J. Trop. Plant Pests Dis.
Vol. 24, No. 2, September 2024
Pages: 284–294

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.224284-294

RESEARCH PAPER

In vitro assessment of antifungal activity of cinnamon leaves extract against the *Colletotrichum* sp. causes of anthracnose on tomato

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Manuscript received: 22 February 2024. Revision accepted: 12 July 2024. Available online: 24 September 2024.

ABSTRACT

Tomato is a horticultural plant widely cultivated in Bali. Over the three years, the productivity and quality of this plant have tended to decrease due to pathogen-derived diseases, such as anthracnose caused by *Colletotrichum* sp. Until recently, control of this pathogenic fungus has relied on chemical-based fungicides, which lead to many long-term complications, including pathogen resistance, environmental pollution, the extinction of non-target microorganisms, and negative impacts on human health. Therefore, alternative methods for plant disease control are urgently needed to combat these pathogen attacks. The use of plant-derived active compounds has been intensively researched worldwide as a more environmentally friendly alternative. The main objective of this research was to investigate the effectiveness of *Cinnamomum burmanii* acetone extract in inhibiting the growth of *Colletotrichum* sp., the causative agent of anthracnose in tomatoes, through an in vitro approach. A non-factorial randomized complete design was applied in the experiment. The results showed that the crude extract of cinnamon leaves inhibited the growth of the *Colletotrichum* sp. with an MIC value of 0.9%, an inhibition zone of 2.55 mm, and an optimal inhibitory concentration of 2%, producing an inhibition zone of 11.10 mm. A GC-MS analysis was conducted to identify the active compounds in the cinnamon leaf extract. Sixteen active compounds were identified, nine of which are known to have antimicrobial activity.

Key words: acetone extract, active compound, botanical fungicide, MIC test, secondary metabolites

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a horticultural plant from the Solanaceae family, which has significant economic value as a vegetable commodity but suffers from low productivity. The low productivity of tomatoes in Indonesia is caused by several factors, one of which is pest and plant disease infection. One of the major plant diseases that infects tomato fruit is anthracnose (Shahriar et al., 2023). Anthracnose disease is caused by pathogenic fungi from the genus *Colletotrichum*. In addition to reducing yield, the pathogen also damages the aesthetic appearance of tomatoes, which reduces their market value (Sopialena et al., 2022). For exported fruit that needs to be stored during shipment, post-harvest infection can result significant economic loss (Chung et al., 2020; da Silva et al., 2020).

To control anthracnose disease, farmers commonly use synthetic fungicides. However, the continuous use of synthetic fungicides can have many long-term negative impacts, such as the development of pathogenic resistance, water and soil pollution, bioaccumulation in organisms, and risk to human health (Gurusinga et al., 2020). Those effects highlight the need for alternative approaches to control anthracnose disease in tomatoes over the long term. One alternative is the use of botanical pesticides, which are more environmentlly sustainable. According to Sutriadi et al. (2020), extract from certain plant species have the potential to be used as botanical pesticides to control pests and diseases in plants. Before being applied in the horticultural industry, plant extract must be tested in vitro to determine their effectiveness so that they can be applied effectively in vivo in the field (Suprapta et al., 2005).

Cinnamon (*Cinnamomum burmanii* (Nees & T. Nees) Blume) is a plant species with the potential to be used as a botanical pesticide. Cinnamon is a popular spice plant and is commonly used as a main ingredient in traditional medicine (Intan et al., 2021). Cinnamon extract has antimicrobial properties that can utilized as a botanical pesticide. Cinnamon leaf extract can inhibit the growth of the *Fusarium lycopersici* fungus, which causes wilt disease in tomatoes, by 41.66% to

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100% (Darmadi et al., 2015). Meanwhile, cinnamon bark extract shows medium to high activity (inhibition diameter 2.33–16.30 mm) in inhibiting the growth of *Candida albicans* bacteria (Prasetyorini et al., 2021). According to Nabila et al. (2021), cinnamon leaf extract also inhibits the growth of *Porphyromonas gingivalis*, a bacterium that causes inflammatory disease in dental integument tissue, with an inhibition percentage of up to 95%. The antimicrobial activity is highly related to the composition of secondary metabolites as its active compounds. According to Rafif et al. (2022), the active compounds in cinnamon essential oil (from bark and leaves) are primarily composed of cinnamaldehyde, along with other compounds such as monoterpenes, phellandrene, linalool, caryophyllene, cinnamaldehyde, hydrocarbon, pinene, benzaldehyde, benzyl benzoate, cinnamyl alcohol, eugenol acetate, cinnamyl acetate, eugenol, and methyl eugenol. These compounds have many medicinal properties, including antimicrobial activity. Because cinnamon demonstrates promising antimicrobial abilities, this study aims to determine the inhibition activity of cinnamon leaf extract against *Colletotrichum* sp., the fungi that cause anthracnose in tomatoes, through an in vitro approach.

MATERIALS AND METHODS

Research Site. The site for tomato fruit sample collection was located in the agricultural area in Petang District, Badung Regency, Bali. The infected and non-infected tomato fruits were preserved separately. The collected samples were then prepared in the Laboratory of Biochemistry. Data collection was carried out in the Laboratory of Biochemistry and the Laboratory of Microbiology, Biology Study Program, Faculty of Mathematics and Natural Sciences, Udayana University.

***Colletotrichum* sp. Isolation.** *Colletotrichum* sp. was isolated from tomatoes showing symptoms of anthracnose disease. The tomatoes were cleaned under running water, rinsed with sterile water, and then cut into 1 × 1 cm pieces. The part of the tomato inoculated was the section with anthracnose symptoms, with the uninfected part serving as a control. These pieces were then incubated in petri dishes containing 10 mL of Potato Dextrose Agar (PDA) media (1 L of PDA requires 250 g of potato, 20 g of dextrose, and 15 g of agar). The fungus that grew on the media was isolated, purified, and identified both macroscopically and microscopically by observing the color, surface and edges of the colony, and the shape of the spores and hyphae. To ensure that the isolated fungus is the cause of anthracnose disease in tomatoes, Koch's Postulate test was carried out.

