## Antifungal activity of wild bergamot (Monarda fistulosa) essential oil against postharvest fungal pathogens of banana fruits.

South African Journal of Botany 144 (2022) 166–174

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

# Antifungal activity of wild bergamot (*Monarda fistulosa*) essential oil against postharvest fungal pathogens of banana fruits

Seema A. Kulkarni[a], Periyar Selvam Sellamuthu[b,*], Santhosh Kumar Nagarajan[c], Thirumurthy Madhavan[c,*], Emmanuel Rotimi Sadiku[d]

[a] *Department of Biotechnology, School of Bioengineering, SRM Institute of Science and Technology, Potheri, Kattankulathur 603203, Chengalpattu District, Tamilnadu, India*
[b] *Department of Food Process Engineering, Postharvest Research Lab, School of Bioengineering, SRM Institute of Science and Technology, Potheri, Kattankulathur 603203, Chengalpattu District, Tamilnadu, India*
[c] *Department of Genetic Engineering, Computational Biology Lab, School of Bioengineering, SRM Institute of Science and Technology, Potheri, Kattankulathur 603203, Chengalpattu District, Tamilnadu, India*
[d] *Institute of NanoEngineering Research (INER), Department of Chemical, Metallurgical and Materials Engineering (Polymer Division), Tshwane University of Technology, Staatsartillerie Rd, 0183, Pretoria West Campus, South Africa*

ARTICLE INFO

*Article History:*
Received 16 February 2021
Revised 17 July 2021
Accepted 9 August 2021
Available online 17 September 2021

Edited by Dr. S.O Amoo.

*Keywords:*
Chitin synthase
Disc volatilisation
Molecular docking
Conceptual DFT
GC-MS

ABSTRACT

This study aimed to examine the antifungal activity of wild bergamot (*Monarda fistulosa*) essential oil on *Colletotrichum musae* and *Lasiodiplodia theobromae*, the causative organisms of anthracnose and crown-rot diseases of banana fruits, respectively. Chitin synthase is a promising target for antifungal compounds, since it is involved in synthesizing chitin, which forms a major proportion of the fungal cell wall. Disc volatilisation method was employed to assess the *in vitro* antifungal activity of the oil. The vapours of this essential oil at 4 $\mu$L per Petriplate exhibited 100% growth inhibition of both the fungal pathogens, and at 66.66 $\mu$LL$^{-1}$ , it significantly (P <0.05) reduced the incidence and severity of anthracnose and crown-rot diseases in artificially wounded and infected fruits. The chemical composition of wild bergamot essential oil was determined by GC-MS technique and the components of the essential oil were docked against chitin synthase to determine the components responsible for antifungal activity. Chitin synthase was modelled by *de novo* approach due to non-availability of the 3D structure. The *in silico* techniques such as molecular docking and conceptual DFT revealed that the major components of the essential oil namely thymol, carvacrol and cinnamyl carbanilate manifested the best antifungal activity. This illustrates effective inhibition of the major postharvest diseases of banana by wild bergamot essential oil.



## 1. Introduction

Banana (*Musa* spp) is a popular tropical climacteric fruit crop predominantly cultivated in Asia, Latin America and Africa, with India being the highest producer of about 29 million tonnes per year on an average (FAO, 2019). Since it is perennially grown, it is an important cash crop too (Okumu et al., 2011). Banana fruits are more prone to fungal attack and decay during postharvest period since they have high sugar level, nutrient content and low pH value. As a result, farmers incur considerable economic losses during the storage and transport (Kalia, 2020). Anthracnose and crown-rot diseases caused by *Colletotrichum musae* and *Lasiodiplodia theobromae*, respectively are two aggressive postharvest diseases of banana that are responsible for the majority of the losses (Li et al., 2019; Guimarães et al., 2019). The disease incidence and damage rate caused by anthracnose is higher than other postharvest diseases and the losses may increase up to 80% if the fruit is not treated (Bill et al., 2014). Most of the postharvest diseases are commercially controlled by dipping clusters of banana in solutions of thiabendazole, imazalil or carbendazim fungicides (Vilaplana et al., 2018). Due to extensive use of these chemical fungicides, most of the pathogenic strains are developing resistance to them (Horsfall, 2012; Hahn, 2014). Additionally, there are reports to manifest the repercussions caused by incessant use of such fungicides, namely- carcinogenesis, residual toxicity and environmental pollution (Komárek et al., 2010; Singh et al., 2016).

Of late, there is a seemingly high demand for fresh produce that are not treated with agrochemicals, particularly the ones applied postharvest, in most of the countries around the globe (Shewfelt et al., 2014). Therefore a promising control measure is imperative to effectively manage major postharvest diseases of banana. Among the safe and natural alternatives to synthetic

* Corresponding authors.
*E-mail addresses:* periyars@srmist.edu.in (P.S. Sellamuthu), thirumum@srmist.edu.in (T. Madhavan).

https://doi.org/10.1016/j.sajb.2021.08.019
0254-6299/

chemicals, the use of plant essential oils has attracted researchers for the management of several diseases of fruits and vegetables for over three decades (Baratta et al., 1998; Soylu et al., 2006; Lee et al., 2020). Essential oils are complex mixtures of volatile components well-known for their therapeutic activity, antifungal, antimicrobial, anti-cancer activity and various other applications. Hence they are gaining increasing interest among the research fraternity due to the features they possess, such as, volatility and safety (Sellamuthu et al., 2013).

The main principle behind this work was effective inhibition of chitin synthase, an enzyme responsible for synthesis of chitin that forms about 20-60% of the cell wall which in turn can lead to impaired cell wall followed by fungal cell membrane destruction due to the lipophilicity of the essential oils leading to whole cell destruction by various mechanisms typical to individual essential oils (Bowman and Free, 2006; Rogg et al., 2012; Nazzaro et al., 2017). To the best of our knowledge, there has been no study previously involved in evaluating the antifungal effects of wild bergamot essential oil components on postharvest banana pathogens.

The objectives of our study were to initially evaluate the antifungal efficacy of wild bergamot essential oil through *in vitro* and *in vivo* methods. Further, to determine the components of wild bergamot essential oil through GC-MS technique and dock the essential oil components against the modelled chitin synthase protein, followed by evaluation of the inhibitory effects of the specific essential oil components responsible for antifungal activity based on the binding affinities and their structure-activity relationship.

## 2. Materials and methods

### 2.1. Essential oil and chemicals

Wild bergamot essential oil (*Monarda fistulosa* L.) was obtained from Cyrus enterprises, Chennai, Tamil Nadu, India, and stored at 4°C. Potato dextrose agar was procured from Himedia Biosciences, India and other chemicals were purchased from SRL Pvt Ltd, Mumbai, India.

### 2.2. Isolation and identification of pathogenic fungi

The banana fruits were collected from a local orchard in Chennai, Tamil Nadu, India. They were harvested at the right maturity stage and were stored at room temperature until spoilage to isolate the fungi, *Colletotrichum musae* and *Lasiodiplodia theobromae*. Identification of these fungi was performed based on their colony and hyphal morphology, conidial structures and also with the help of available literature describing their characteristic features (Sutton and Waterson, 1970; Sangeetha et al., 2012). Pure cultures were maintained on potato dextrose agar (PDA) at 25°C.

