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Antifungal activity and phytochemical analysis of *Miliusa sessilis* twig extract to control anthracnose disease in mango (*Mangifera indica*)

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Abstract The efficacy of crude extract of *Miliusa sessilis* to control *Colletotrichum* sp. causing anthracnose disease of mango *in vitro* is determined. The *M. sessilis* twigs were extracted with four different organic solvents (hexane (Hex), ethanol (EtOH), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH). In a preliminary study, crude extracts at various concentrations from 1000-7000 ppm were tested by dual culture assay. The result showed that Hexane and EtOAc crude extracts at 7000 ppm had a high percentage of mycelial growth inhibition. For further study, Hexane and EtOAc crude extracts were carried out to investigate the diameter of mycelia growth inhibition by poisoned food technique at different concentrations (1000, 5000 and 10000 ppm). The inhibition toward mycelial growth of *Colletotrichum* sp. was increased with increasing concentrations of Hexane and EtOAc crude extract as compared with control. The highest percentage of inhibitory control of 90.07% and 78.52% were obtained with the efficacy of EtOAc and Hexane crude extract at 10000 ppm, respectively. The minimum inhibitory concentration value of Hexane and EtOAc crude extract was 125 µg mL^{-1} against *Colletotrichum* sp. Phytochemical investigation of Hexane and EtOAc crude extracts was performed by NMR spectroscopic techniques and thin layer chromatography (TLC) profiling. ^{1}H NMR spectra of Hex and EtOAc crude extracts exhibited that neolignans were predominant in the extracts. TLC profiling of the crude extracts constituted different coloured phytochemical compounds with different R_f values. The present study provided an evidence that Hexane and EtOAc crude extracts of *M. sessilis* contained bioactive compounds that promising for cytotoxic effects against *Colletotrichum* sp.

Keywords: *Colletotrichum* sp., Phytochemical, Biological control, ^{1}H-NMR, TLC profiling

Introduction

Anthracnose disease is an important disease in Thailand (Dinh *et al*., 2003, Sangchote, 1987). *Colletotrichum* sp. is a causal agent of a fungal disease that causes a majority of postharvest losses in all mango producing areas of the world. In tropical regions, the fungal spores (conidia) disperse and cause fast

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dissemination as a quiescent infection on fruit in the field (Simmond, 1941). Mango fruit is reduced in quality and quantity due to this disease (Cannon *et al*., 2012, Dinh, 2002, Dodd *et al*., 1991). For postharvest disease management, fungicides are widely used and are known to be highly effective. However, chemical strategies have led to the resistant strains and proliferation of fungal pathogens, including the negative effects on human health and the environment from chemical residues (Murray *et al*., 2017). In this regard, various plant extracts have been examined based on natural compounds as alternative choices to reduce synthetic fungicides and be friendly environment (Yazdani *et al*., 2011, Zhou *et al*., 2018). Plant extracts of various native plants from different plant parts revealed antimicrobial efficacy to control phytopathogens (Gurjar *et al*., 2012).

Miliusa sessilis (Bai-Biaw-Dam-Kwan) was discovered in the southern forest of Thailand. This plant species was classified as Annonaceae, which included approximately 50 species (Chaowasku and Kessler, 2013). In previously reported studies, many reports have demonstrated the antimicrobial and cytotoxic activities of *Miliusa* species (Jumana *et al*., 2000, Wongsa *et al*., 2017). *Miliusa* spp. has been investigated for biologically active molecules which possess antibiotic properties such as acetogenins, flavonoids, tannins, phenolics, quinones, and lignans (Compean and Ynalvez, 2014, Jumana *et al*., 2000).

This study was to evaluate the antifungal activities of *M. sessilis* using its twig extract against *Colletotrichum* sp. *in vitro* and analyse the bioactive properties to reveal the source of antifungal activities. The present study is reported the antifungal activities of *M. sessilis* extract against anthracnose disease for the first time in Thailand.

Materials and methods

Fungal pathogen isolation and identification

Infected mango fruits were randomly collected from Huahin market, Prachuapkirikun province, Thailand and isolated using tissue transplanting method. First, the mango fruit skin was surface steriled with 70% ethanol. The anthracnose lesions were cut out, approximately 3mm^2 pieces of infected tissue. Tissues were surface disinfected with sodium hypoclorite 1% for 5 minutes then rinsed in sterile distilled water for 2-3 times. The sterilized tissues were placed on sterile paper and plated on Potato Dextrose Agar (PDA). After 48-72 hours of incubation at room temperature, the mecelia grown on a PDA plate are transferred to a new PDA plate for purification. Identification of *Colletotrichum*

sp. was carried out by using morphological studies (Alexopoulos *et al*., 2002, Barnett and Hunter, 1986).