Cinnamon Leaves Extraction. The leaves were collected from a local cinnamon plantation with the following criterias: (1) the leaves must be free of pests and diseases; (2) the leaves must be in mature phase, so the 3rd and 4th leaves were collected; and (3) the tree must be more than 5 years old and regularly harvested. To extract the active compounds, the cinnamon leaves were chopped into small pieces (approximately 1 × 1 cm), then air-dried at room temperature. The dried leaves were then blended into powder. One hundred grams of cinnamon leaf powder were then macerated with 1 L of acetone PA (Pro Analysis) for 72 hours in a dark place, room-temperature environment. Four layers of gauze and Whatman filter paper No. 1 were used to filter the macerated powder. The residue obtained was macerated again with 1 L of acetone, repeated twice. The filtrates were combined and then evaporated using a vacuum rotary evaporator (Iwaki, Japan) at 40 ºC to obtain the crude extract.

Test of Antifungal Activity.

Well diffusion test. Fungal suspensions were prepared by subculturing pure fungal cultures in PDA media. Seven-day-old fungal cultures were added to 10 mL of sterile distilled water and then scraped with a hook needle to release the fungal conidia and mycelium. The next step was filtering, which was carried out using gauze. The fungal suspension was then diluted to reach a concentration of 2×10^5 conidia/mL. Petri dishes containing 10 mL of PDA media and 200 µL of fungal suspension were allowed to solidify. Once solid, a diffusion well was made using a cork borer. Each diffusion well was filled with 20 µL of cinnamon crude extract using a micropipette. After 72 hours, the diameter of the inhibition zone around the diffusion well was measured. According to Flanagan and Steck (2017), an inhibition zone ≥ 20 mm is categorized as very strong inhibition, 10–20 mm as strong inhibition, 5–10 mm as medium inhibition, and < 5 mm as weak inhibition.

Minimum Inhibitory Concentration (MIC) *test.* To determine the Minimum Inhibitory Concentration (MIC) of the crude leaf extract, the study was conducted using the well-diffusion method. The crude extract concentrations tested were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, and 0.0% (sterile distilled water was used as a control). The incubation period was 72 hours,

after which the inhibition diameter was measured. The research used a completely randomized design with 11 treatments and three replications (n = 33) (Suprapta et al., 2005).

Test of optimization of antifungal activity. The optimal concentration of cinnamon leaf crude extract that can inhibit the growth of *Colletotrichum* sp. was determined after identifying the MIC value of the extract. The concentration of cinnamon leaf crude extract tested for optimizing antifungal activity were 1, 2, 3, 4 and 5% (v/v). Sterile water was used as the negative control, while the synthetic fungicide Nystatin 5% served as the positive control. The method for determining the optimal concentration of cinnamon leaf crude extract that can inhibit the growth of *Colletotrichum* sp. was the same as the method used for the antifungal activity test.

Active Compound Identification. The identification of active compounds in the plant leaf extracts that inhibit *Colletotrichum* sp. was carried out using Gas Chromatography–Mass Spectroscopy (GC-MS QP2010 Ultra Shimadzu). For GC-MS, the eluent used was a 40:60 ratio of MeOH and H_2O, with a Wakosil ODS/5C18-200 column (4.6 × 200 mm), an eluent flow rate of 1 mL/min, a temperature of 250 ºC, and detection using UV light at 254 nm. The results were analyzed by matching the molecular weights and fragmentation patterns of the isolated compounds with data in the GC-MS library (Zayed et al., 2019).

RESULTS AND DISCUSSION

Isolation of *Colletotrichum* sp. *Colletotrichum* is a concerning pathogen responsible for anthracnose disease in several types of plants, particularly horticultural commodities (Cannon et al., 2012). Tomato is one of the horticultural plants affected by anthracnose infection, leading to reduced productivity and decreased market value (Prasetyo, 2017). Controlling anthracnose is challenging due to the unclear host association of *Colletotrichum* with the crop plants, which can be either host-specific or involve multiple hosts (Talhinhas & Baroncelli, 2021; Zakaria, 2021). In tomatoes, anthracnose disease is caused by several species of the genus *Colletotrichum,* including *C. cocodes, C. dematium* and *C. gloeosporioides* (Kumar et al., 2018). Symptoms of anthracnose first appear as small, circular, slightly sunken lesions on the surface of ripe fruit (Dowling et al., 2020; Zakaria, 2021). In some cases, *Colletorichum* can induce secondary conidiation and infect immature fruit without showing symptoms (also known as quiescent infections), with anthracnose symptoms appearing when the fruit ripens (Dowling et al., 2020).

Tomato fruit infected by anthracnose shows typical symptoms, particularly lesions (rot) on the fruit surface, as shown in Figure 1. The infected fruit, when isolated, exhibited the formation of white-hyphae (Figure 1C), which was identified as the colony of *Colletotrichum* sp. Specifically, the colony of *Colletotrichum* sp. has a cream-white color with grey to black edges. Sudirga et al. (2022) confirmed that after a certain period (more than 15 days), the culture develops black spots on the surface of the colony. This pure isolate of the fungus was used in a confirmation test, which was proven through Koch's Postulate. The results of the confirmation test are presented in Figure 2.

The Koch's postulate test, as shown in Figure 2, with the pathogen isolated from tomatoes with

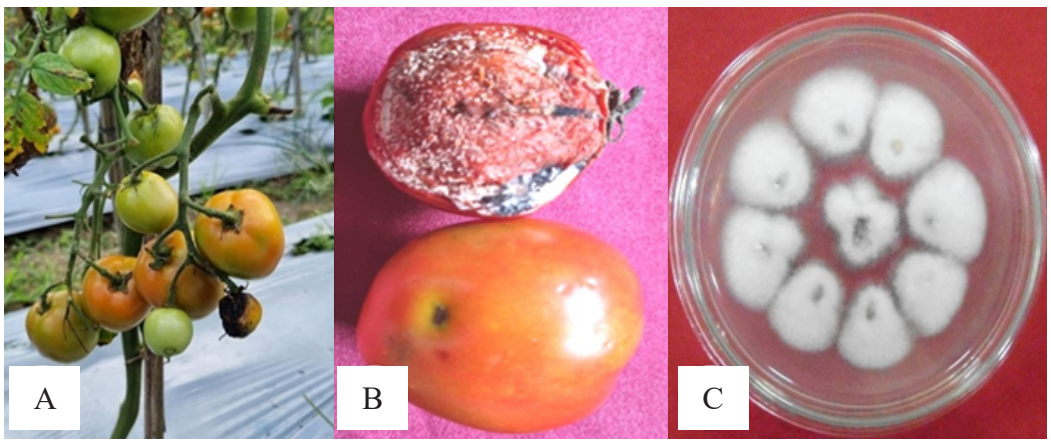

Figure 1. Isolation of pathogenic fungi from tomato fruit showing symptoms of anthracnose. A. Tomato plants; B. Tomato fruit showing symptoms of anthracnose; C. Pure isolate of fungus isolated from tomato fruit showing anthracnose disease.

