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Original Article

Chemical constituents from *Melodorum fruticosum* Lour. flowers against plant pathogenic fungi

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ABSTRACT

The antifungal activity of hexane, dichloromethane and methanol extracts of 45 Thai plants were *in vitro* screened against plant phytopathogenic fungi (*Alternaria porri*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Phytophthora parasitica*). Seven extracts strongly inhibited the mycelial growth of fungi. The plant extracts with highest antifungal activity were *Limnophila aromatic*, *Eupatorium odoratum*, *Melodorum fruticosum* and *Alpinia galanga* with 70%, 58%, 74% and 100% inhibition, respectively. The potent dichloromethane extract from *M. fruticosum* flowers was separated using bioassay guided against *P. parasitica*. Eight compounds: 1-hexacosanol (**1**), 5-hydroxy-7-methoxyflavone (**2**), β-sitosterol (**3**), melodorinone B (**4**), benzoic acid (**5**), chrysin (**6**), melodorinol (**7**) and melodorinone A (**8**) could be isolated. Among the isolated compounds, benzoic acid (**5**) and melodorinol (**7**) exhibited strong activity against mycelial growth of *P. parasitica* at 100% and 93% inhibition with the half maximal inhibitory concentration (IC_{50}) values of 108 μg/mL and 130 μg/mL, respectively. This plant could be exploited for eco-friendly management control of plant diseases.

Introduction

The agricultural problems from plant diseases caused by fungi have resulted in extensive loss of yield and quality in many products (Oreke et al., 1994). Plant diseases caused by fungi can spread very rapidly and become widespread when prescribed control measures are not undertaken (Ishnava et al., 2011). Four fungi were selected in this study—*Alternaria porri* (purple blotch), *Colletotrichum gloeosporioides* (anthracnose), *Fusarium oxysporum* (Fusarium wilt) and *Phytophthora parasitica* (heart or root rot). Many synthetic fungicides have been used to control plant diseases at both pre- and post-cultivation. However, the use of fungicides may have adverse effects including their residual toxicity to humans and organisms, pollution of the environment, a long degradation period and accumulation in the food chain; therefore, fungicides have been restricted (Bernabò et al., 2016). Moreover, plant pathogens can become resistant to fungicides; thus, it is important that novel antifungal agents be identified and developed (Mukalazi et al., 2001; Lee et al., 1999).

Plants have long been recognized as a potential source of bioactive compounds, which may be used against phytopathogenic fungi (Parveen et al., 2013; Bhagwat and Datar, 2013). Thailand is located in the tropical region with vast biodiversity and an abundance of natural products where a large number of medicinal plants have been used for traditional treatment in the primary health care and used for agrochemicals (Manosroi et al., 2006; Cespedes et al., 2014). Plant extracts may also be useful to certain farmers who may not be able to afford commercial fungicides which may also not be acceptable in organic agricultural crops (Mahlo et al., 2010). Therefore, research into natural products involving plants is targeted toward the goal of studying biological activity and developing environmentally safe controls for plant disease.

Melodorum fruticosum belongs to the Annonaceae family and its common names in Thailand are devil tree, white cheesewood and lamduan (Pripdeevech and Chukeatirote, 2010). This plant has been used as a tonic, a mild cardiac stimulant, to reduce fever and as a hematinic to resolve dizziness (Rujjanawate et al., 2008). The chemical constituents of *M. fruticosum* have been studied with