Preparation of plants for extraction

The twigs of *Miliusa sessilis* Chaowasku & Kessler sp. nov. (Annonaceae) were collected and deposited as described by Pootaeng-on *et al*., 2020. The dried, ground twigs of *Miliusa sessilis* (4.8 kg) were extracted with extracted with 95% ethanol (EtOH) (3 × 7 l) at room temperature. The filtrate was evaporated under reduced pressure to obtain the EtOH extract (239 g) as dark green-brown gum. The crude extract was prepared using the sequential liquid-liquid extraction method by dissolving in water and then successively partitioned with *n*-hexane (Hex), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH). Hex, EtOAc, n-BuOH, H_2O soluble, and H_2O insoluble crude extract yields are 4.96, 31.7, 25.6, 67.1, and 105.8 g, respectively.

Screening for crude extract from Miliusa sessilis to control Colletotrichum sp. in vitro

Crude extract was tested for preliminary screening to inhibit mycelial growth of *Colletotrichum* sp. A mycelial disc of *Colletotrichum* sp., obtained from a 7-day-old culture, was placed at the center of the PDA plate. Steriled filter paper discs (6 mm. in diameter, Whatman) loaded with each crude extract at different concentrations (0, 1000, 2000, 3000, 4000, 5000, 6000 and 7000 ppm) were placed on a PDA plate between the peripheral region and 2 cm from the *Colletotrichum* sp. agar plug (Jamal *et al*., 2015). *Colletotrichum* sp. plate without any crude extract and dimethyl sulfoxide (DMSO) were used as positive and negative controls, respectively. The crude extract treatment, which had a high percentage of mycelial growth inhibition, was performed for further study. The percentage of mycelial growth inhibition was calculated after 7 days of incubation at room temperature using the following formula (Sivakumar *et al*., 2000):

Percentage inhibition (I) = $[C - T]/C \times 100$

Where C is a radial growth measurement of the pathogen in control and T is a radial growth measurement of the pathogen in treatments.

All treatments were carried out with 4 replications and designed in a completely randomized design (CRD). Treatment means were compared with Duncan's multiple range test (DMRT). The statistical analysis was performed by using R statistical software.

In vitro test of crude extract from Miliusa sessilis against Colletotrichum sp.

The antifungal efficacy of hexane and ethyl acetate crude extracts to control *Colletotrichum* sp. was determined using the poisoned food technique (Al-Samarrai *et al*., 2012) at different concentrations (1000, 5000, and 10000 ppm). Each filtered crude extract was mixed into the PDA medium. A mycelial disc, *Colletotrichum* sp., was placed in the center of the PDA plate. Mycelial growth diameter was measured after 14 days of incubation at room temperature and calculated the percentage of mycelial growth inhibition using the formula as above. Also, morphological structures were observed under a light microscope.

The experiment was designed by using a 2 factor factorial experiment in a Completely Randomized Design (CRD) with 4 replications. Treatment means were compared with Duncan's multiple range test (DMRT). The statistical analysis was performed by using R statistical software.

Determination of minimum inhibitory concentration (MIC) of the extract

Hexane and ethyl acetate crude extracts of *M. sessilis* were determined MIC values with different concentrations (125, 250, 500, 750, 1000, 3000 and 5000 µg mL^{-1}). *Colletotrichum* sp. was initially prepared in PDA broth and shaking incubated at 26℃ for 10 days. The hexane and ethyl acetate crude extracts were added to *Colletotrichum* sp. broth in each sterile test tube. Dimethyl sulfoxide (DMSO) and *Colletotrichum* sp. broth were used as negative and positive controls, respectively. The MIC values were taken as the lowest concentration and low turbidity in the sterile test tube after being incubated at 26℃ for 5 days. The effect of crude extracts on the growth of *Colletotrichum* sp. was determined under a light microscope.

Phytochemical analysis

Thin layer chromatography analysis (TLC analysis)

For the TLC analysis, a plate with Silica gel 60 F254 TLC (Merck, Germany) was cut into 4x5 cm and marked with soft pencil. Glass capillaries were used to spot the sample for TLC, which applied a sample volume of 10-µl of sample by using capillary at a distance of 0.8 cm at 5 tracks, for EtOH, Hex, EtOAc, *n*-BuOH, and H_2O crude extract, respectively. Two different mobile phase systems for developing TLC plates are Hexane: EtOAc acid (4:1) and CH_2Cl_2: MeOH: H_2O (30:3:1, lower layer). After pre-saturation, the mobile phase was used for development for 20 min. After the development, the plates

are dried and observed under UV light (254 nm) and sprayed with dye (1% $CeSO_4$ in 10% aqueous H_2SO_4) followed by heating. The movement compounds were expressed by retention factor (R_f) values were calculated for different samples.

$$R_f = \frac{\text{distance traveled by the solute}}{\text{distance traveled by the solvent front of TLC plate}}$$

NMR spectroscopic analysis

On a Brüker AVANCE 300 MHz (300 MHz for ^{1}H NMR) spectrometer, ^{1}H NMR spectroscopic data of hexane and EtOAc crude extracts were recorded in $CDCl_3$ solutions. Chemical shifts are in δ (ppm) with tetramethylsilane (TMS) as an internal standard.