anthracnose disease, confirms *Colletotrichum* sp. as the cause of anthracnose in tomato fruit. The genus *Colletotrichum* consists of approximately 200 species that cause anthracnose in plants worldwide (Mongkolporn & Taylor, 2018). Isolation and identification of pathogens in tomatoes showing symptoms of anthracnose are carried out to determine the specific pathogen infecting the tomatoes. Koch's postulates were used to confirm that fungal isolates isolated from tomatoes exhibited symptoms of anthracnose disease (Sudirga, 2016). According to Herliyana et al. (2020), Koch's Postulate testing procedure to confirm pathogens consists of several stages: isolation, inoculation, reisolation and identification of pathogens.

Cinnamon Leaf Extraction and Test of Antifungal Activity. Crude extract of cinnamon leaves was obtained by extracting the leaves using the maceration method with acetone. The results of the cinnamon leaf extraction are presented in Figure 3. The crude extract was then stored in a dark bottle at room temperature. The well diffusion method was used to test the effectiveness of the crude extract of cinnamon leaves against *Colletotrichum* sp. The results of the antifungal activity test are presented in Figure 4. The control treatment using sterile water showed no inhibition, while the 33% cinnamon leaf crude extract demonstrated clearinhibition. As inhibition was observed, the test was continued to determine the lowest concentration that could inhibit the growth of *Colletotrichum* sp.

The Minimum Inhibitory Concentration (MIC) test was carried out, and the results are presented in Table 1. Based on the MIC test, the minimum concentration of cinnamon crude extract required to inhibit *Colletotrichum* sp. was 0.9%. The result imply that concentrations 1% or higher show inhibitory performance. To determine the optimum concentration for inhibition, an optimization test was conducted using cinnamon crude extract at five concentrations (1%, 2%, 3%, 4%, and 5%) and compared to sterile water as a negative control and 5% Nystatin fungicide as a positive control. The results of the optimization test was presented in Table 2.

Based on the results in Table 2, the 2% of cinnamon crude extract (11.10 ± 0.57 mm) showed better inhibition than the 5% Nystatin (10.48 ± 0.28 mm). The highest inhibition occurred with the 5% cinnamon crude extract, showing 16.44 ± 0.35 mm inhibition, indicating its potential use as a natural antifungal.

Secondary metabolites such as terpenoids, alkaloids, and phenol compounds found in plants act as defense compounds that have the potential to inhibit the growth of pathogenic fungi. Extraction is a common method used to isolate secondary metabolites from plant parts. Based on the results presented in Figures

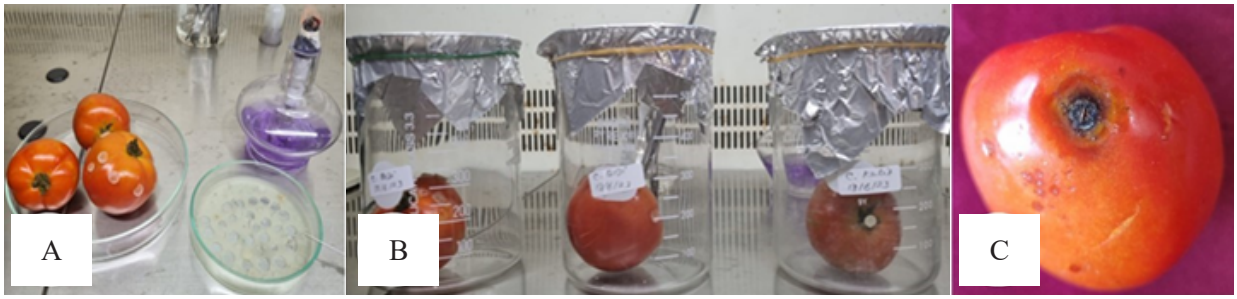

Figure 2. Koch's Postulate Test for isolated pure *Colletotrichum* sp. A. Fresh tomatoes inoculated with isolated the fungus; B. Incubation of tomatoes that have been infected with isolated the fungus; C. Tomato fruit shows symptoms of anthracnose.

Figure 3. Extraction of cinnamon leaves. A. Cinnamon plants; B. Maceration of cinnamon leaf powder with PA acetone; C. Crude extract of cinnamon leaves.

3 and Figure 4, the crude extract of cinnamon leaves effectively inhibited the growth of *Colletotrichum* sp., the fungus causing anthracnose in tomatoes, with an inhibition zone of 25 mm. According to Flanagan & Steck (2017), the inhibition zone reflects the ability of the active compounds in plant extracts to inhibit

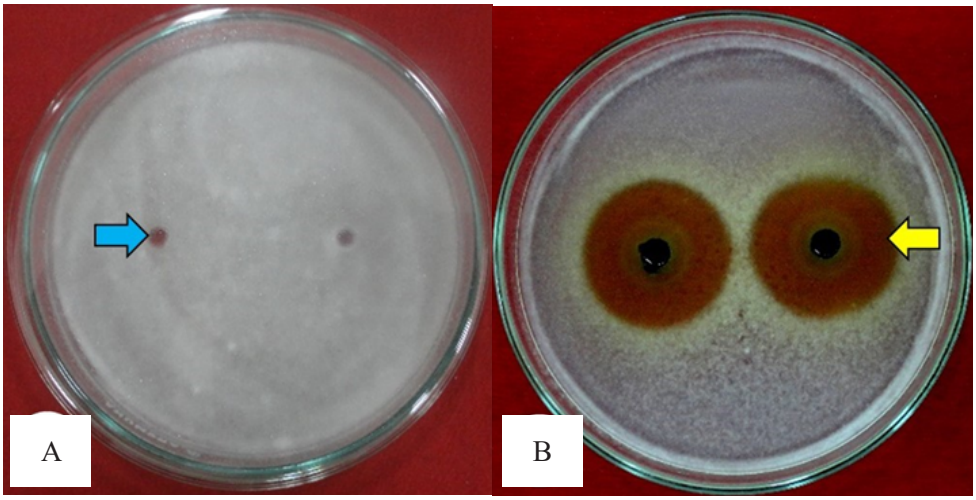

Figure 4. Inhibition of cinnamon leaf crude extract against the fungus *Colletotrichum* sp. A. Control treatment by using sterile water. The blue arrow showing the diffusion well without inhibition; B. Treatment of 33% cinnamon leaf crude extract. The yellow arrow showing the inhibition zone.