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the major constituents being heptene derivatives—melodienone, *iso*-melodienone, acetylmelodorinol, dichamanetin, pinocembrin, polycarpol, benzyl benzoate, a mixture of stigmasterol and β-sitosterol, melodorinol, homomelodienone, 7-hydroxy-6-hydro melodinone and homoiso-melodienone (Jung et al., 1990a, 1991). Bioactive butenolides from the leaves and branches have been identified as (4*Z*)-6-acetoxy-7-benzoyloxy-2,4-heptadien-4-olide, (4*E*)-6-acetoxy-7-benzoyloxy-2,4-heptadien-4-olide, (4*E*)-7-ben zoyloxy-6-hydroxy-2,4-hepta- dien-4-olide, (4*Z*)-6-benzoyloxy-7-hydroxy-2,4-heptadien-4-olide, chrysin and benzoic acid (Tuchinda et al., 1991). In addition, 5,7-dimethoxyflavone, melodorinone A, melodorinone B, tautomelodorinone and acetylpolycarpol have been noted as constituents of the flowers (Tiyaworanan, 1998; Juengwatanakul, 2000; Chaichantipyuth et al., 2001). The cytotoxicity, antitumor and plant growth regulation of isolated compounds from this plant have been scrutinized (Jung et al., 1990a, 1991; Tuchinda et al., 1991) and antifungal activity against *Colletotrichum* sp. and the antioxidant activities of the essential oil and various extracts have been reported (Pripdeevech and Chukeatirote, 2010). However, there are no reports concerning anti-phytopathogenic activity from the flowers; thus, this paper focused on the isolation of the constituents possessing anti-phytopathogenic fungal activity.

Materials and methods

Samples of 45 Thai medicinal plants and weeds were collected from Cha-Am district, Phetchaburi province or were purchased from the “Chow Krom Per” and “Tai-Hua-Chan” medical herbal shops, Bangkok, Thailand in 2013.

Plant extraction

The fresh plants were cut into small pieces, dried, milled to powder and extracted using a Soxhlet apparatus. The extraction was started with hexane for the leaves and CH_2Cl_2 for the other parts of samples and the residue was further extracted using MeOH. The extracts were filtered and evaporated using a rotatory evaporator.

Preliminary antiphytopathogenic fungi screening

The obtained extracts were subjected to antifungal activity screening against four fungi: *A. porri* DOAC 1601, *C. gloeosporioides* DOAC 2047, *F. oxysporum* DOAC 1258 and *P. parasitica* DOAC 2052. These fungi were supplied by the Division of Plant Disease and Microbiology, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. *P. parasitica* was cultured on carrot agar at 28 °C (Poopaiboon et al., 2003) whereas the others were separately cultured and maintained on potato dextrose agar (PDA) slant stored at 4 °C until used. The screening antifungal activity used the agar incorporation method (Tangjitcharoenkul, 2010). All fungi were cultivated on PDA in Petri dishes for 7 d before use. The crude extracts from plants were dissolved in dimethylsulfoxide (DMSO), 100 μL of each solution extract was aseptically added and mixed with 9.9 mL of melted PDA to obtain the final concentration of 1000 μg/mL and after mixing well was poured into a Petri dish and allowed to cool and solidify. The disc mycelial (8 mm diameter) growth of tested fungi was picked up from the culture and placed in the center of each Petri dish. Agar plates amended with DMSO were used as a negative control and with metalaxyl (100 μg/mL active ingredient) were used as a positive control. After the tested plates were incubated for 5–7 d at 27 °C, radial mycelial measurements were taken when the fungi of the control plate reached the edge of the plate. Colony diameters were measured and calculated as mycelial growth inhibition (Eq. (1)):

$$\text{Mycelial growth inhibition } (\%) = [(dc - dt)/dc] \times 100 \quad (1)$$

where d*c* is the average diameter of the fungal colony of control plate and d*t* is the average diameter of the fungal colony of the treatment plate (Tegegne et al., 2008). All treatments were replicated three times.

Isolation and bioassay guide

The CH_2Cl_2 extract (150 g) from the flowers of *M. fruticosum* was fractionated using a quick column with silica gel (Kieselgel 60 Art 7729; Merck; Darmstadt, Germany). A stepwise elution was conducted using 100% hexane and the polarity was increased with EtOAc and finally with 10% MeOH in EtOAc. The fractions were collected and combined according to thin layer chromatography behavior and then evaporated to obtain 10 subfractions (Mf1–Mf10). All fractions were tested against *P. parasitica* at 1000 μg/mL. The active fractions were subjected to a silica gel column, Sephadex LH-20 and chromatotron. The elution system used for this isolation was EtOAc in hexane with a gradual increase in the polarity using EtOAc. The highest active compounds were further tested for antifungal activity against all selected fungi at various concentrations and the half maximal inhibitory concentration (IC_{50}) values were calculated.