Results

Fungal pathogen isolation and identification

Colletotrichum sp. was isolated from infected mango fruit that showed the symptoms as rounded black lesions on the fruit surface (Figure 1, A). In an advanced stage of the disease, the fungus produces orange conidial masses on the lesions. To study morphological characteristics, the colony appears to have a light gray to dark gray mycelium with an irregular margin and produces orange conidial masses (Figure 1, B). The vegetative hyphae were smooth-walled, hyaline and septate. Conidia were hyaline, cylindrical shapes with both ends rounded and smooth-walled (Figure 1, C).

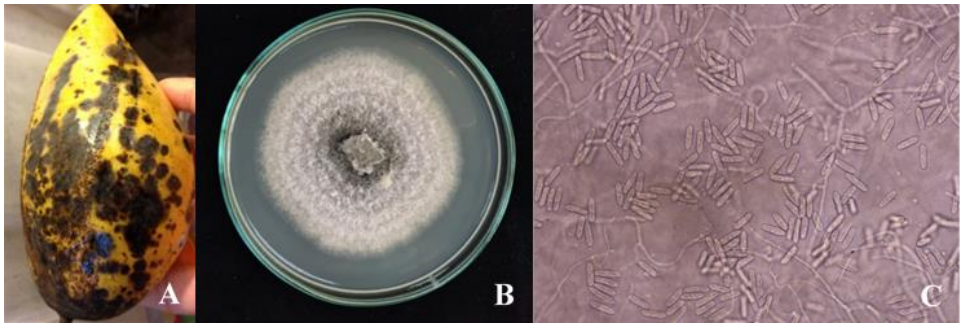

Figure 1. Macroscopic and microscopic studies of *Colletotrichum* sp. A; Anthracnose symptom on mango fruit, B; Colony on PDA, C; Conidial characteristics

Screening for crude extract from Miliusa sessilis to control Colletotrichum sp. *in vitro*

The preliminary screening of antifungal activity of crude extract from *M. sessilis* against *Colletotrichum* sp. was described in the dual culture assay. The result revealed that Hex and EtOAc crude extract at 7000 ppm had a strong antifungal activity against *Colletotrichum* sp., with a growth inhibition rate of 29.87% and 24.18%, respectively (Table 1).

Table 1. Effect of *Miliusa sessilis* extracts on the mycelial growth of *Colletotrichum* sp.

Crude extract	Concentration (ppm)	Growth inhibition (%)[/1]
DMSO	–	11.87d
water soluble (WS)	1000	16.13abcd
	2000	18.50abcd
	3000	18.50abcd
	4000	19.45abcd
	5000	21.82abcd
	6000	21.82abcd
	7000	22.29abcd
water insoluble (WI)	1000	12.34cd
	2000	12.81cd
	3000	14.23abcd
	4000	14.71abcd
	5000	15.66abcd
	6000	16.60abcd
	7000	17.55abcd
ethanol (EtOH)	1000	14.71abcd
	2000	15.18abcd
	3000	16.13abcd
	4000	20.39abcd
	5000	20.87abcd
	6000	20.87abcd
	7000	23.24abcd

Table 1. (Con.)

Crude extract	Concentration (ppm)	Growth inhibition (%)[1]
n-butanol (*n*-BuOH)	1000	11.87d
	2000	12.81cd
	3000	13.76bcd
	4000	13.76bcd
	5000	18.50abcd
	6000	18.50abcd
	7000	19.45abcd
ethyl acetate (EtOAc)	1000	17.55abcd
	2000	19.45abcd
	3000	19.45abcd
	4000	21.34abcd
	5000	22.29abcd
	6000	22.29abcd
	7000	24.18abcd
haxane (Hex)	1000	18.50abcd
	2000	22.76abcd
	3000	26.08abcd
	4000	26.55abcd
	5000	27.98abc
	6000	28.92ab
	7000	29.87a
C.V. (%)		36.12

[1]/The letters indicate significant differences between different treatments within the same column ($P<0.01$)

In vitro test of hexane and ethyl acetate crude extract from Miliusa sessilis against Colletotrichum sp.