Table 1. Minimum inhibitor concentration (MIC) of cinnamon leaf extract against the fungus *Colletotrichum* sp. which causes anthracnose disease on tomatoes.

No.	Extract concentration (%)	Inhibition zone (mm)
1.	Sterile water (control)	0
2.	0.1	0
3.	0.2	0
4.	0.3	0
5.	0.4	0
6.	0.5	0
7.	0.6	0
8.	0.7	0
9.	0.8	0
10.	0.9	2.55
11.	1.0	9.64

Table 2. Inhibitory effect of cinnamon leaf extract treatment on the *Colletotrichum* sp. which causes anthracnose disease on tomatoes in vitro.

No.	Treatment	Average of inhibition (mm)
1.	Sterile water (negative control)	0.00 ± 0.00a
2.	Nystatin 5% (positive control)	10.48 ± 0.28c
3.	Cinnamon crude extract 1%	9.64 ± 0.40b
4.	Cinnamon crude extract 2%	11.10 ± 0.57d
5.	Cinnamon crude extract 3%	13.42 ± 0.19e
6.	Cinnamon crude extract 4%	14.45 ± 0.21f
7.	Cinnamon crude extract 5%	16.44 ± 0.35g

Annotation: the average value in the same column is significantly different at the 5% error ($p \leq 0.05$) if followed by different letters.

the growth of pathogenic fungi. If the inhibition zone exceeds 20 mm, it indicates that the active compound is very effective at inhibiting the pathogenic fungus. According to Liang et al. (2019), crude cinnamon leaf extract can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria, with inhibition zones of 23.31 mm and 20.61 mm, respectively. Additionally, cinnamon bark extract can inhibit the growth of *Colletotrichum musae* and prevent spore germination in *C. gloeosporioides* (Kowalska et al., 2021). The antifungal mechanism of the extract works by damaging the fungal cell walls (Darmadi et al., 2022).

Active Compound of Cinnamon Leaf Crude Extract. The ability of cinnamon leaf extract to inhibit the growth of *Colletotrichum* sp. is due to the presence of secondary metabolites within it. The analysis of secondary metabolite content in the cinnamon leaf extract, perfomed using GC-MS, is presented as a chromatogram in Figure 5, and the identified active compounds are listed in Table 3. Based on the analysis, 16 compounds were identified in the acetone extract of cinnamon leaves. Four of these compounds, while having different retention time (RT), are still included in the Table 3: (1) 2,6-Dimethyl-6-nitro-2-hepten-4-one; (2) 2-Propenal, 3-phenyl-; (3) 1-Nonadecene; and (4) 1-Heptacosanol. Among the 16 active compounds, nine are recognized for their antimicrobial potential, marked with an asterisk (*) in Table 3.

The compound 2-Propenal, 3-phenyl- is known as cinnamaldehyde, a well-known active compound in cinnamon that effectively inhibits various microorganisms, including bacteria, yeasts, and moulds, and also prevents these microorganisms from producing toxin (Doyle & Stephens, 2019). In terms of antifungal activity, cinnamaldehyde and its derivatives have been reported to inhibit several pathogenic fungus. Cinnamaldehyde can inhibit the growth of *Streptococcus mutans* (He et al., 2019), and *Staphylococcus epidermidis* (Albano et al., 2019), and it has also been shown to inhibit fungi from the genera *Aspergillus, Trichophyton, Coriolus, Laetiporus,* and *Candida* (Doyle & Stephens, 2019). Albano et al. (2019), further revealed that cinnamaldehyde can affect cell membrane permeability and reduce microbial biofilm formation, suggesting that it not only has potential as an antimicrobial agent but also as an antibiofilm agent for industrial disinfection.

The derivative of cinnamaldehyde identified in this study with antimicrobial activity is 2-Propen-1-ol, 3-phenyl- (CAS:cinnamyl), also known as cinnamyl alcohol. Cinnamyl alcohol is produced through the selective hydrogenation of cinnamaldehyde and is mainly used in industries as a key precursor for flavouring, perfumes, and pharmaceutical compounds (Bonita et al., 2020; Lv et al., 2020). According to Vasconcelos et al. (2018), cinnamyl alcohol, as an antimicrobial compound, inhibits microbial growth by preventing cell division, ATP synthesis, and microbial motility. Cinnamyl alcohol can also be used to modify chitosan oligosaccharides to enhance their antimicrobial activity, particularly as an antibacterial agent (Yue et al., 2020).

The compound 2H-1-Benzopyran-2-one (CAS: coumarin) is known as coumarin. Coumarin and its derivatives are naturally found in various plant species and are widely used as antimicrobial agents and even as anticancer compounds (Akkol et al., 2020; Annunziata et al., 2020; Carneiro et al., 2021; Sahoo et al., 2021). In terms of antifungal activity, coumarin is a promising antifungal agent that significantly inhibits the growth of *Candida albicans, Fusarium oxysporum, Aspergillus flavus, Aspergillus niger* and