Statistical analysis

All data were analyzed using the SPSS statistic analytical analysis software (Windows version 20.0; SPSS Inc; Chicago, Il, USA) and comparison of means used Duncan's Multiple Range Test at the level $p < 0.05$. The experiment was designed as a general linear model within a completely randomized design with triplications.

Results and discussion

Preliminary antiphytopathogenic fungi screening

The antifungal activity screening results of the 45 Thai plant extracts belonging to 32 families exhibited different extents depending on the phytopathogenic fungi (Table 1). Some selected plants displayed promising antifungal activity at 1000 μg/mL. The essential oil of *Limnophila aromatica* (Scrophulariaceae) revealed the highest activity against *A. porri* with 70% mycelial growth inhibition. Eighteen species moderately inhibited *A. porri* (41–62% mycelial growth inhibition). In previous studies, the biological activities from *Limnophila* species such as antimicrobial, anti-inflammatory, antioxidant, wound healing, antitubercular, cytotoxic and anthelmintic were investigated (Reddy et al., 1991; Brahmachari, 2008). For the antifungal activity against the mycelial growth of *C. gloeosporioides*, the CH_2Cl_2 extracts from Compositae (*Eupatorium odoratum* and *Ageratum conyzoides*) showed moderate activity against tested organisms (58% and 49%). The CH_2Cl_2 extracts of *M. fruticosum* and *Alpinia galanga* showed strong inhibition against *F. oxysporum* with 74% and 70% mycelial growth inhibition, respectively. All selected plants in the Zingiberaceae, except *Zingiber cassumunar*, showed high activity against *P. parasitica*. In particular, the CH_2Cl_2 extract of *A. galanga* completely inhibited mycelial growth followed by *Cinnamomum porrectum*, *Acorus calamus*, *M. fruticosum*, *Kaempferia galanga*, *Boesenbergia rotunda*, *Mansonia gagei* and *E. odoratum* (70–87% mycelial growth inhibition). In addition, the MeOH extracts showed less antifungal activity than the CH_2Cl_2 extracts (Table 1).

Table 1
Screening antifungal activity of Thai plant extracts against plant pathogenic fungi at 1000 μg/mL concentration.