The results of hexane and ethyl acetate (EtOAc) crude extract with different concentrations (1000, 5000 and 10000 ppm) were determined for fungal growth inhibition. The EtOAc crude extract showed highest percentage of mycelial growth inhibition (90.07%), followed by inhibitions of 58.12% and 28.16% at concentrations of 5000 and 1000 ppm, respectively, when compared

to the control. However, Hexane crude extract reduced fungal growth inhibition by up to 78.52% at 10000 ppm, whereas the Hex crude extract concentrations at 5000 and 1000 ppm showed 56.50% and 37.00% inhibition, respectively (Figure 2, A). Furthermore, there was a visible hyphae deformation. The hyphal morphology under the light microscope revealed alterations, including swelling, distortion (Figure 2, B).

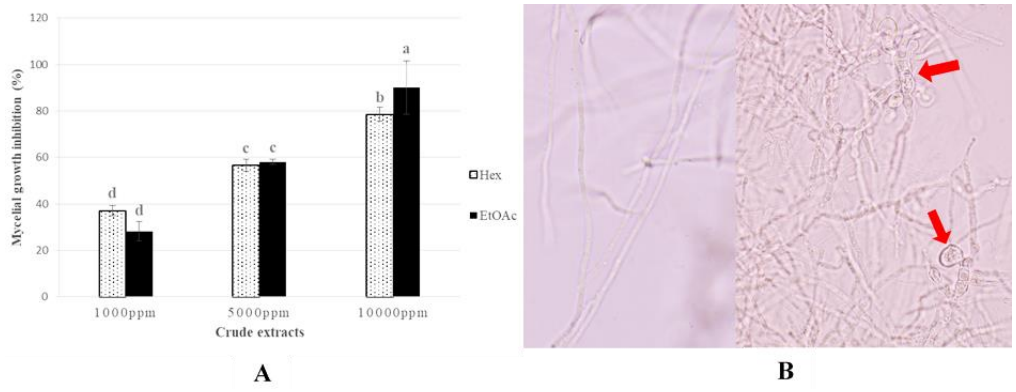

Figure 2. The effect of hexane (Hex) and ethyl acetate (EtOAc) crude extract from *Miliusa sessilis* against *Colletotrichum* sp. at various concentrations. A; Mycelial growth inhibition (%) of Hex and EtOAc crude extracts, B; Hyphal morphology, control (left) and inhibitory effect of EtOAc crude extract at 10000 ppm (right)

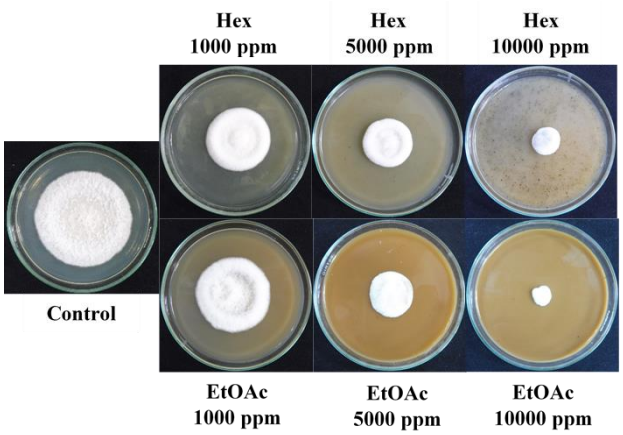

Figure 3. Inhibitory effect of *Miliusa sessilis* crude extracts on mycelial growth of *Colletotrichum* sp. at different concentrations (control, 1000, 5000 and 10000 ppm) after 14 days of incubation

Determination of minimum inhibitory concentration (MIC) of the crude extract

The minimum inhibitory concentration (MIC) of hexane and ethyl acetate (EtOAc) crude extracts from *M. sessilis* were examined using a serial dilution of the original extract. After incubation, the MIC values were taken as the lowest concentration and turbidity in the sterile test tube. When compared to other treatments, the concentration of 5000 μg mL^{-1} had a significant effect on inhibiting visible growth of *Colletotrichum* sp. hyphae by slight turbidity in the sterile test tube. Both Hex and EtOAc crude extracts gave the MIC value of 125 μg mL^{-1} against *Colletotrichum* sp. Furthermore, the inhibitory effect of crude extracts on fungal hyphae revealed that the hyphae were deformed, including swelling and hyphal lysis. However, DMSO had no effect on hyphal mycelia (Figure 7).