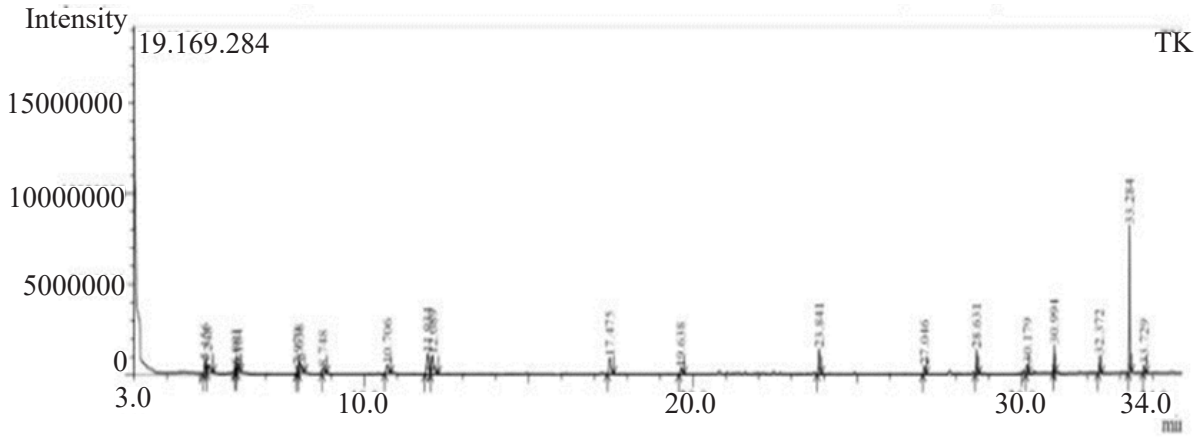

Figure 5. Chromatogram of GC-MS analysis of cinnamon leaf extract with the highest inhibition against *Colletotrichum* sp.

Cryptococcus neoformans (Annunziata et al., 2020). Coumarin also exhibits antiviral properties. Mishra et al. (2020) compiled the potential of coumarin and its derivatives as antiviral compounds, showing efficacy against hepatitis viruses, HIV, influenza virus, dengue virus, chikungunya virus, and others.

1-Nonadecene has been reported to exhibit antifungal activity against *Fusarium* sp. and antibacterial activity against Gram-positive bacteria (Smaoui et al., 2012). This compound can be extracted from plants, such as in the methanolic extract of *Ageratum conyzoides* (Ferdosi at al., 2021), or from fungi like *Streptomyces* species (Smaoui et al., 2012) or *Aspergillus arcoverdensis* (Skanda & Vijayakumar, 2021). 1-Nonadecene not only acts as an antimicrobial agent but also possesses antioxidative and anti-inflammatory properties (Skanda & Vijayakumar, 2021). Another compound from the extract, phytolisomer, also exhibits antifungal activity against *Alternaria infectoria* and *Aspergillus fumigatus* (Silva et al., 2018).

Hexadecanoic acid, commonly referred to as palmitic acid, is a methyl ester compound with antimicrobial properties, particularly as an antibacterial and antifungal agent (Akpuaka et al., 2013; Skanda & Vijayakumar, 2021). According to Shaaban et al. (2021), hexadecanoic acid, when combined with silver nanoparticles, can inhibit drug-resistant microbes. Another ester compound found in the acetone extract of cinnamon leaves is 1,2-Benzenedicaboxylic acid, mono(2-ethyl). The antifungal activity of this compound was reported by Singh et al. (2015) in tests using mango bark extract. Other research has confirmed the biological activities of 1,2-Benzenedicaboxylic acid, such as antimicrobial, antifungal, antioxidative, and anticancer properties (Zayed et al., 2019; Kumar et al., 2022a). It also shows potential for use as a larvicidal and pupicidal agent for mosquito control (Kumar et al., 2022b).

1-Heptacosanol is an important compound in plants that plays a vital role in protecting against pathogenic fungal infections (Chowdhary & Kaushik, 2018; Hawar et al., 2023). 1-Heptacosanol has various uses in plants and fungi and also holds pharmaceutical value for humans, including antifungal, antimicrobial, antinematode, anticancer, and antioxidative properties

Table 3. The identified active compounds in cinnamon leaf extract have the potential to inhibit the growth of *Colletotrichum* sp.

ID	Compound	Retention Time (min)	Peak Area (%)	Molecular Formula	Molecular Weight
1	2,6-Dimethyl-6-nitro-2-hepten-4-one	5.156	699691	C9H15NO3	185
	2,6-Dimethyl-6-nitro-2-hepten-4-one	5.249	1369408	C9H15NO3	185
2	Guanidine, monothiocyanate	6.091	225905	C2H4N4S	116
3	2,4-Pentanediol, 2-methyl-(CAS)2-methyl-2	6.161	274629	C6H14O2	118
4*	2-Propenal, 3-phenyl-	7.978	244541	C9H8O	132
	2-Propenal, 3-phenyl-	8.037	996450	C9H8O	132
5*	2-Propen-1-ol,3-phenyl-(CAS) cinnamyl	8.747	247154	C9H10O	134
6	1-Tetradecene	10.708	246503	C14H28	196
7*	2H-1-Benzopyran-2-one (CAS) coumarin	11.934	1405717	C9H6O2	146
8	2-Propen-1-ol, 3-phenyl-, acetate	12.091	819863	C11H12O2	176
9	1-Hexadecene (CAS) cetene	17.477	347334	C16H32	224
10	5-Methyl-3-phenylcyclopent-2-en-1-one	19.637	174909	C12H12O	172
11*	1-Nonadecene	23.841	332848	C19H38	266
	1-Nonadecene	28.631	262511	C19H38	266
12*	Hexadecanoic acid, methyl ester	27.045	245458	C17H34O2	270
13*	Phytolisomer	30.179	129999	C20H40O	296
14*	n-Tetracosanol-1	30.994	189795	C24H50O	354
15*	1-Heptacosanol	32.372	134649	C27H56O	396
	1-Heptacosanol	33.728	74499	C27H56O	396
16*	1,2-Benzenedicarboxylic acid, mono(2-ethyl)	33.284	3439668	C16H22O4	278

Asterix (*) after the number indicating the active compounds with antimicrobial potential.

(Raman et al., 2012; Skanda & Vijayakumar; 2021). n-Tetracosanol-1, is also known as lignoceric alcohol, is classified as a primary alcohol, with its main function being an antioxidative compound (Farida et al., 2021). According to research by Talie et al. (2020), n-Tetracosanol-1, found in the essential oil of *Rhizopogon* species, exhibits antifungal activity against *Penicillium chrysogenum, Aspergillus niger,* and *Alternaria alternata,* which cause post-harvest disease in apple plants.