### 2.3. In vitro *antifungal assay*

Disc volatilisation method was employed to determine the antifungal activity of wild bergamot essential oil against *C. musae* and *L. theobromae* (Reyes-Jurado et al., 2020). Fungal plugs from 14 days old actively growing culture, measuring 6 mm in diameter were inoculated in Petriplates containing 15 mL of fresh Potato Dextrose Agar (PDA) medium. Further, sterile Whatman filter paper discs of about the same size as fungal plugs were fixed to the inner surface of the Petriplate lid using double-sided sticky tape. Thereafter, aliquots of undiluted essential oils ranging from 1 $\mu$L to 10 $\mu$L were added onto the discs. Sterile distilled water served as the control and carbendazim, which is a systemic fungicide commonly used to dip-treat banana fruits, served as the positive control. The Petriplates were sealed with parafilm immediately after adding essential oil to ensure effective exposure of vapours to the fungal mycelia followed by incubation at 25°C for 7 days. This assay per test pathogen was triplicated to derive statistically sound conclusion. Poison-food technique was followed for carbendazim treatment since it is not volatile in nature to employ disc volatilization technique. The percentage inhibition of fungal growth is an index of antifungal efficacy of the oil. It is expressed as: IRMG (%) = [(dc - dt)/dc]* 100, where IRMG % is the percentage inhibition of radial mycelial growth, dc and dt are the radial mycelial growth measurements in control and test Petriplates, respectively (Zhang et al., 2019).

### 2.4. Antifungal efficacy of essential oil on artificially inoculated banana fruits

Freshly harvested untreated banana fruits (cv. Poovan) were procured from a local orchard in Chennai, India. The *in vivo* study was performed at the right stage of maturity of the fruits. The peel colour of the fruits were pale green to light yellow, the angularity on the peel had disappeared, the length of the fruits were roughly 16 cm and the diameter was about 38 mm. The fruits were disinfected using 1.5% sodium hypochlorite solution, followed by washing with water and then air-dried.

Spore suspensions of the two fungal pathogens were prepared by flooding 14-day-old culture with 5 mL of sterile distilled water containing 0.05% (v/v) Tween-80 and the surface of the culture was gently scraped using an L-rod. The suspensions were filtered through a three-layered muslin cloth for the removal of residual mycelia, and the spore concentration was adjusted to $10^8$ spores $mL^{-1}$ using a haemocytometer. The fruits were wounded uniformly (3 mm deep and 3 mm wide) with a sterile cork borer at the crown-end and equatorial regions, followed by inoculation using spore suspensions (20 $\mu$L) of *L. theobromae* and *C. musae* at the respective regions. The fruits were then divided into five groups and placed in airtight boxes measuring 27 × 20 × 9 cm, with 90% relative humidity. The groups were: i) Control group: untreated inoculated fruits; ii) Positive control: inoculated fruits treated (dip treatment for 5 minutes) with synthetic fungicide carbendazim (0.05%). The rest of the three groups were inoculated fruits treated with essential oil at iii) 16.66 $\mu LL^{-1}$; iv) 33.33 $\mu LL^{-1}$; v) 66.66 $\mu LL^{-1}$, which was calculated based on the volume of essential oil required to inhibit the organisms at *in vitro* level. The highest volume of the essential oil was fixed at 66.66 $\mu LL^{-1}$ of the boxes in which the fruits were placed for *in vivo* testing. This was based on the volume of essential oil required to completely inhibit the fungal pathogens in Petridishes with a total volume of 60 mL (which was 4 $\mu$L per 60 mL). The rest of the two concentrations were consecutively halved for comparison purpose. The experiment was performed twice, with 12 fruits for each volume of the essential oil. The fungal growth was recorded on day 4 and day 7. The disease severity for each of the diseases was expressed as a direct measure of the lesion diameter around the wound (mm) for 24 fruits and the average measure was calculated. The disease incidence was calculated according to Perumal et al. (2017) as

$$Disease\ incidence\ (\%) = \frac{Number\ of\ infected\ wounds}{Total\ number\ of\ inoculated\ wounds} \times 100$$

### 2.5. GC-MS analysis of essential oil

Agilent 7890B gas chromatograph (equipped with split/splitless inlet) was employed to perform GC analysis coupled with an Agilent 5977A MSD for compound identification (Adams, 2007). The separation column made up of HP-5MS 5% phenyl methyl silox material with following dimensions 30 m × 250 $\mu$m × 0.25 $\mu$m was used for compound separation (Agilent part number G4513A-G3440B-G7038A). Helium was used as carrier gas with a flow rate of 0.8 ml/min and at a constant pressure of 65 kPa. One microliter of essential oil (10% concentration using n-hexane as a solvent) was injected into the column with a split

**Table 1**
Effect of wild bergamot essential oil on *Colletotrichum musae* and *Lasiodiplodia theobromae* as determined by disc volatilisation method.

| Concentration of oil ($\mu$L per plate) | Wild bergamot essential oil (Inhibition %) *C. musae* | *L. theobromae* |
|---|---|---|
| 1 | $0.0 \pm 0^a$ | $0.0 \pm 0^a$ |
| 2 | $56.1 \pm 0.9^b$ | $76.6 \pm 1.1^b$ |
| 3 | $77.7 \pm 1.1^c$ | $87.7 \pm 1.1^c$ |
| 4 | $100 \pm 0^d$ | $100 \pm 0^d$ |
| 5 | $100 \pm 0^d$ | $100 \pm 0^d$ |
| 6 | $100 \pm 0^d$ | $100 \pm 0^d$ |
| 7 | $100 \pm 0^d$ | $100 \pm 0^d$ |
| 8 | $100 \pm 0^d$ | $100 \pm 0^d$ |
| 9 | $100 \pm 0^d$ | $100 \pm 0^d$ |
| 10 | $100 \pm 0^d$ | $100 \pm 0^d$ |
| Control (sterile distilled water) | $0.0 \pm 0^a$ | $0.0 \pm 0^a$ |
| Carbendazim (0.05%) | $100 \pm 0^d$ | $100 \pm 0^d$ |

ratio of 25:1 with the injector temperature set to 250°C. The temperature programme ranged from 60-240°C with a ramp of 3°C/min and a total run time of 60 min. The interface temperature was set to 300°C. The mass spectra were read at 70ev, under positive electron impact ionisation, with a mass range from 50 to 550 amu (David et al., 2002). The identities of the compounds were verified by comparison with the mass spectra in National Institute of Standards and Technology (NIST) 14 library and also with available literature (Perumal et al., 2016).

### 2.6. De novo *protein structure modelling and validation*

Since 3D structure of chitin synthase protein was not available in Protein Data Bank (PDB), homology modelling technique could not be employed. Suitable templates for threading method were not generated when the sequences of the protein were subjected to Basic Local Alignment Search Tool (BLAST) against PDB structures. Therefore the chitin synthase protein structures had to be modelled through *De novo* approach for both the fungal organisms, because the number and type of amino acid residues in their respective sequences were subtly different. The UniProt accession number of chitin synthase sequence of *C. musae* and *L. theobromae* are J7HAE1 and A0A2U9IY26, respectively. *De novo* modelling of protein structure was previously performed by Kulkarni et al. (2021) using QUARK web server (Xu and Zhang 2013). QUARK employs an algorithm to build protein structures through small peptides by replica exchange Monte Carlo simulation.