Phytochemical analysis

Thin layer chromatographic studies

Phytochemical constituents of sequential extracts (EtOH, hexane, EtOAc, *n*-BuOH, and H_2O crude extracts) were analyzed by thin-layer chromatography. The chromatogram developed in the Hexane: EtOAc acid (4:1) mobile system, showed nine compounds (bands **1**-**9**) with R_f values in the range of 0.81-0.14. The chromatogram in a higher polarity mobile phase system, CH_2Cl_2: MeOH: H_2O (30:3:1, lower layer) exhibited nine higher polarity compounds (bands **10**-**18**) with R_f values in the range of 0.70-0.05 (Figure 4 and Table 2). Fourteen substances exhibited strong absorbtion of UV light except for bands **4**, **9**, **15**, and **18**, suggesting that these strong absorbion UV light substances should have a structure with a conjugated double bond system or have a structure of aromatic rings. Interestingly, these compounds (bands **2**-**10**, **12**-**17**) that absorb UV are similar characters in color reagent (1% $CeSO_4$) to dark gray-purple, indicating that these compounds are similar in structure. The main constituents are band **6**; lesser substances are bands **11**, **9**, **8**, **7**, **5**, **4**, and **3**. From the chromatograms, hexane extract and EtOAc extract contain similar chemical compositions, but ethyl acetate extract contains higher polar compounds in a higher ratio. *n*-BuOH and H_2O crude extracts contain high polarity chemical compositions so that they cannot move on the chromatogram in the highly polar mobile phase system. This showed that most of the consituents of *n*-BuOH and H_2O crude extracts are saccharides.

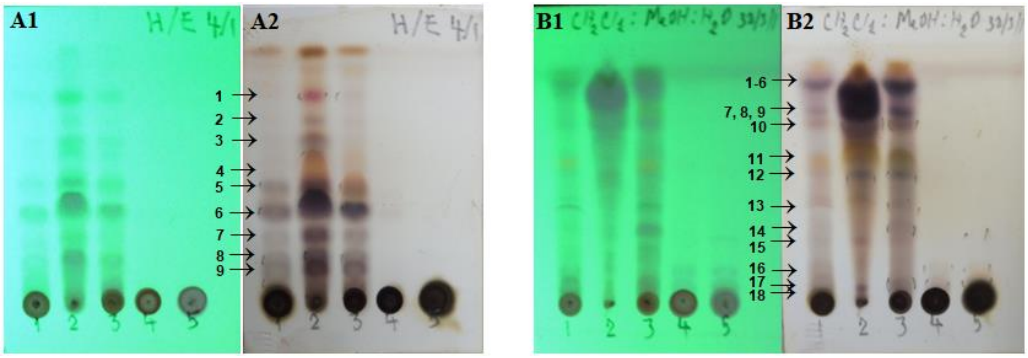

Figure 4. TLC profile for EtOH (lane 1), hexane (lane 2), EtOAc (lane 3), *n*-BuOH (lane 4), and H_2O (lane 5) soluble extract of *Miliusa sessilis*. TLC Chromatograms developed in mobile phase system Hexane: EtOAc acid (4:1) (Figure A1 and A2) and CH_2Cl_2: MeOH: H_2O (30:3:1, lower layer) (Figure B1 and B2). Observation developed chromatograms under UV light (254 nm) (Figure A1 and B1) and after spraying with dye (1% $CeSO_4$ in 10% aqueous H_2SO_4) following by heating (Figure A2 and B2).

Table 2. R_f values of TLC profile exhibited the phytochemical constituents of Crude extracts from twigs of *M. sessilis* using different mobile phase systems

Band	Rf value	The present of chemical compositions fromCrude extract									
		EtOH		Hexane		EtOAc		n-BuOH		H_2O	
		UV	dye	UV	dye	UV	dye	UV	dye	UV	dye
Mobile phase system Hexane: EtOAc acid (4:1)											
1	0.81	+	+	+	+						
2	0.73	+	+	+	+						
3	0.66	+	+	+	+						
4	0.51	-	+	-	++++	-	++				
5	0.47	++	++	+++	+++	++	++				
6	0.35	++	++	+++++	+++++	+++	++++	+	+		
7	0.27	+	+	+++	+++	+	+				
8	0.19	+	+	+++	+++	+	+				
9	0.14	-	+	-	+++		++				
Mobile phase system CH_2Cl_2: MeOH: H_2O (30:3:1, lower layer)											
10	0.70	+	+	++	++	+++	+++				
11	0.57	+	+	++	++	++	++				
12	0.51	+	+	++	++	++	++				
13	0.43	+	+	+	+	++	++				
14	0.30	+	+			++	++	+		+	
15	0.24	-	+	-	+	-	+				
16	0.14	+	+			+	+	++		++	
17	0.08	+	+			+++	+++	++		++	
18	0.05	-		-	+						