No.	Family	Scientific name	Plant part	Fraction	Inhibition (%)			
					A. por	*C. glo*	*F. oxy*	*P. par*[a]
1	Acanthaceae	*Acanthus ilicifolius*	Leaf	CH_2Cl_2	4.5 ± 1.0	3.7 ± 1.2	37.8 ± 1.0	38.9 ± 0.0
2		*Andrographis paniculata*	Leaf	CH_2Cl_2	18.3 ± 0.0	31.6 ± 0.6	24.1 ± 0.0	21.1 ± 0.0
				MeOH	28.3 ± 1.5	18.4 ± 2.6	9.2 ± 2.0	20.6 ± 1.0
3	Annonaceae	*Melodorum fruticosum*	Flower	CH_2Cl_2	49.5 ± 1.5	31.1 ± 0.0	74.4 ± 1.2	83.3 ± 3.7
				MeOH	47.1 ± 1.5	11.1 ± 0.0	26.7 ± 1.2	51.4 ± 1.6
4	Araceae	*Acorus calamus*	Rhizome	CH_2Cl_2	50 ± 0.0	43.7 ± 1.2	66.7 ± 0.0	85.6 ± 8.9
				MeOH	26.7 ± 1.2	10.3 ± 1.2	13.8 ± 3.5	32.2 ± 1.0
5	Bignoniaceae	*Oroxylum indicum*	Bark	CH_2Cl_2	23.1 ± 1.2	0.0 ± 0.0	45.5 ± 7.5	58.9 ± 2.3
				MeOH	40 ± 1.7	3.3 ± 0.0	44.1 ± 3.2	51.4 ± 1.0
			Wood	CH_2Cl_2	10.0 ± 0.0	0.0 ± 0.0	41.4 ± 11.9	43.3 ± 3.7
				MeOH	2.5 ± 0.0	0.0 ± 0.0	22.2 ± 1.0	5.5 ± 1.6
6	Boraginaceae	*Heliotropium indicum*	Whole Plant	CH_2Cl_2	3.1 ± 1.2	2.3 ± 1.5	7.4 ± 3.1	17 ± 1.5
				MeOH	4.6 ± 3.1	3.2 ± 3.1	11.4 ± 2.1	12.2 ± 1.0
7	Caesalpiniaceae	*Cassia siamea*	Leaf	CH_2Cl_2	−8.7 ± 1.2	0.9 ± 1.5	33.3 ± 0.0	3.5 ± 0.0
				MeOH	−7.7 ± 1.0	−1.4 ± 1.6	32.2 ± 0.0	14.4 ± 1.2
			Stem	MeOH	43.3 ± 3.2	24.1 ± 2.1	13.8 ± 1.2	0.0 ± 0.0
8	Compositae	*Ageratum conyzoides*	Whole Plant	Hexane	32.2 ± 1.6	48.9 ± 2.1	15.2 ± 3.2	50.0 ± 0.0
				CH_2Cl_2	33 ± 0.0	45.6 ± 2.2	26.7 ± 1.4	51.9 ± 0.6
				MeOH	17 ± 1.2	40.0 ± 0.0	19.7 ± 2.1	17.4 ± 0.6
9		*Eupatorium odoratum*	Leaf	Hexane	57.0 ± 1.2	57.8 ± 0.0	48.1 ± 0.6	56.7 ± 1.0
				CH_2Cl_2	58.1 ± 0.6	56.7 ± 0.0	50.0 ± 0.0	70.0 ± 0.0
				MeOH	34.8 ± 0.6	40.0 ± 0.0	16.7 ± 0.0	12.6 ± 1.6
			Stem	Hexane	38.9 ± 1.7	46.7 ± 0.0	27.0 ± 4.0	35.6 ± 0.0
				CH_2Cl_2	44.1 ± 1.5	50.0 ± 0.0	26.3 ± 0.6	59.2 ± 0.0
				MeOH	17.4 ± 0.6	31.1 ± 0.0	13.3 ± 0.0	0.0 ± 0.0
10		*Grangea maderaspatana*	Leaf	CH_2Cl_2	9.0 ± 1.5	−9.3 ± 1.5	−3.1 ± 0.0	0.0 ± 0.0
11		*Tagetes erecta*	Flower	CH_2Cl_2	31.4 ± 1.5	16.1 ± 1.0	54.4 ± 1.2	20.0 ± 0.0
				MeOH	21.7 ± 1.0	21.2 ± 1.0	19.5 ± 1.2	0.0 ± 0.0
12	Cucurbitaceae	*Momordica charantia*	Leaf	CH_2Cl_2	18.1 ± 0.0	28.8 ± 1.0	10.0 ± 5.4	−15.2 ± 0.7
				MeOH	25.6 ± 0.0	16.7 ± 12.9	30.6 ± 1.0	−13.3 ± 0.0
			Stem	CH_2Cl_2	27.3 ± 2.1	14.4 ± 1.6	0.0 ± 0.0	−16.3 ± 1.2
				MeOH	22.2 ± 0.0	15.3 ± 0.0	8.9 ± 0.0	−13.0 ± 1.5
13		*Momordica cochinchinensis*	Leaf	CH_2Cl_2	−12.8 ± 1.6	3.7 ± 1.5	22.2 ± 0.0	8.9 ± 0.0
				MeOH	−3.7 ± 1.2	17.0 ± 6.1	11.1 ± 0.0	27.8 ± 0.0
			Seed	CH_2Cl_2	0.0 ± 0.0	28.9±	17.2 ± 4.7	14.4 ± 0.0
				MeOH	22.2 ± 0.0	24.8 ± 2.5	11.1 ± 0.0	13.9 ± 2.6