The models generated were previously validated using Ramachandran plot, ERRAT, Verify 3D and ProSA Z-score. Additionally, ERRAT graph was also plotted to evaluate the residue-wise overall quality of the protein structure.

**Table 3**
GC-MS analysis of wild bergamot essential oil.

| Peak no | Name of the compound | Retention time | Area (%) | Similarity index (%) |
|---|---|---|---|---|
| 1 | Tricyclene | 5.575 | 0.010 | 93 |
| 2 | $\beta$-Thujene | 5.698 | 0.008 | 90 |
| 3 | $\alpha$-Pinene | 5.898 | 5.498 | 81 |
| 4 | Camphene | 6.331 | 0.142 | 96 |
| 5 | 1,1,2,3-Tetramethylcyclohexane | 6.820 | 0.011 | 90 |
| 6 | $\beta$-Pinene | 7.209 | 5.393 | 86 |
| 7 | cis-p-Menthane | 7.620 | 0.260 | 93 |
| 8 | 3-Carene | 8.331 | 0.095 | 95 |
| 9 | $\alpha$-Terpinene | 8.575 | 0.073 | 87 |
| 10 | o-Cymene | 8.786 | 0.435 | 85 |
| **11** | **Cinnamyl carbanilate** | 8.942 | **17.547** | 94 |
| 12 | D-Limonene | 9.042 | 0.131 | 95 |
| 13 | Eucalyptol | 9.142 | 0.381 | 85 |
| 14 | Isopropyltoluene | 9.408 | 0.145 | 85 |
| 15 | $\gamma$-Terpinene | 10.186 | 0.144 | 86 |
| 16 | Linalool oxide | 10.786 | 0.023 | 94 |
| 17 | 2-Carene | 11.375 | 0.103 | 95 |
| 18 | Linalool | 11.975 | 8.251 | 89 |
| 19 | Camphenol | 12.986 | 0.009 | 94 |
| 20 | Dihydrolinalool | 13.363 | 0.084 | 88 |
| 21 | Pinocarveol | 13.541 | 0.071 | 92 |
| 22 | L-camphor | 13.752 | 0.016 | 94 |
| 23 | Verbenol | 13.852 | 0.042 | 93 |
| 24 | D-menthone | 14.174 | 0.019 | 94 |
| 25 | L-4-terpineol | 15.230 | 0.250 | 90 |
| 26 | L-$\alpha$-Terpineol | 15.885 | 0.038 | 88 |
| 27 | Myrtenol | 16.130 | 0.108 | 92 |
| 28 | (R)-(+)-$\beta$-Citronellol | 17.619 | 0.420 | 93 |
| 29 | L-Carvone | 18.252 | 0.043 | 92 |
| **30** | **Thymol** | 20.696 | **23.438** | 94 |
| **31** | **Carvacrol** | 21.129 | **23.438** | 91 |
| 32 | $\alpha$-Cubebene | 22.685 | 0.054 | 94 |
| 33 | Citronellyl acetate | 23.018 | 4.249 | 90 |
| 34 | $\alpha$-Copaene | 23.796 | 0.186 | 94 |
| 35 | Caryophyllene | 25.651 | 7.183 | 96 |
| 36 | Humulene | 27.051 | 0.773 | 93 |
| 37 | $\delta$-Cadinene | 29.928 | 0.092 | 92 |
| 38 | Caryophyllene oxide | 31.050 | 0.031 | 93 |
| 39 | Epoxycaryophyllene | 32.250 | 0.740 | 93 |
| 40 | Humulene oxide II | 33.283 | 0.050 | 86 |
| 41 | Beta-Caryophyllene epoxide | 35.716 | 0.018 | 93 |

*The components with high area % have been highlighted.

### 2.7. *Ligand preparation*

The components of wild bergamot essential oil (as determined through GC-MS) were the ligands to be docked in the chitin synthase protein. The aliphatic components have been eliminated since they generate many conformers owing to their structural flexibility, which may led to unrealistic scores. The ligand structures (3D) and carbendazim structure (control) were downloaded from PUBCHEM database (pubchem.ncbi.nih.nlm.gov), which is maintained by NIH, NCBI. The

**Table 2**
Effect of wild bergamot essential oil on incidence and severity of anthracnose and crown-rot diseases in inoculated banana fruits (cv. Poovan).

| Concentration of wild bergamot essential oil ($\mu$LL$^{-1}$) | Incidence of Crown-rot (%) | | Severity of Crown-rotLesion diameter (mm) | | Incidence of Anthracnose (%) | | Severity of AnthracnoseLesion diameter (mm) | |
|---|---|---|---|---|---|---|---|---|
| | Day 4 | Day 7 | Day 4 | Day 7 | Day 4 | Day 7 | Day 4 | Day 7 |
| 16.66 | $55.56 \pm 0.1^d$ | $66.67 \pm 0^d$ | $10.3 \pm 1.4^c$ | $12.2 \pm 1.1^d$ | $55.56 \pm 0.2^c$ | $66.67 \pm 0.1^c$ | $9.7 \pm 1.5^c$ | $11.4 \pm 0.5^d$ |
| 33.33 | $44.45 \pm 0.3^c$ | $55.56 \pm 0.1^c$ | $8.5 \pm 0.9^b$ | $11.1 \pm 1.3^c$ | $33.34 \pm 0.3^b$ | $44.45 \pm 0.2^b$ | $8.6 \pm 1.6^b$ | $10.2 \pm 0.9^c$ |
| 66.66 | $11.12 \pm 0.1^a$ | $22.23 \pm 0.2^a$ | $6.7 \pm 0.6^a$ | $7.3 \pm 0.9^a$ | $11.12 \pm 0.2^a$ | $22.23 \pm 0.2^a$ | $5.9 \pm 1.1^a$ | $6.7 \pm 1.4^a$ |
| Control (sterile distilled water) | $100 \pm 0^e$ | $100 \pm 0^e$ | $12.2 \pm 1.1^d$ | $15.0 \pm 1.4^e$ | $100 \pm 0^d$ | $100 \pm 0^d$ | $13.3 \pm 1.1^d$ | $18.5 \pm 0.8^e$ |
| Carbendazim (0.05%) | $22.23 \pm 0.2^b$ | $33.34 \pm 0.3^b$ | $7.1 \pm 0.6^a$ | $8.5 \pm 0.9^b$ | $11.12 \pm 0.2^a$ | $22.23 \pm 0.3^a$ | $6.2 \pm 0.9^a$ | $7.8 \pm 0.6^b$ |

Values in the table are denoted as mean ± SD, where n=72. Different letters in the superscript indicate significant difference (P < 0.05) as analysed by Duncan's multiple range test (SPSS v.22) ligand compounds were loaded into the PyRx virtual screening software (Dallakyan and Olson, 2015) through OpenBabel control in SDF file format. Energy minimization of the ligands was performed using Universal Force Field (UFF) by adopting the conjugate gradient algorithm, and further they were converted to PDBQT format. The ligands were assigned charges.

### 2.8. Molecular docking

In this study, docking calculations have been performed using AutoDock Vina in PyRx virtual screening open source software (Trott and Olson, 2010). The protein is prepared prior to docking and energy is minimized. To characterize the active site, the modelled protein structures were submitted to active site prediction server maintained by SCFBio, IIT-D (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp). The grid was fixed on the cavity by selecting the active site residues and the grid dimensions were manually adjusted to cover the complete site. Further, AutoDock Vina was run by specifying eight repeats for each ligand. The binding affinities (kcal/mol) were displayed in the results tab. The results table was exported as a CSV (comma separated value) file for reference. The docked poses were visualized along with the non-covalent interactions using PyMOL visualization tool (http: //www. pymol. org).