CONCLUSION

The crude extract of cinnamon leaves (*Cinnamomum burmanii*) can inhibit the growth of the pathogenic fungus *Colletotrichum* sp., which causes anthracnose in tomatoes, with a very strong inhibition zone. A 2% (w/v) concentration of cinnamon extract effectively inhibits *Colletotrichum* sp. in vitro. Sixteen active compounds were identified in the acetone extract of cinnamon leaves, nine of which are antimicrobial compounds: 2-Propenal, 3-phenyl; 2-Propen-1-ol, 3-phenyl-(CAS cinnamyl); 2H-1-Benzopyran-2-one (CAS coumarin); 1- Nonadecene; Hexadecanoic acid, methyl ester; Phytolysomer; n-Tetracosanol-1; 1-Heptacosanol; and 1,2-Benzenedicaboxylic acid, mono(2-ethyl).

ACKNOWLEDGMENTS

We would like to thank Udayana University, particularly the Institute for Research and Community Service, for providing the grant that funded this research (No. B/1.487/UN14.4.A/PT.01.03/2023, date 2 May 2023).

FUNDING

This research was funded by Udayana University PNBP funds under the research implementation assignment agreement No. B/1.487/UN14.4.A/PT.01.03/2023, dated 2 May 2023.

AUTHORS' CONTRIBUTIONS

SKS and AAKD design the research structure and methods. IMSW and DAY analyzed the data and conducted cross-references for bioactive compounds. All author were involved in data collection and manuscript writing. SKS and IMSW proofread the manuscript and prepared the final version. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors declare that there is no potential conflict of interest.

REFERENCES

Akkol EK, Genç Y, Karpuz B, Sobarzo-Sánchez E, & Capasso R. 2020. Coumarins and coumarin-related compounds in pharmacotherapy of cancer. *Cancers.* 12(7): 1959. https://doi.org/10.3390/cancers12071959

Akpuaka A, Ekwenchi MM, Dashak DA, & Dildar A. 2013. Biological activities of characterized isolates of n-hexane extract of Azadirachta indica A.Juss (neem) leaves. *N Y Sci J*. 6(6): 119–124.

Albano M, Crulhas BP, Alves FCB, Pereira AFM, Andrade BFMT, Barbosa LN, Furlanetto A, Lyra LPdS, Rall VLM, & Júnior AF. 2019. Antibacterial and anti-biofilm activities of cinnamaldehyde against *S. epidermidis. Microb. Pathog.* 126: 231–238. https://doi.org/10.1016/j.micpath.2018.11.009

Annunziata F, Pinna C, Dallavalle S, Tamborini L, & Pinto A. 2020. An overview of coumarin as a versatile and readily accessible scaffold with broad-ranging biological activities. *Int. J. Mol. Sci.* 21(13): 4618. https://doi.org/10.3390/ijms21134618

Bonita Y, Jain V, Geng F, O'Connell TP, Ramos NX, Rai N, & Hicks JC. 2020. Hydrogenation of cinnamaldehyde to cinnamyl alcohol with metal phosphides: Catalytic consequences of product and pyridine doping. *Appl. Catal. B: Environ*. 277: 119272. https://doi.org/10.1016/j.apcatb.2020.119272

Carneiro A, Matos MJ, Uriarte E & Santana L. 2021. Trending topics on coumarin and its derivatives in 2020. *Molecules*. 26(2): 501. https://doi.org/10.3390/molecules26020501

Cannon PF, Damm U, Johnston PR, & Weir BS. 2012. *Colletotrichum*: Current status and future directions. *Stud. Mycol*. 73(1): 181–213. https://doi.org/10.3114/sim0014

Chowdhary K & Kaushik N. 2018. Biodiversity study and potential of fungal endophytes of peppermint

and effect of their extract on chickpea rot pathogens. *Arch. Phytopathol. Pflanzenschutz*. 51(3-4): 139–155. https://doi.org/10.1080/03235408.2018.1440707

Chung PC, Wu HY, Wang YW, Ariyawansa HA, Hu HP, Hung TH, Tzean SS, & Chung CL. 2020. Diversity and pathogenicity of *Colletotrichum* species causing strawberry anthracnose in Taiwan and description of a new species, *Colletotrichum miaoliense* sp. nov. *Sci. Rep.* 10: 14664. https://doi.org/10.1038/s41598-020-70878-2

da Silva LL, Moreno HLA, Correia HLN, Santana MF, & de Queiroz MV. 2020. *Colletotrichum*: Species complexes, lifestyle, and peculiarities of some sources of genetic variability. *Appl. Microbiol. Biotechnol*. 104: 1891–1904. https://doi.org/10.1007/s00253-020-10363-y

Darmadi AAK, Suprapta DN, Temaja IGRM, & Swantara IMD. 2015. Leaf extract of Cinnamomum burmanni Blume effectively suppress the growth of *Fusarium oxysporum* f.sp. lycopersici the cause of fusarium wilt disease on tomato. *J Biol Agric Healthcare.* 5(4): 131–137.

Darmadi AAK, Suriani NL, Ginantra IK, & Sudirga SK. 2022. Effectiveness of cinnamon leaf extract to control anthracnose disease on large chilies in Bali, Indonesia. *Biodiversitas*. 23(6): 2859–2864. https://doi.org/10.13057/biodiv/d230611

Dowling M, Peres N, Villani S, & Schnabel G. 2020. Managing *Colletotrichum* on fruit crops: A 'complex' challenge. *Plant Dis*. 104(9): 2301–2316. https://doi.org/10.1094/PDIS-11-19-2378-FE

Doyle AA & Stephens JC. 2019. A review of cinnamaldehyde and its derivatives as antibacterial agents. *Fitoterapia* 139: 104405. https://doi.org/10.1016/j.fitote.2019.104405

Farida Y, Azela W, Lestari ME, & Pratami DK. 2021. The quality parameters, total flavonoids determination and antioxidant activity compound of andaliman fruit andaliman fruit (*Zanthoxylum acanthopodium* DC.) extract. *Int. J. Appl. Pharm*. 13(2): 34–40. https://doi.org/10.22159/ijap.2021.v13s2.07