14	Dipterocarpaceae	*Croton bonplandianus*	Leaf	CH_2Cl_2	−15.7 ± 2.0	−1.8 ± 2.1	30.0 ± 1.0	0.0 ± 0.0
15	Euphorbiaceae	*Vatica diospyroides*	Leaf	CH_2Cl_2	21.4 ± 2.1	12.8 ± 0.0	60.6 ± 8.9	20.0 ± 1.6
				MeOH	23.9 ± 2.1	28.9 ± 0.0	50.0 ± 0.6	50.0 ± 2.4
16	Flacourtiaceae	*Hydnocarpus anthelminthicus*	Seed	CH_2Cl_2	47.1 ± 0.0	17.8 ± 0.0	51.7 ± 1.0	7.8 ± 1.6
17	Gentianaceae	*Fagraea fragrans*	Flower	CH_2Cl_2	45.6 ± 0.6	17.8 ± 1.0	18.9 ± 2.6	16.7 ± 0.0
				MeOH	52.2 ± 0.6	26.11.0	17.0 ± 0.6	16.7 ± 0.0
			Bark	CH_2Cl_2	62.6 ± 1.5	29.2 ± 1.5	49.7 ± 1.0	34.4 ± 0.0
				MeOH	57.8 ± 1.2	28.1 ± 1.0	22.2 ± 0.0	21.9 ± 0.6
			Wood	CH_2Cl_2	58.0 ± 3.5	26.7 ± 1.0	15.6 ± 1.7	19.2 ± 0.6
				MeOH	51.9 ± 1.2	18.9 ± 0.0	10.0 ± 1.0	17.0 ± 0.6
18	Gramineae	*Cymbopogon nardus*	Leaf	CH_2Cl_2	−6.8 ± 2.1	22.2 ± 1.0	37.8 ± 0.0	23.3 ± 0.0
19	Lauraceae	*Cinnamomum porrectum*	Wood	CH_2Cl_2	26.2 ± 3.1	20.7 ± 0.0	13.8 ± 2.3	86.7 ± 0.0
				MeOH	26.6 ± 2.3	8.9 ± 0.0	6.7 ± 2.0	79.3 ± 0.6
20	Leguminosae	*Erythrina variegata*	Leaf	CH_2Cl_2	27.9 ± 1.6	17.1 ± 1.0	56.1 ± 3.0	12.2 ± 1.6
				MeOH	24.3 ± 0.6	14.4 ± 1.6	57.2 ± 1.2	13.3 ± 0.6
21	Loranthaceae	*Helixanthera cylindrica*	Leaf	CH_2Cl_2	37.9 ± 1.6	25.0 ± 0.0	30.0 ± 0.0	0.0 ± 0.0
				MeOH	−19.1 ± 2.5	−13.9 ± 2.6	28.9 ± 0.0	25.6 ± 1.0
22	Malvaceae	*Hibiscus sabdariffa*	Fruit	MeOH	36.8 ± 1.0	−6.9 ± 2.0	40.0 ± 2.0	68.9 ± 0.0
23	Moraceae	*Artocarpus heterophyllus*	Leaf	CH_2Cl_2	16.7 ± 13.2	17.8 ± 1.5	−21.4 ± 1.6	−15.3 ± 0.2
				MeOH	−10.5 ± 1.2	−1.8 ± 2.0	−28.6 ± 0.6	−17.1 ± 0.6
			Root	CH_2Cl_2	3.3 ± 0.6	5.6 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
				MeOH	22.6 ± 4.5	16.7 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
24	Myrtaceae	*Syzygium cumini*	Leaf	Hexane	40.4 ± 2.1	20.0 ± 0.0	55.6 ± 1.0	26.7 ± 2.1
				CH_2Cl_2	27.1 ± 4.7	19.4 ± 0.6	55.6 ± 1.2	21.7 ± 1.5
				MeOH	35.4 ± 1.5	17.8 ± 1.0	49.4 ± 2.3	15.6 ± 1.6
			Bark	CH_2Cl_2	51.4 ± 0.0	18.9 ± 2.1	55.0 ± 4.7	25.4 ± 0.6
				MeOH	52.1 ± 0.6	12.8 ± 1.2	53.3 ± 2.6	15.6 ± 0.6
25	Passifloraceae	*Passiflora foetida*	Leaf	CH_2Cl_2	41.4 ± 4.5	41.4 ± 0.0	51.9 ± 0.6	56.7 ± 0.6
				MeOH	53.3 ± 0.0	28.7 ± 2.1	53.3 ± 0.0	44.8 ± 0.5
			Stem	CH_2Cl_2	20.7 ± 3.9	30.8 ± 1.6	56.7 ± 3.9	58.9 ± 0.6
				MeOH	45.0 ± 0.5	17.2 ± 2.1	60.5 ± 1.2	25.9 ± 1.2
26	Piperaceae	*Areca catechu*	Seed	CH_2Cl_2	44.3 ± 1.6	21.1 ± 1.0	53.3 ± 1.5	22.2 ± 2.1
				MeOH	10.0 ± 3.8	5.8 ± 1.0	9.2 ± 0.0	21.1 ± 6.2
27		*Piper betle*	Leaf	Hexane	26.4 ± 1.0	18.3 ± 1.6	54.4 ± 0.6	40.0 ± 2.1
				CH_2Cl_2	46.7 ± 0.6	29.9 ± 9.4	44.8 ± 0.0	65.6 ± 4.0
28	Piperaceae	*Piper ribesoides*	Leaf	CH_2Cl_2	30.8 ± 0.0	48.3 ± 1.0	47.7 ± 1.0	5.0 ± 1.0