### 2.9. Conceptual DFT

Conceptual density functional theory was employed for the interpretation/prediction of experimental/theoretical reactivity data based on a series of reactivity descriptors which in turn determines the chemical behavior of a molecule (Domingo et al., 2016). They are, namely total energy, molecular dipole moment, lowest unoccupied molecular orbital (LUMO), highest occupied molecular orbital (HOMO), energy gap, global softness, absolute hardness, electronegativity, electrophilicity index and chemical potential. These descriptors have been calculated based on the electron density of molecules in each orbital using Fukui's molecular orbital theory (Fukui, 1982). From the statistical results obtained, the structure-activity relationship of all the molecules has been analyzed.

### 2.10. Statistical analysis

The fungal growth inhibition percentage is expressed as mean of the three records with standard deviation. Significance of mean differences was statistically compared using Duncan's multiple range test at $P < 0.05$. The experimental data were subjected to one way analysis of variance (ANOVA) using the Statistical Package for Social Scientists (SPSS Inc., Chicago, IL, USA) 22.0 software package.

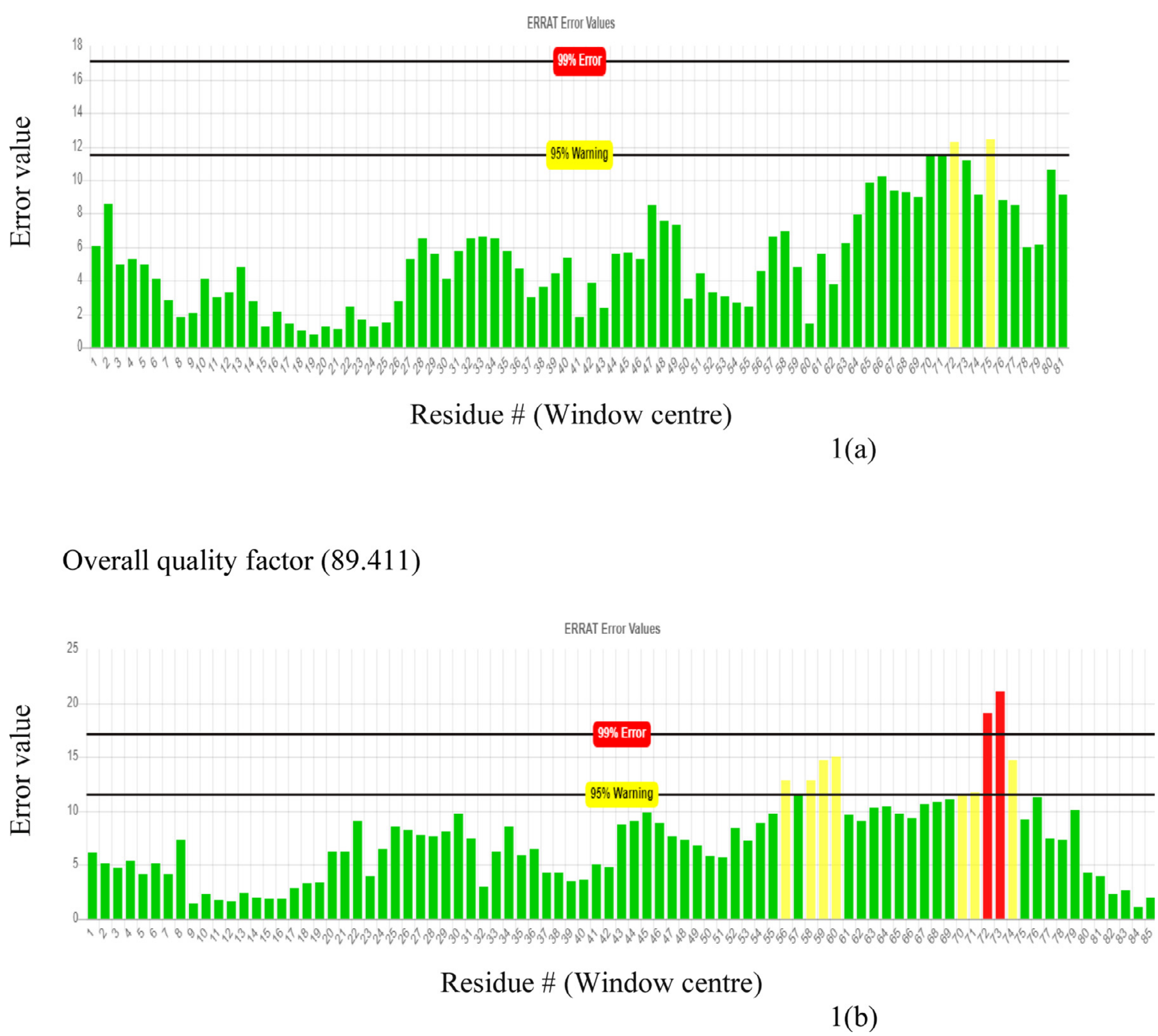


**Fig. 1.** (a) ERRAT plot for chitin synthase (*Lasiodiplodia theobromae*) and (b) ERRAT plot for chitin synthase (*Colletotrichum musae*).

**Table 4**
Binding affinity of the ligands against chitin synthase of *Lasiodiplodia theobromae* and *Colletotrichum musae*. Carbendazim (Standard synthetic fungicide) is used as a control.

| Compound name | Binding affinity (kcal/mol) | |
|---|---|---|
| | *L. theobromae* | *C .musae* |
| Carbendazim | -5.9 | -5.0 |
| $\beta$-Thujene | -4.3 | -4.3 |
| $\alpha$-Pinene | -4.7 | -4.5 |
| Tricyclene | -3.9 | -4.3 |
| Camphene | -4.6 | -4.6 |
| 1,1,2,3-Tetramethylcyclohexane | -4.1 | -5.0 |
| $\beta$-Pinene | -4.4 | -4.5 |
| cis-p-Menthane | **-5.4** | -4.6 |
| $\alpha$-Terpinene | -5.0 | -4.9 |
| o-Cymene | -5.2 | -5.2 |
| **Cinnamyl carbanilate** | **-7.5** | **-7.0** |
| D-Limonene | -4.9 | -4.9 |
| Eucalyptol | -3.8 | -4.4 |
| $\gamma$-Terpinene | **-5.3** | -5.0 |
| Camphenol | -4.3 | -4.6 |
| Pinocarveol | -4.5 | -4.7 |
| L-camphor | -3.9 | -4.3 |
| Verbenol | -4.6 | -4.7 |
| D-menthone | -4.5 | -4.4 |
| L-4-terpineol | **-5.3** | -4.8 |
| L-$\alpha$-terpineol | **-5.4** | -4.9 |
| L-Carvone | -5.1 | -5.2 |
| **Thymol** | **-5.6** | **-5.6** |
| **Carvacrol** | **-5.6** | **-5.6** |
| $\alpha$-Cubebene | -5.2 | -3.3 |
| $\alpha$-Copaene | -5.2 | -3.6 |
| Caryophyllene | -4.4 | **-5.4** |
| Humulene | -4.3 | -5.1 |
| $\delta$-Cadinene | -4.8 | **-6.0** |
| Caryophyllene oxide | -4.5 | -5.2 |
| Humulene oxide II | -4.2 | **-5.4** |

*The highlighted compounds have high binding affinity towards chitin synthase of the fungal organisms.