Ferdosi MFH, Javaid A, Khan IH, Fardosi MFA & Munir A. 2021. Bioactive components in methanolic flower extract of *Ageratum conyzoides*. *Pak. J. Weed Sci. Res.* 27(2): 181–190. https://doi.org/10.28941/pjwsr.v27i2.954

Flanagan JN & Steck TR. 2017. The relationship between agar thickness and antimicrobial susceptibility testing. *Indian J. Microbiol*. 57(4): 503–506. https://doi.org/10.1007/s12088-017-0683-z

Gurusinga RE, Retnowati L, Wiyono S, & Tondok ET. 2020. Dampak penggunaan fungisida sintetik pada kelimpahan cendawan endofit tanaman padi [The impact of synthetic fungicides application on abundance of endophytic fungi of rice plants]. *JIPI.* 25(3): 432–439. https://doi.org/10.18343/jipi.25.3.432

Hawar SN, Taha ZK, Hamied AS, Al-Shmgani HS, Sulaiman GM, & Elsilk SE. 2023. Antifungal activity of bioactive compounds produced by the endophytic fungus *Paecilomyces* sp. (JN227071.1) against *Rhizoctonia solani*. *Int. J. Biomater.* 20: 2411555. https://doi.org/10.1155/2023/2411555

He Z, Huang Z, Jiang W, & Zhou W. 2019. Antimicrobial activity of cinnamaldehyde on *Streptococcus mutans* biofilms. *Front. Microbiol.* 10: 2241. https://doi.org/10.3389/fmicb.2019.02241

Herliyana EN, Sakbani L, Herdiyeni Y, & Munif A. 2020. Identifikasi cendawan patogen penyebab penyakit pada daun jabon merah (*Anthocephalus macrophyllus* (Roxb.) Havil) [Identification fungi pathogen cause of disease in red leaf jabon (*Anthocephalus macrophyllus* (Roxb.) Havil)]. *Jurnal Silvikultur Tropika*. 11(3): 154–162. https://doi.org/10.29244/j-siltrop.11.3.154-162

Intan K, Diani A, & Nurul ASR. 2021. Aktivitas antibakteri kayu manis (*Cinnamomum burmanii*) terhadap pertumbuhan *Staphylococcus aureus* [Antibacterial activity of cinnamon (*Cinnamomum burmanii*) against the growth of *Staphylococcus aureus*]. *Jurnal Kesehatan Perintis*. 8(2): 121–127. https://doi.org/10.33653/jkp.v8i2.679

Kowalska J, Tyburski J, Matysiak K, Jakubowska M, Łukaszyk J, & Krzyminska J. 2021. Cinnamon as a useful preventive substance for the care of human and plant health. *Molecules*. 26(17): 5299. https://doi.org/10.3390/molecules26175299

Kumar SP, Srinivasulu A, & Babu KR. 2018.

Symptomology of major fungal diseases on tomato and its management. *J. Pharmacogn. Phytochem*. 7(6): 1817–1821.

Kumar A, Kaur S, Dhiman S, Singh PP, Bhatia G, Thakur S, Tuli HS, Sharma U, Kumar S, Almutary AG, Alnuqaydan AM, Hussain A, Haque S, Dhama K, & Kaur S. 2022a. Targeting Akt/NF-kB/p53 pathway and apoptosis inducing potential of 1,2-Benzenedicarboxylic acid, bis (2-Methyl propyl) ester isolated from *Onosma bracteate* Wall. against human osteosarcoma (MG-63) cells. *Molecules.* 27(11): 3478. https://doi.org/10.3390/molecules27113478

Kumar PS, Reegan AD, Rajakumari K, Asharaja AC, Balakrishna K, & Ignacimuthu S. 2022b. Bio-efficacy of soil actinomycetes and an isolated molecule 1,2-Benzenedicarboxylic acid from *Nonomuraea* sp. against *Culex quinquefasciatus* Say and *Aedes aegypti* L. mosquitoes (Diptera: Culicidae). *Appl. Biochem. Biotechnol*. 194: 4765–4782. https://doi.org/10.1007/s12010-021-03766-8

Liang Y, Li Y, Sun A, & Liu X. 2019. Chemical compound identification and antibacterial activity evaluation of cinnamon extracts obtained by subcritical n-butane and ethanol extraction. *Food Sci. Nutr*. 7(6): 2186–2193. https://doi.org/10.1002/fsn3.1065

Lv Y, Han M, Gong W, Wang D, Chen C, Wang G, Zhang H, & Zhao H. 2020. Fe-Co alloyed nanoparticles catalysed efficient hydrogenation of cinnamaldehyde to cinnamyl alcohol in water. *Angew. Chem. Int. Ed*. 59(52): 23521–23526. https://doi.org/10.1002/anie.202009913

Mishra S, Pandey A, & Manvati S. 2020. Coumarin: An emerging antiviral agent. *Heliyon.* 6(1): e03217. https://doi.org/10.1016/j.heliyon.2020.e03217

Mongkolporn O & Taylor PWJ. 2018. Chili anthracnose: *Colletotrichum* taxonomy and pathogenicity. *Plant Pathol.* 67(6): 1255–1263. https://doi.org/10.1111/ppa.12850

Nabila R, Purnamasari CB & Alhawaris. 2021. Uji aktivitas antibakteri ekstrak etanol daun kayu manis (*Cinnamomum Burmannii* Blume) terhadap pertumbuhan bakteri *Porphyromonas gingivalis* dengan metode disc diffusion [Test of the antibacterial activity of ethanol extract of cinnamon leaves (*Cinnamomum Burmannii* Blume) on the growth of *Porphyromonas gingivalis* bacteria using the disc diffusion method]. *J. Ked. Mulawarman.* 8(2): 64–72.

Prasetyo A. 2017. Pemanfaatan kitosan untuk pengendalian penyakit antraknosa (*Colletotrichum* sp.) pada cabai (*Capsicum annuum* L.) Skripsi. Bogor: Fakultas Pertanian, Institut Pertanian Bogor.