Table 1 (*continued*)

No.	Family	Scientific name	Plant part	Fraction	Inhibition (%)			
					A. por	*C. glo*	*F. oxy*	*P. par*[a]
				MeOH	40.0 ± 0.6	31.0 ± 1.0	23.0 ± 0.0	0.0 ± 0.0
29	Plamae	*Piper nigrum*	Seed	CH_2Cl_2	57.1 ± 0.0	51.7 ± 0.0	49.7 ± 0.0	55.6 ± 0.6
				MeOH	57.6 ± 1.0	52.2 ± 0.6	54.4 ± 1.6	48.9 ± 1.2
30	Plantaginaceae	*Plantago major*	Leaf	CH_2Cl_2	−7.8 ± 1.0	−11.5 ± 0.6	33.3 ± 0.0	0.0 ± 0.0
31	Pteridaceae	*Acrostichum aureum*	Leaf	CH_2Cl_2	−22.8 ± 2.0	0.9 ± 0.6	30.0 ± 1.5	2.2 ± 2.0
32	Rubiaceae	*Gardenia angusta*	Flower	CH_2Cl_2	8.5 ± 0.0	35.1 ± 0.0	−12.7 ± 0.0	36.2 ± 4.4
				MeOH	−8.5 ± 1.0	5.2 ± 0.6	1.6 ± 0.6	19.4 ± 2.3
			Branch	CH_2Cl_2	−7.0 ± 0.0	22.1 ± 0.0	−10.6 ± 0.0	48.2 ± 7.1
				MeOH	−3.8 ± 0.0	14.3 ± 0.0	−13.8 ± 0.0	4.5 ± 0.6
33		*Gardenia jasminoides*	Flower	CH_2Cl_2	1.4 ± 0.0	19.5 ± 0.0	−6.3 ± 0.0	0.0 ± 0.0
				MeOH	9.4 ± 1.2	−14.3 ± 0.0	−8.4 ± 0.0	17.1 ± 1.6
34	Rutaceae	*Zanthoxylum limonella*	Stem	CH_2Cl_2	55.4 ± 0.6	31.9 ± 0.0	44.4 ± 0.0	37.8 ± 1.0
				MeOH	54.7 ± 1.5	26.9 ± 3.6	33.3 ± 0.0	48.9 ± 1.0
35	Sapindaceae	*Nephelium lappaceum*	Seed	hexane	12.2 ± 1.0	15.2 ± 0.6	5.0 ± 0.0	0 ± 0.0
				CH_2Cl_2	26.7 ± 1.0	16.7 ± 0.0	2.6 ± 0.6	3.3 ± 1.0
				MeOH	50.0 ± 0.6	25.9 ± 3.5	13.0 ± 0.6	13.7 ± 3.5
36	Scrophulariaceae	*Limnophila aromatica*	Whole Plant	Essential oil	70.3 ± 0.6	11.1 ± 0.0	46.3 ± 0.6	54.4 ± 5.5
37	Stercubaceae	*Mansonia gagei*	Wood	CH_2Cl_2	53.3 ± 1.3	25.3 ± 1.5	54.0 ± 2.3	73.3 ± 1.0
				MeOH	48.3 ± 1.7	21.8 ± 1.1	50.6 ± 2.1	26.7 ± 1.0
38	Verbenaceae	*Clerodendrum inerme*		CH_2Cl_2	35.0 ± 3.5	28.7 ± 1.2	23.0 ± 1.0	22.2 ± 20.2
				MeOH	31.7 ± 6.0	23.0 ± 2.1	11.5 ± 2.1	0.0 ± 0.0
39		*Tectona grandis*	Wood	CH_2Cl_2	53.3 ± 0.0	42.2 ± 1.0	20.0 ± 0.0	60.8 ± 2.1
				MeOH	49.7 ± 2.5	37.4 ± 1.5	19.7 ± 0.6	55.2 ± 0.6
40	Zingiberaceae	*Alpinia galanga*	Rhizome	CH_2Cl_2	42.5 ± 1.0	33.3 ± 2.0	70.1 ± 1.5	100.0 ± 0.0
				MeOH	28.3 ± 0.6	8.1 ± 0.0	3.4 ± 0.0	0.0 ± 0.0
41		*Boesenbergia rotunda*	Rhizome	CH_2Cl_2	51.7 ± 1.0	43.7 ± 0.0	46.0 ± 1.5	20.0 ± 2.0
				MeOH	48.3 ± 0.0	42.5 ± 1.2	51.7 ± 1.0	80.6 ± 2.5
42		*Curcuma longa*	Rhizome	CH_2Cl_2	50.0 ± 0.0	39.1 ± 1.0	64.4 ± 1.6	64.4 ± 14.2
43		*Kaempferia galanga*	Rhizome	CH_2Cl_2	61.7 ± 0.0	44.8 ± 0.2	46.0 ± 0.0	81.7 ± 1.5
				MeOH	45.0 ± 1.0	27.6 ± 1.6	37.9 ± 1.0	42.2 ± 1.0
44		*Kaempferia parviflora*	Rhizome	CH_2Cl_2	39.2 ± 1.0	57.5 ± 0.0	59.2 ± 4.9	46.7 ± 11.4
				MeOH	38.3 ± 0.6	24.1 ± 2.5	47.1 ± 2.1	32.2 ± 1.0
45		*Zingiber cassumunar*	Rhizome	CH_2Cl_2	31.7 ± 0.0	24.1 ± 1.0	47.1 ± 1.6	0.0 ± 0.0
				MeOH	27.5 ± 1.0	28.1 ± 1.0	20.7 ± 0.0	0.0 ± 0.0

