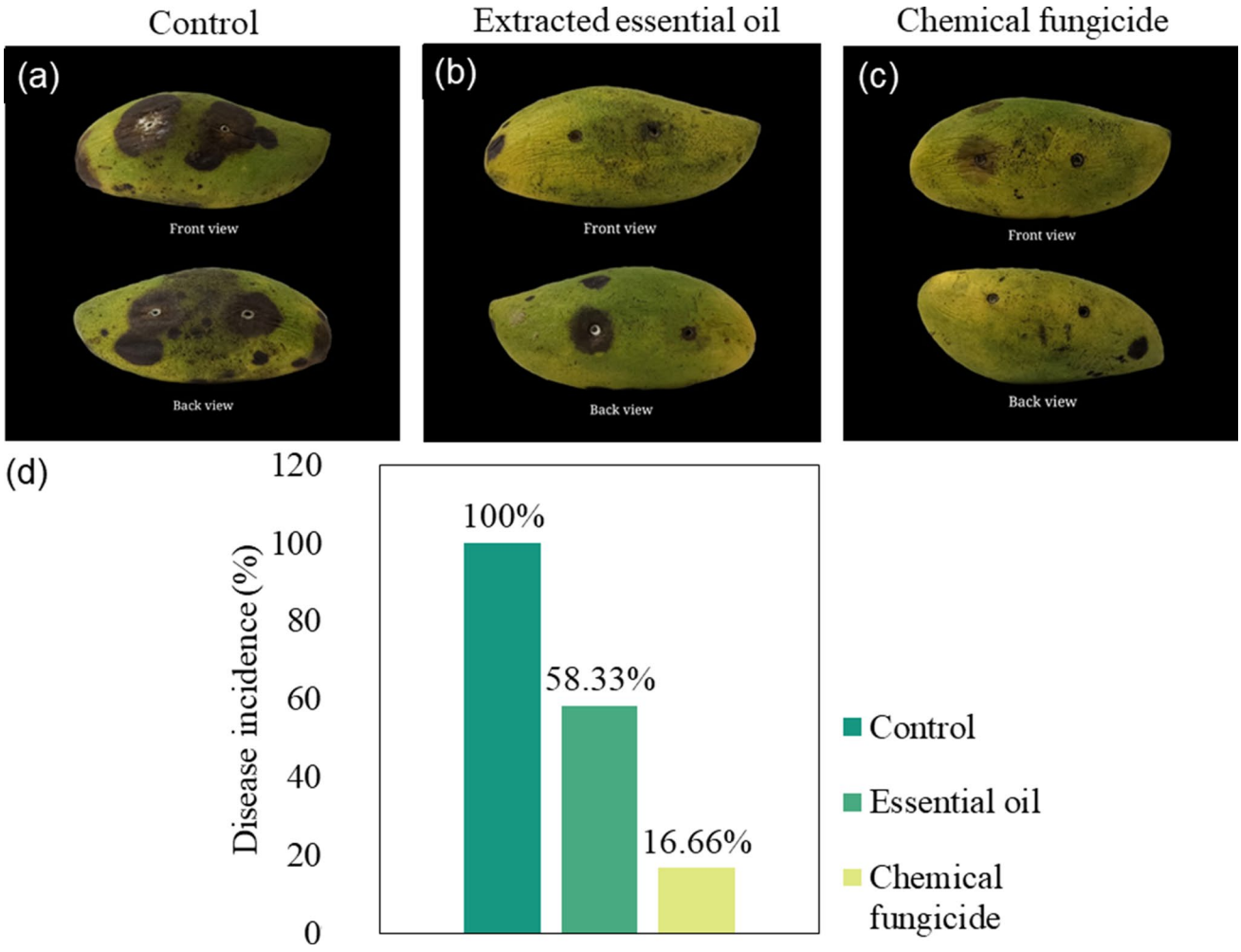


Fig. 5 *Aspergillus niger* development at the wound site of inoculated mangoes. Mango treated with **a** Control (distilled water); **b** Essential oil extracted from *Coleus aromaticus*; **c** Chemical fungicide (Globus 5.5); **d** Effect of different treatments on disease incidence

Table 4 Evaluation on the quality of mangoes with treatment

Treatment	Colour Index	SSC (%)	Weight loss (%)	Firmness (*g/f*)
Control (distilled water)	−3.41 ± 1.35	24.57 ± 0.21	45.07 ± 0.39	558.70 ± 34.15
Essential oil *C. aromaticus*	−4.47 ± 2.21	24.6 ± 0.1	28.73 ± 0.44	581.41 ± 16.65
Chemical fungicide (Globus 5.5)	−2.81 ± 1.3	24.67 ± 0.12	30.75 ± 0.64	605.19 ± 23.90
ANOVA analysis (*P* value)	**0.126002**	**0.718173**	**7.55E-05**	**0.003464**
Probability	***P>0.05***	***P>0.05***	***P<0.05***	***P<0.05***

Effects of control, essential oil of *Coleus aromaticus* and chemical fungicide on weight loss (%), colour index, SSC (%) and firmness (*g/f*) are shown

the improvement in terms of weight loss reduction (Sánchez-González et al. 2011) by forming a continuous matrix around the fruit and thus, minimises the water loss in fruits through the formation of water barrier on the surface.

Conclusions

The present study was designed to produce a green fungicide from essential oil from *Coleus aromaticus*, which is eco-friendly, to replace the uses the chemical fungicide and minimise the postharvest disease in mango (*Mangifera indica*). The essential oil extracted by solvent-free microwave extraction from *C. aromaticus* was found to contain functional groups of O–H, C–O, CH, and C=C. From the result of GC–MS, there were six major compounds and some others that were noticed from the essential oil of *C. aromaticus*. From in vivo and in vitro studies, essential oil extracted from *C. aromaticus* showed more than 50% of inhibition rate of *Aspergillus niger* infection on the mangoes. Additionally, the application of essential oil on unwounded mangoes did not affect the soluble solids content and colour index; however, it did affect positively the weight and firmness of the mangoes. This study demonstrates the effectiveness of direct application of essential oils on fruit as a postharvest control.

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