[1]/(+) =Relative degree of the presentation

NMR spectroscopic analysis

Based upon TLC profile and antifungal activity, indicating that the hexane and EtOAc extracts contain interesting chemical constituents.The ^{1}H NMR spectroscopic data of hexane and EtOAc extracts exhibited similar pattern signals (Figure 5-6 and Table 3). The predominant ^{1}H NMR signal both hexane and EtOAc extracts showed the signals of one 1,3,4,5-tetrasubstituted aromatic ring [δ 6.49 (1H, d, *J* = 1.8 Hz, H-2) and 6.40 (1H, d, *J* = 1.8 Hz, H-6) one 1,3,5-trisubstituted aromatic ring [δ 6.80 (1H, d, *J* = 1.8 Hz, H-2′); 6.89 (1H, d, *J* = 8.1 Hz, H-5′) and 6.71 (1H, dd, *J* = 8.1, 1.8 Hz, H-6′)], and two allylic groups [δ 3.24 (2H, d, *J* = 6.6 Hz, H-7); 5.93 (1H, m Hz, H-8); 5.06 (2H, m, H-9) and 3.38 (2H, d, *J* = 6.6 Hz, H-7′); 5.93 (2H, m Hz, H-8′); 5.06 (1H, m, H-9′). In addition, the ^{1}H NMR spectroscopic data showed two methoxyl groups at δ_H 3.89 (3H, s) ×2 were observed.

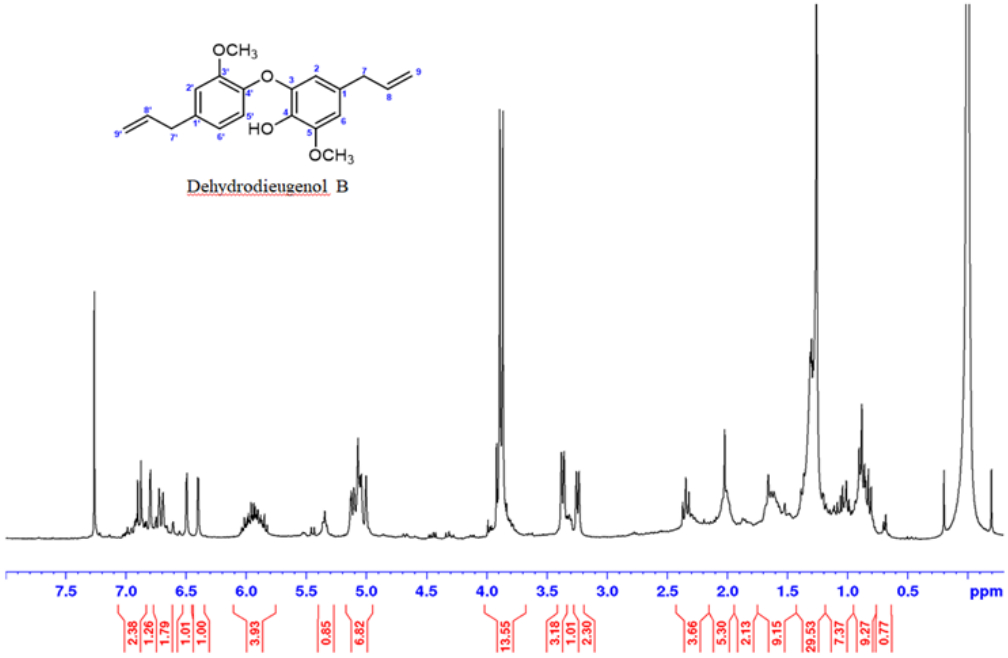

Figure 5. ^{1}H NMR spectrum of hexane crude extract from twigs of *M. sessilis* (300 MHz, $CDCl_3$)

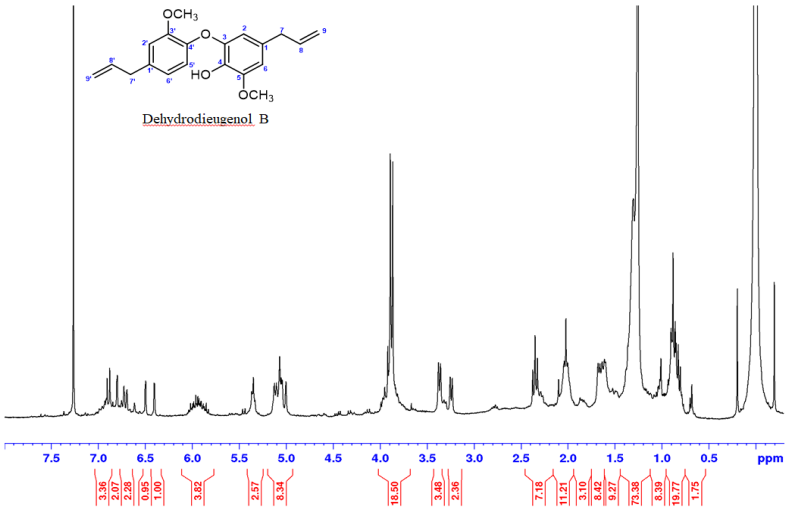

Figure 6. ^{1}H NMR spectrum of ethyl acetate crude extract from twigs of *M. sessilis* (300 MHz, $CDCl_3$)