## 3. Results

### 3.1. In vitro *antifungal assay*

The *in vitro* antifungal activity of wild bergamot essential oil on both the fungal pathogens has been depicted in Table 1. There was a rise in growth inhibition percentage with increase in concentration of the oil. The vapours of the oil could inhibit the pathogen growth completely at a concentration of 4 $\mu$L per Petriplate and onwards. The fungicidal effect of the oil on both the pathogens was confirmed by transferring the essential oil treated mycelial plug to a fresh plate with PDA. No growth was observed on fresh plates.

### *3.2. Effect of wild bergamot essential oil vapour on artificially infected banana fruits*

The banana fruits treated with wild bergamot essential oil have shown significant ($P < 0.05$) reduction in the severity and incidence of crown-rot and anthracnose diseases, with increase in oil concentration. Effective control of the diseases was observed at 66.66 $\mu LL^{-1}$ concentration of the oil when compared to lower concentrations of the oil, with the untreated control fruits, and also with the positive control. The results have been tabulated in Table 2 and the picture depicting antifungal efficacy of wild bergamot essential oil on artificially inoculated banana fruits has been provided as a supplementary material (Fig.S1). Although there was an increase in disease incidence and severity on day 7, compared to the day 4, the vapours of the essential oil controlled the postharvest diseases better than the commercial treatment (5 min dip treatment using 0.05% carbendazim).

### *3.3. Composition of wild bergamot essential oil*

The results of GC-MS analysis revealed that there were 41 components in the wild bergamot essential oil, out of which, thymol (23.43%), carvacrol (23.43%) and cinnamyl carbanilate (17.54%) were the major components as shown in Table 3. The similarity index (%) of the components, quantitatively indicates the difference between the spectrum of the unknown sample and that of the library. It was calculated using an equation stated by Gumbi et al., 2019.

### *3.4. De novo chitin synthase model generation and validation*

ERRAT validation graph was plotted (Fig. 1) to evaluate the overall quality factor of the two models. The plots indicated excellent overall quality factors of 97.530 and 89.411 for chitin synthase structure of *L. theobromae* and *C. musae* respectively, with negligible warnings and errors.

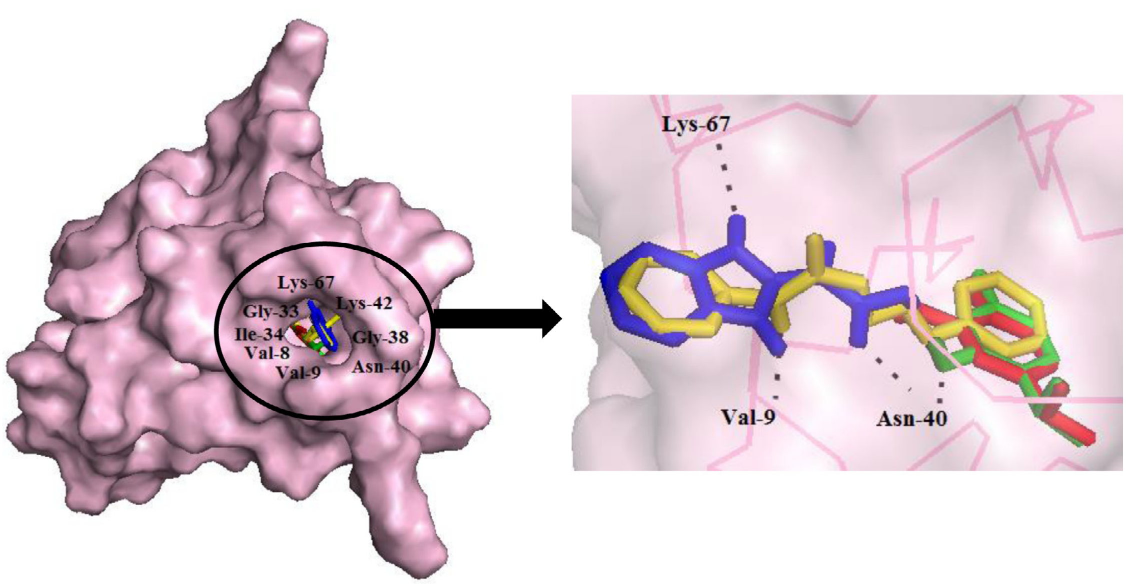


**Fig. 2.** Docked ligands thymol (green), carvacrol (red), cinnamyl carbanilate (yellow) and carbendazim (blue) exhibiting hydrogen bonding with residues in the binding pocket of chitin synthase of *Lasiodiplodia theobromae.*

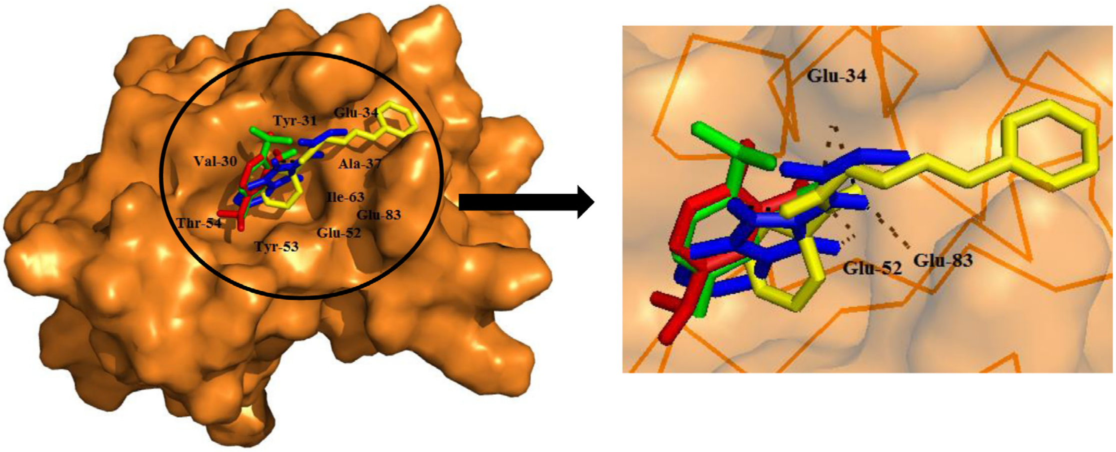


**Fig. 3.** Docked ligands thymol (green), carvacrol (red), cinnamyl carbanilate (yellow) and carbendazim (blue) exhibiting hydrogen bonding with residues in the binding pocket of chitin synthase of *Collectotrichum musae.*

### *3.5. Molecular docking*

In this study, based on the binding affinities with least RMSD, we can conclude that thymol, carvacrol, cinnamyl carbanilate were better inhibitors of chitin synthase protein of both the organisms, compared to the rest of the compounds (Table 4). The binding affinities of these components were comparable to that shown by carbendazim. In case of chitin synthase of *C. musae*, these components scored better than the control. Some components such as cis-p-menthane, $\gamma$-Terpinene, L-$\alpha$-terpineol and L-4-terpineol were specifically effective inhibitors of chitin synthase of *L. theobromae.* Similarly, caryophyllene, $\delta$-Cadinene and Humulene oxide II specifically inhibited chitin synthase of *C. musae*.