[a] *A. por* = *A. porri*; *C; glo* = *C. gloeosporioides*; *F. oxy* = *F. oxysporum*; *P. par* = *P. parasitica*.

Among the selected plants, only seven plant species (*A. galanga*, *C. porrectum*, *A. calamus*, *M. fruticosum*, *K. galanga*, *B. rotunda*, and *M. gagei*) displayed strong antifungal activity (more than 70% mycelial growth inhibition). The CH_2Cl_2 extracts of *A. galanga*, *M. fruticosum* and *M. gagei* displayed strong inhibition against the mycelial growth of *P. parasitica* and the other tested fungi. Recently, the bioactive compounds from the rhizomes of *A. galanga* against plant pathogenic fungi have been reported and the active compound was 1′-acetoxychavicol acetate (Mongkol et al., 2015).

Antifungal activity of M. fruticosum flowers

This paper reports further investigation on the isolation and characterization of bioactive compounds from the flowers of *M. fruticosum*. The CH_2Cl_2 and MeOH extracts of this plant were tested for antifungal assay against all fungi at 1000 μg/mL. The CH_2Cl_2 extract revealed a higher antifungal activity than the MeOH extract (Fig. 1) against *C. gloeosporioides*, *A. porri*, *F. oxysporum* and *P. parasitica* with inhibition of 31%, 50%, 74% and 83%, respectively (Fig. 1).