Prasetyorini, Utami NF, Yulianita, Novitasari N, & Fitriyani W. 2021. Potensi ekstrak refluks kulit batang kayu manis (*Cinnamomum burmannii*) sebagai antijamur *Candida albicans* dan *Candida tropicalis* [The potent of cinnamon bark reflux extract (*Cinnamomum burmannii*) as an anti-fungal *Candida albicans* and *Candida tropicalis*]. *Jurnal Fitofarmaka*. 11(2):164–178. https://doi.org/10.33751/jf.v11i2.2722

Rafif KA, Intan ST, Hamida HR, Cinta A, & Yan A. 2022. Beneficial impacts and phytocomponents of cinnamomum in Indonesia. *Res. J. Biotech.* 17(10): 114–123. https://doi.org/10.25303/1710rjbt1140123

Raman BV, Samuel LA, Saradhi PM, Rao NB, Krishna NVA, Sudhakar M, & Radhakrishnan TM. 2012. Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum. Asian J. Pharm. Clin. Res.* 5(2): 99–106.

Sahoo CR, Sahoo J, Mahapatra M, Lenka D, Sahu PK, Dehury B, Padhy RN, & Paidesetty SK. 2021. Coumarin derivatives as promising antibacterial agent(s). *Arab J. Chem*. 14(2): 102922. https://doi.org/10.1016/j.arabjc.2020.102922

Shaaban MT, Ghaly MF, & Fahmi SM. 2021. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. *J. Basic Microbiol.* 61(6): 557–568. https://doi.org/10.1002/jobm.202100061

Shahriar SA, Husna A, Paul TT, Eaty MNK, Quamruzzaman M, Siddique AB, Rahim MdA, Ahmmed ANF, Uddain J, & Siddiquee S. 2023. *Colletotrichum truncatum* causing anthracnose of tomato (*Solanum lycopersicum* L.) in Malaysia. *Microorganisms.* 11(1): 226. https://doi.org/10.3390/microorganisms11010226

Singh R, Singh SK, Maharia RS & Garg AN. 2015. Identification of new phytoconstituents and antimicrobial activity in stem bark of *Mangifera*

indica (L.). *J. Pharm Biomed Anal.* 105: 150–155. https://doi.org/10.1016/j.jpba.2014.12.010

Silva P, Fernandes C, Barros L, Ferreira ICFR, Pereira L, & Gonçalves T. 2018. The antifungal activity of extracts of *Osmundea pinnatifida*, an edible seaweed, indicates its usage as a safe environmental fungicide or as a food additive preventing post-harvest fungal food contamination. *Food Funct*. 9(12): 6187–6195. https://doi.org/10.1039/C8FO01797B

Skanda S & Vijayakumar BS. 2021. Antioxidant and anti-inflammatory metabolites of a soil-derived fungus *Aspergillus arcoverdensis* SSSIHL-01. *Curr. Microbiol.* 78: 1317–1323. https://doi.org/10.1007/s00284-021-02401-3

Smaoui S, Mathieu F, Elleuch L, Coppel Y, Merlina G, Karray-Rebai I, & Mellouli L. 2012. Taxonomy, purification and chemical characterization of four bioactive compounds from new *Streptomyces* sp. TN256 strain. *World J. Microbiol. Biotechnol.* 28: 793–804. https://doi.org/10.1007/s11274-011-0872-6

Sopialena S, Subiono T, Rosyidin AU, & Tantiani D. 2022. Control of anthracnose disease in tomato (*Solanum lycopersicum*) using endophytic fungi. In First Asian PGPR Indonesia Chapter International e-Conference 2021, *KnE Life Sciences.* pp. 393–408. https://doi.org/10.18502/kls.v7i3.11147

Sudirga SK. 2016. Isolasi dan identifikasi jamur *Colletotrichum* spp. isolat PCS penyebab penyakit antraknosa pada buah cabai besar (*Capsicum annuum* L.) di Bali [Isolation and Identification of the fungus *Colletotrichum* sp. PCS isolates causing anthracnose disease in large chili fruits (*Capsicum annuum* L.) in Bali]. *Jurnal Metamorfosa*. III(1): 23–30.

Sudirga SK, Wijaya IMS, & Darmadi AAK. 2022. First testimony of new host plant of *Colletotrichum magnum* (S.F. Jenkins & Winstead) Rossman & W.C. Allen causing anthracnose in *Carica papaya* L. fruits in Bali, Indonesia. *SABRAO J. Breed. Genet*. 54(4): 834–841. https://doi.org/10.54910/sabrao2022.54.4.14

Suprapta DN, Sudarma M, Arya N, & Ohsawa K. 2005. Plants extract to control wilt disease in banana seedlings. *J. ISSAAS.* 11(2): 84–90.

Sutriadi M, Harsanti ES, Wahyuni S, & Wihardjaka A. 2020. Pestisida nabati: Prospek pengendali hama ramah lingkungan [Botanical pesticides: The prospect of environmentally friendly pest control]. *Jurnal Sumberdaya Lahan.* 13(2): 89–101.

Talhinhas P & Baroncelli R. 2021. *Colletotrichum* species and complexes: geographic distribution, host range and conservation status. *Fungal Diversity*. 110: 109–198. https://doi.org/10.1007/s13225-021-00491-9

Talie MD, Wani AH, Lone BA, & Bhat MY. 2020. Chemical composition and antifungal activity of essential oil of *Rhizopogon* species against fungal rot of apple. *JABS.* 14(3): 296–308.

Vasconcelos NG, Croda J, & Simionatto S. 2018. Antibacterial mechanisms of cinnamon and its constituents: A review. *Microb. Pathog.* 120: 198–203. https://doi.org/10.1016/j.micpath.2018.04.036

Yue L, Sun D, Khan IM, Liu X, Jiang Q, & Xia W. 2020. Cinnamyl alcohol modified chitosan oligosaccharide for enhancing antimicrobial activity. *Food Chem.* 309: 125513. https://doi.org/10.1016/j.foodchem.2019.125513

Zakaria L. 2021. Diversity of *Colletotrichum* species associated with anthracnose disease in tropical fruit crops — A Review. *Agriculture.* 11(4): 297. https://doi.org/10.3390/agriculture11040297

Zayed MK, Wu A, & Sallam S. 2019. Comparative phytochemical constituents of *Leucaena leucocephala* (Lam.) leaves, fruits, stem barks, and wood branches grown in Egypt using GC-MS method coupled with multivariate statistical approaches. *BioRes.* 14(1): 996–1013. https://doi.org/10.15376/BIORES.14.1.996-1013