Four active cavities were suggested by the active site prediction server SCFBio. Out of which, one was selected, based on the binding affinities and bond formation, which were better in this active site compared to the others. The residues in the catalytically important active site of chitin synthase of *L. theobromae* are Val-8, Val-9, Gly-33, Ile-34, Gly-38, Asn-40, Lys-42 and Lys-67. Thymol, carvacrol, cinnamyl carbanilate and carbendazim displayed hydrogen bonding with residues Val-9, Asn-40 and Lys-67 (Fig. 2). Similarly, the residues in the catalytically important active site of chitin synthase of *C. musae* are Tyr-31, Glu-34, Ala-37, Glu-52, Tyr-53, Thr-54, Ile-63, Glu-83 and Val-30. Thymol, carvacrol, cinnamyl carbanilate and carbendazim displayed hydrogen bonding with residues Glu-34, Glu-52 and Glu-83 (Fig. 3).

### *3.6. Conceptual DFT*

The statistical values of the descriptors are presented in Table 5. The difference between $E_{HOMO}$ and $E_{LUMO}$ is the energy gap ($\Delta$E), which is inversely proportional to the molecular reactivity. Out of the six components that exhibited high binding affinity, thymol and carvacrol had the lowest energy gap with values 5.32 and 5.86 respectively, indicating high molecular reactivity. Molecular dipole moment is directly proportional to chemical reactivity of a molecule. Cinnamyl carbanilate displayed the highest dipole with value 2.24 Debye, followed by carvacrol, thymol and L-$\alpha$-Terpineol with almost similar values. Electronegativity ($\chi$) is an important index to assess the ability of a molecule to accept electrons. Lower the electronegative index, higher is the inhibitory activity of the molecule. Chemical potential ($\mu$) of a molecule indicates the energy absorbed or released by a molecule during a chemical reaction. It is expressed as negative of electronegativity of a molecule. The selected EO components (Table 5) were all almost equally electronegative with values in the range -2.44 to -2.89. Other descriptors such as global softness ($\sigma$), absolute hardness ($\eta$), electrophilicity index ($\omega$) were also calculated using $E_{HOMO}$ and $E_{LUMO}$ values. The descriptors suggest that thymol, carvacrol and cinnamyl carbanilate were chemically active, which is in line with the docking results. The electron density maps of these selected components have been presented in Fig. 4.

## 4. Discussion

In view of consumer safety with regard to consumption of fruits and vegetables that are treated with synthetic fungicides, essential oils have been explored over the past few decades as a natural and safe alternative. In addition to being detrimental to human health, chemical fungicides bring about deleterious repercussions on the environment too. Postharvest antifungal activities of several essential oils on a diverse range of fungi that affect climacteric fruits have been well documented by various postharvest researchers (Sivakumar and

**Table 5**
Statistics of DFT based molecular descriptors of selected components of wild bergamot essential oil.

| Compound | Total Energy (E $\gamma$) (in eV) | Molecular dipole moment (Debye) | $E_{HOMO}$ | $E_{LUMO}$ | HOMO/LUMO Gap ($\Delta$E) | Absolute Hardness ($\eta$) | Global Softness ($\sigma$) | Electronegativity ($\chi$) | Chemical potential ($\mu$) | Electrophilicity index ($\omega$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Carvacrol | -12644.57 | 1.44 | -5.82 | 0.04 | 5.86 | 2.93 | 0.17 | -2.89 | 2.89 | 1.42 |
| Cinnamyl-carbanilate | -22417.11 | 2.24 | -6.12 | 1.16 | 7.28 | 3.64 | 0.14 | -2.48 | 2.48 | 0.85 |
| cis-p-menthane | -10697.22 | 0.11 | -7.40 | 2.57 | 9.97 | 4.99 | 0.10 | -2.42 | 2.42 | 0.59 |
| $\delta$-Cadinene | -15945.95 | 0.25 | -5.69 | 0.64 | 6.33 | 3.16 | 0.16 | -2.52 | 2.52 | 1.01 |
| L-$\alpha$-Terpineol | -12710.32 | 1.42 | -5.76 | 0.88 | 6.64 | 3.32 | 0.15 | -2.44 | 2.44 | 0.90 |
| Thymol | -12644.62 | 1.42 | -5.28 | 0.04 | 5.32 | 2.66 | 0.19 | -2.62 | 2.62 | 1.29 |