Table 3. Comparative analysis of ^{1}H NMR (300 Hz) data for predominant signals of hexane and EtOAc extracts from twigs of *M. sessilis* in $CDCl_3$ with Dehydrodieugenol B (*J* in Hz in parentheses)

Position	**Hexane extract δ (ppm)**	**EtOAc extract δ (ppm)**	**Dehydrodieugenol B[1] δ (ppm)**
2	6.49 (1H, d, 1.8)	6.49 (1H, d, 1.8)	6.49 (1H, d, 1.8)
6	6.40 (1H, d, 1.8)	6.40 (1H, d, 1.8)	6.40 (1H, d,1.8)
7	3.24 (2H, d, 6.6)	3.24 (2H, d, 6.6)	3.24 (1H, d, 6.6)
8	5.93 (1H, m)	5.93 (1H, m)	5.92 (1H, m)
9	5.06 (2H, m)	5.06 (2H, m)	5.06 (2H, m)
2′	6.80 (1H, d, 1.8)	6.80 (1H, d, 1.8)	6.79 (1H, d, 2.0)
5′	6.89 (1H, d, 8.1)	6.89 (1H, d, 8.1)	6.89 (1H, d, 8.1)
6′	6.71 (1H, dd, 8.1, 1.8)	6.71 (1H, dd, 8.1, 1.8)	6.70 (1H, dd, 8.1, 2.0)
7′	3.36 (1H, d, 6.6)	3.37 (1H, d, 6.6)	3.36 (1H, d, 6.6)
8′	5.93 (1H, m)	5.94 (1H, m)	5.93 (1H, m)
9′	5.06 (2H, m)	5.06 (2H, m)	5.06 (2H, m)
OCH_3-5	3.89 (3H, s)	3.89 (3H, s)	3.89 (3H, s),
OCH_3-3′	3.86 (3H, s)	3.86 (3H, s)	3.86 (3H, s,)

[1]/Costa-Silva *et al.*, 2015

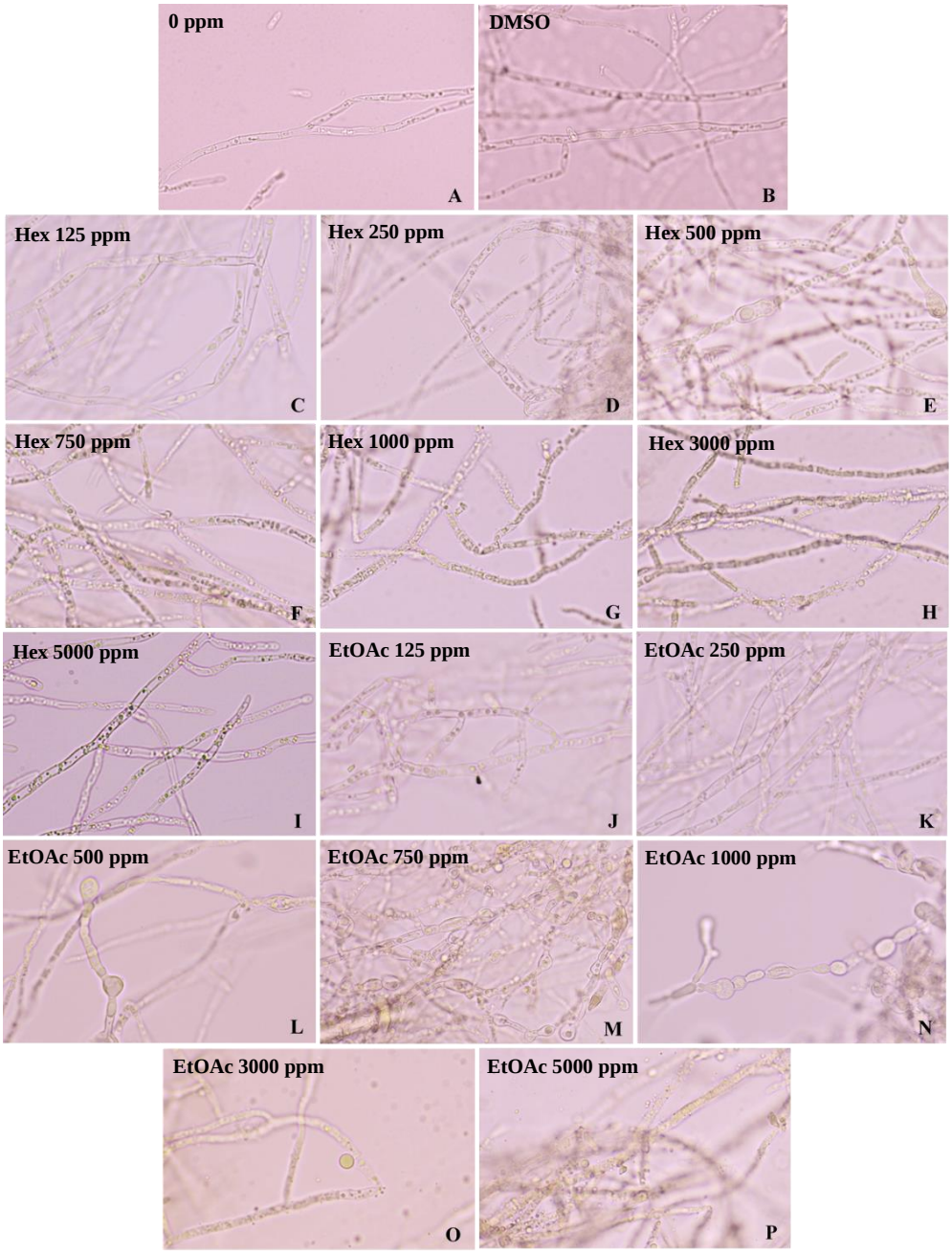

Figure 7. The minimum inhibitory concentration (MIC) values of *Miliusa sessilis* crude extracts against *Colletotrichum* sp. at varied concentrations of 125, 250, 500, 750, 1000, 3000 and 5000 µg mL^{-1} in hexane and ethyl acetate solvents compared with the control

Discussion

The mango anthracnose disease is a serious problem with yield quality worldwide. The symptoms showed brown to black spots appeared on the fruit's skin, especially during ripening of mature fruit (Scot, 2008). Anthracnose disease, a fungal infection of tropical fruit crops in Asia and the Pacific region, is caused by *Colletotrichum* sp. (Altendorf, 2018, Ploetz, 1999). As a result of this study, the morphological characteristics of the fungal pathogen and symptoms on mango fruit were confirmed as *Colletotrichum* sp. (Alexopoulos *et al*., 2002, Barnett and Hunter, 1986).

Antifungal activity of the twigs of *M. sessilis* extracts revealed the inhibitory effect against *Colletotrichum* sp. using the different solvents. For preliminary screening, the trend of fungal growth inhibitory effect of *M. sessilis* crude extracts seems to be of low activity, and it would need a much higher concentration. The EtOAc and Hex extracts showed the highest percentage of inhibitory control by up to 90.07% using the poisoned food assay. In this study, the aqueous extracts of *M. sessilis* crude extracts showed less antifungal activity than solvent extracts that were related to the reports of De Zoysa *et al*. (2019) and Wigmore *et al*. (2016). The concentration of crude extracts and the rate of inhibition on mycelial growth of *Colletotrichum* sp. had a positive relationship. According to Tschesche (1970), the bioactive compound can also cause agitation in the fungal cell wall. This research investigated that the crude extracts caused abnormal hyphal structures.

The sequential extraction from the EtOH crude extract obtained hexane and EtOAc crude extract, which only had 2.1% and 13.3% extraction percentages. The TLC profile and antifungal activity suggests that most of the interesting chemical compositions which have bioactive compounds are present in the hexane and EtOAc crude extracts.

From the information of ^{1}H NMR, it was indicated that hexane and EtOAc crude extracts contained neolignan named dehydrodieugenol B by comparing its spectroscopic data with literature values (De Diaz *et al*., 1980, Da Costa-Silva *et al*., 2015). The bioactive chemicals of the twigs of *M. sessilis* plant extracts revealed that the presence of neolignans was predominant in the Hex and EtOAc crude extracts. However, each part of the plant had different bioactive chemical compounds (Uchimahali *et al*., 2019).

In addition, the data from the TLC profiles of the two extracts above, we considered the chemical shift value range 3.2-6.9, which integral proton value compared to the signal at δ 6.40. The ratio of integral proton signals on aromatic rings of dehydrodieugenol B at δ 6.40, 6.49, 6.71, 6.80, and 6.89 were 1:1:1:1:1 but for the signal of the hexane extract was 1.0:1.0:1.8:1.3:2.4 and the

EtOAc extract was 1.0:1.0:2.3:2.1:3.4. Together with the ratio of integral proton signals on allylic groups of dehydrodieugenol B at δ 3.24, 3.38, 5.06, and 5.93 were 2:2:4:2 while the hexane extract was 2.3:3.2:6.8:3.9, and the EtOAc extract was 2.4:3.5:8.3:3.8. In addition to dehydrodieugenol B, other neolignans are also present in the hexane and EtOAc extracts.

This phytochemical compound was firstly described as an antifungal agent purified from the twigs of *M. sessilis* that had antifungal action against *Colletotrichum* sp.

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