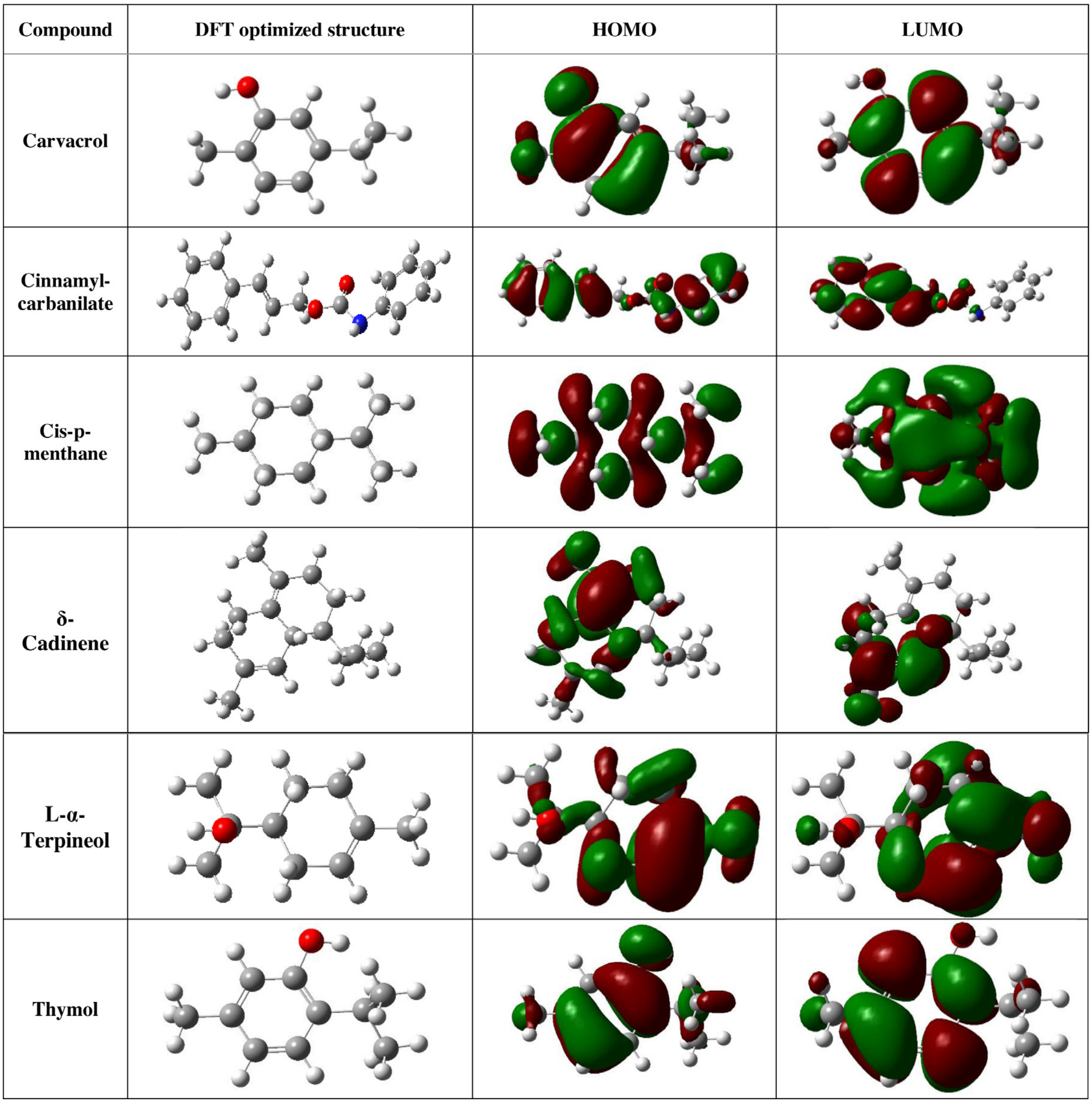


**Fig. 4.** Electron density maps of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of phytochemicals.

Bautista-Baños, 2014; Taghavi et al., 2018; Kumar and Kudachikar, 2018). Apart from testing the antifungal activity of the essential oils, the mechanism of action, and also the components responsible for the inhibitory activity provides more insight into the study. Conceptual DFT is an *in silico* technique that is useful in predicting the components of the essential oil responsible for high activity based on various molecular descriptors. Structure information of the target as well as the ligands is important for portraying interactions and binding affinities, if not, the protein has to be modelled by alternative ways (Abbass et al., 2013; Franca, 2015). The non-covalent interaction between the compounds and the target protein can be clearly envisioned with the help of visualization tools. Many authors have reported that the antifungal activities of essential oils are evidently related to their chemical composition, percentage constitution of individual components and also to their structure (Dorman and Deans, 2000; Radulovic et al., 2013).

Terpenes and terpenoids have been reported to have high antifungal activity compared to the other components of essential oils. It has been noted that compounds with a phenol ring structure have better antifungal activity; however, addition of hydroxyl group enhances their activity (Hyldgaard et al., 2012). Therefore thymol which is a monoterpenoid, and carvacrol, which is an isomer of thymol and L-$\alpha$-terpineol, which are similar in structure to thymol exhibit the best activity, which was clearly noted in our study too. In contrast, menthol which is an aliphatic analog of thymol and carvacrol lack the activity. Components like cis-p-menthane, $\delta$-Cadinene, L-4-terpineol, $\gamma$-terpinene, had good activity due to the presence of phenol ring. Terpenoids with aldehyde and alcohol groups are believed to basically disrupt the cell wall, followed by a cascade of events such as binding to membrane proteins and changing their conformation, disrupting ergosterol biosynthesis pathway, altering the membrane potential and impairing membrane integrity, finally, diminishing the mitochondrial content which results in altered level of reactive oxygen species (ROS) and ATP generation, although the mechanism is not clearly understood yet (Kishore and Pande 2005; Haque et al., 2016; Sant et al., 2016).

Wild bergamot essential oil used in our study was rich in monoterpenoids (majorly thymol and carvacrol) which indicates a fair inference that this oil has potential antifungal properties. Although, the composition of this oil has been determined before, the GC-MS analysis has been performed again to note the variations in the composition of the oil due to various factors such as method of essential oil extraction, place and season (Malankina et al., 2020). Moreover, thymol, carvacrol and cinnamyl carbanilate had docked well in a binding pocket with complementary binding, and one or more hydrogen bonding with the residues in the pocket which is an evidence of good inhibition. The parameters evaluated in conceptual DFT study also suggested that thymol, carvacrol and cinnamyl carbanilate are highly active based on the molecular descriptor values. The oil could inhibit the growth of both the pathogens completely at 4 $\mu$L per Petriplate, and could significantly control the postharvest diseases at *in vivo* level. This is an advantage since vapours from a single oil is sufficient to control major postharvest diseases of banana.

## 5. Conclusion

In this study, composition of wild bergamot essential oil was determined by GC-MS technique. Through *in vitro* disc volatilization method, we found that the oil could completely inhibit the growth of *C. musae* and *L. theobromae* at 4 $\mu$L per Petriplate. At 66.66 $\mu$LL$^{-1}$ concentration, the essential oil could significantly ($P < 0.05$) reduce the incidence and severity of anthracnose and crown-rot diseases of the banana fruits. The components of the oil were docked against the *de novo* modelled chitin synthase. The docking results and conceptual DFT descriptors suggested that thymol, carvacrol and cinnamyl carbanilate exhibited the best antifungal activity. It can therefore be deduced that wild bergamot essential oil has potent antifungal activity against major pathogenic fungi affecting banana fruits.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2021.08.019.

## References


Abbass, J., Nebel, J.C., Mansour, N., Elloumi, M., Zomaya, A.Y., 2013. Ab initio protein structure prediction: methods and challenges. Biological Knowledge Discovery Handbook. John Wiley and Sons, Inc, Hoboken, New Jersey, pp. 703–724.

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry (Vol. 456). Allured Publishing Corporation, Carol Stream, IL.

Baratta, M.T., Dorman, H.D., Deans, S.G., Figueiredo, A.C., Barroso, J.G., Ruberto, G., 1998. Antimicrobial and antioxidant properties of some commercial essential oils. Flavour Frag. J. 13, 235–244.

Bill, M., Sivakumar, D., Korsten, L., Thompson, A.K., 2014. The efficacy of combined application of edible coatings and thyme oil in inducing resistance components in avocado (*Persea americana* Mill.) against anthracnose during post-harvest storage. Crop Prot. 64, 159–167.

Bowman, S.M., Free, S.J., 2006. The structure and synthesis of the fungal cell wall. Bioessays 28, 799–808.

Dallakyan, S., Olson, A.J., 2015. Small-molecule library screening by docking with PyRx. Chemical Biology. Humana Press, New York, NY, pp. 243–250.

David, F., Sandra, P., Wylie, P.L., 2002. Improving the Analysis of Fatty Acid Methyl Esters Using Retention Time Locked Methods and Retention Time Databases. Agilent Technologies-Application.

Domingo, L.R., Ríos-Gutiérrez, M., Pérez, P., 2016. Applications of the conceptual density functional theory indices to organic chemistry reactivity. Molecules 21, 748.

Dorman, H.D., Deans, S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88, 308–316.

FAO, 2019. http://www.fao.org/economic/est/est-commodities/bananas/en/

França, T.C.C., 2015. Homology modeling: an important tool for the drug discovery. J. Biomol. Struct. Dyn. 33, 1780–1793.

Fukui, K., 1982. The role of frontier orbitals in chemical reactions (Nobel Lecture). Angewandte Chemie Int. Ed. English 21, 801–809.

Guimarães, J.E., de la Fuente, B., Pérez-Gago, M.B., Andradas, C., Carbó, R., Mattiuz, B.H., Palou, L., 2019. Antifungal activity of GRAS salts against *Lasiodiplodia theobromae in vitro* and as ingredients of hydroxypropyl methylcellulose-lipid composite edible coatings to control Diplodia stem-end rot and maintain postharvest quality of citrus fruit. Int. J. Food Microbiol. 301, 9–18.

Gumbi, B.P., Moodley, B., Birungi, G., Ndungu, P.G., 2019. Target, suspect and non-target screening of silylated derivatives of polar compounds based on single Ion monitoring GC-MS. Int. J. Environ. Res. Public Health 16, 4022.

Hahn, M., 2014. The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. J. Chem. Biol. 7, 133–141.

Haque, E., Irfan, S., Kamil, M., Sheikh, S., Hasan, A., Ahmad, A., Lakshmi, V., Nazir, A., Mir, S.S., 2016. Terpenoids with antifungal activity trigger mitochondrial dysfunction in *Saccharomyces cerevisiae*. Microbiology 85, 436–443.

Horsfall, J.G., 2012. Plant Disease: An Advanced Treatise: How Disease Is Managed. Elsevier.

Hyldgaard, M., Mygind, T., Meyer, R.L., 2012. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Microbiol. 3, 12.

Kalia, A., 2020. Microbiology of fresh bananas and processed banana products. Handbook of Banana Production. Postharvest Sci., Process. Technol. Nutrition 245–267.

Kishore, G.K., Pande, S., 2005. Natural fungicides for management of phyto-pathogenic fungi. Ann. Rev. Plant Pathol. 3, 331–356.

Komárek, M., Čadková, E., Chrastný, V., Bordas, F., Bollinger, J.C., 2010. Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. Environ. Int. 36, 138–151.

Kulkarni, S.A., Sellamuthu, P.S., Anitha, D.P.M., Madhavan, T., 2021. *In vitro* and *in silico* evaluation of antifungal activity of cassia (*Cinnamomum cassia*) and holy basil (*Ocimum tenuiflorum*) essential oils for the control of anthracnose and crown-rot postharvest diseases of banana fruits. Chem. Pap. 75, 2043–2057.

Kumar, A., Kudachikar, V.B., 2018. Antifungal properties of essential oils against anthracnose disease: a critical appraisal. J. Plant Dis. Protection. 125, 133–144.

Lee, J.E., Seo, S.M., Huh, M.J., Lee, S.C., Park, I.K., 2020. Reactive oxygen species mediated-antifungal activity of cinnamon bark (*Cinnamomum verum*) and lemongrass (*Cymbopogon citratus*) essential oils and their constituents against two phytopathogenic fungi. Pestic. Biochem. Physiol. 168, 104644.

Li, T., Wu, Q., Zhu, H., Zhou, Y., Jiang, Y., Gao, H., Yun, Z., 2019. Comparative transcriptomic and metabolic analysis reveals the effect of melatonin on delaying anthracnose incidence upon postharvest banana fruit peel. BMC Plant Biol. 19, 1–15.

Malankina, E.L., Kuzmenko, A.N., Zaitchik, B.T., Ruzhitskiy, A.O., Evgrafov, A.A., Kozlovskaya, L.N., 2020. Content and composition of wild bergamot (*Monarda fistulosa* L.) essential oil at different phenological phases. Moscow Univ. Chem. Bull. 75, 391–394.

Nazzaro, F., Fratianni, F., Coppola, R., Feo, V.D., 2017. Essential oils and antifungal activity. Pharmaceuticals 10, 86.

Okumu, M.O., van Asten, P.J., Kahangi, E., Okech, S.H., Jefwa, J., Vanlauwe, B., 2011. Production gradients in smallholder banana (cv. Giant Cavendish) farms in Central Kenya. Scientia Horticult. 127, 475–481.

Perumal, A.B., Sellamuthu, P.S., Nambiar, R.B., Sadiku, E.R., 2017. Effects of essential oil vapour treatment on the postharvest disease control and different defence responses in two mango (*Mangifera indica L.*) cultivars. Food Bioprocess Technol. 10, 1131–1141.

Perumal, A.B., Sellamuthu, P.S., Nambiar, R.B., Sadiku, E.R., 2016. Antifungal activity of five different essential oils in vapour phase for the control of *Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae in vitro* and on mango. Int. J. Food Sci. Technol. 51, 411–418.

Radulovic, N.S., Blagojevic, P.D., Stojanovic-Radic, Z.Z., Stojanovic, N.M., 2013. Antimicrobial plant metabolites: structural diversity and mechanism of action. Curr. Med. Chem. 20, 932–952.

Reyes-Jurado, F., Navarro-Cruz, A.R., Ochoa-Velasco, C.E., Palou, E., López-Malo, A., Ávila-Sosa, R., 2020. Essential oils in vapor phase as alternative antimicrobials: A review. Crit. Rev. Food Sci. Nutr. 60, 1641–1650.

Rogg, L.E., Fortwendel, J.R., Juvvadi, P.R., Steinbach, W.J., 2012. Regulation of expression, activity and localization of fungal chitin synthases. Med. Mycol. 50, 2–17.

Sangeetha, G., Anandan, A., Rani, S.U., 2012. Morphological and molecular characterisation of *Lasiodiplodia theobromae* from various banana cultivars causing crown rot disease in fruits. Arch. Phytopathol. Plant Protection 45, 475–486.

Sant, D.G., Tupe, S.G., Ramana, C.V., Deshpande, M.V., 2016. Fungal cell membrane - Promising drug target for antifungal therapy. J. Appl. Microbiol. 121, 1498–1510.

Sellamuthu, P.S., Sivakumar, D., Soundy, P., Korsten, L., 2013. Essential oil vapours suppress the development of anthracnose and enhance defence related and antioxidant enzyme activities in avocado fruit. Postharvest Biol. Technol. 81, 66–72.

Shewfelt, R.L., Prussia, S.E., Sparks, S.A., 2014. Challenges in handling fresh fruits and vegetables. Postharvest Handling. Academic Press, pp. 11–30.

Singh, S., Singh, N., Kumar, V., Datta, S., Wani, A.B., Singh, D., Singh, K., Singh, J., 2016. Toxicity, monitoring and biodegradation of the fungicide carbendazim. Environ. Chem. Lett. 14, 317–329.

Sivakumar, D., Bautista-Baños, S., 2014. A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. Crop Prot. 64, 27–37.

Soylu, E.M., Soylu, S., Kurt, S., 2006. Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent Phytophthora infestans. Mycopathologia 161, 119–128.

Sutton, B.C., Waterston, J.M., 1970. *Colletotrichum musae*. [Descriptions of Fungi and Bacteria]. IMI Descriptions of Fungi and Bacteria, (23).

Taghavi, T., Kim, C., Rahemi, A., 2018. Role of natural volatiles and essential oils in extending shelf life and controlling postharvest microorganisms of small fruits. Microorganisms 6, 104.

Trott, O., Olson, A.J., 2010. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31, 455–461.
Vilaplana, R., Pazmiño, L., Valencia-Chamorro, S., 2018. Control of anthracnose, caused by *Colletotrichum musae*, on postharvest organic banana by thyme oil. Postharvest Biol. Technol. 138, 56–63.
Xu, D., Zhang, Y., 2013. Toward optimal fragment generations for ab initio protein structure assembly. Proteins Struct. Funct. Bioinf. 81, 229–239.
Zhang, J., Ma, S., Du, S., Chen, S., Sun, H., 2019. Antifungal activity of thymol and carvacrol against postharvest pathogens *Botrytis cinerea*. J. Food Sci. Technol. 56, 2611–2620.