Different metabolites such as essential oils, alkaloids, saponins, terpenes, flavonoids, peptides and proteins exhibit antimicrobial activity (Sati and Joshi, 2011). However, it could not be specified which compound was responsible with regard to the tested fungi until the bioactive phytochemicals were isolated. Therefore, the bioactive compounds were investigated using spectroscopic techniques. From separation using quick column chromatography and the antifungal activity screening results, Mf5-6 completely inhibited *P. parasitica* at a concentration of 1000 μg/mL followed by Mf8 (85%), Mf7 (69%) and Mf9 (59%) (data not shown). Mf1, 3, 4 and 10 slightly inhibited the mycelial growth of *P. parasitica* (16%, 9%, 28% and 11%, respectively). However, Mf2 resulted in mycelial growth rather than inhibition when compared with the control. Finally, compounds were isolated from these active fractions and compared with the ^{1}H and ^{13}C nuclear magnetic resonance data in previous reports (Jung et al., 1990b, 1991; Tiyaworanan, 1998). All isolated compounds were identified—1-hexacosanol (**1**), 5-hydroxy-7-methoxyflavone (**2**), β-sitosterol (**3**), melodorinone B (**4**), benzoic acid (**5**), chrysin (**6**), melodorinol (**7**), and melodorinone A (**8**)—as shown in Fig. 2. All compounds were evaluated for antifungal activity against *P. parasitica* at 1000 μg/mL except for compound (**2**). Fig. 3 shows that benzoic acid (**5**) completely inhibited *P. parasitica*, followed by melodorinol (**7**) with 93%

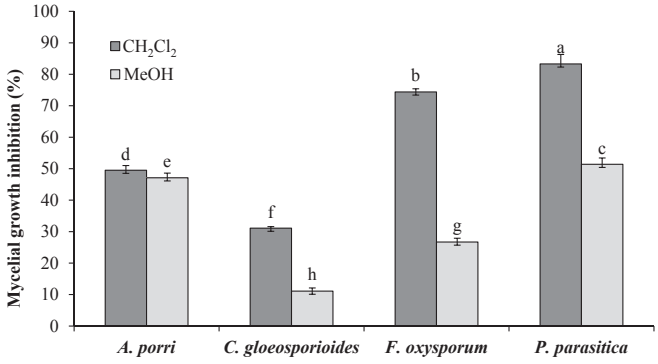

Fig. 1. Mycelial growth inhibition of *M. fruticosum* flowers extract against various fungi (Error bars show mean ± SD; different lower case letters for each column indicate a significant difference at $p < 0.05$).

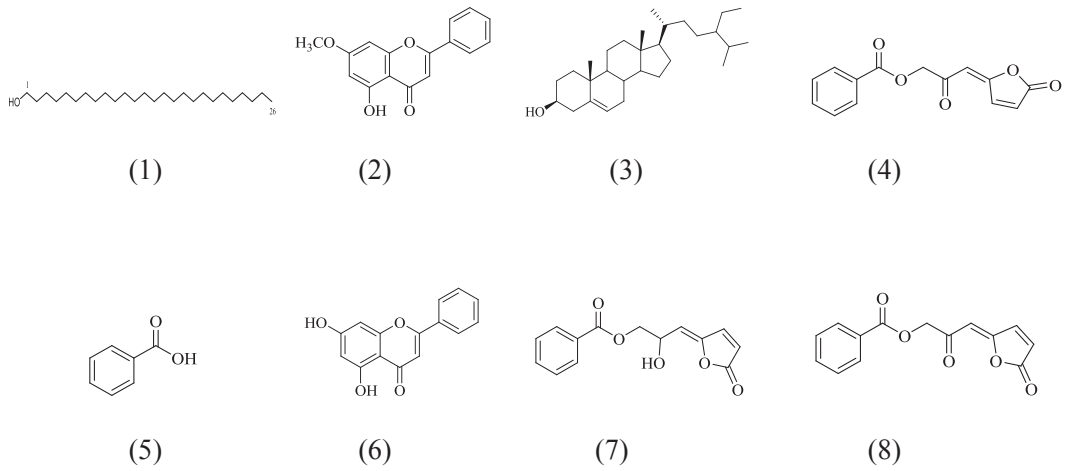

Fig. 2. Structures of isolated compounds from *M. fruticosum* flowers: hexacosanol (**1**); 5-hydroxy-7-methoxyflavone (**2**); β-sitosterol (**3**); melodorinone B (**4**); benzoic acid (**5**); chrysin (**6**); melodorinol (**7**); melodorinone A (**8**).

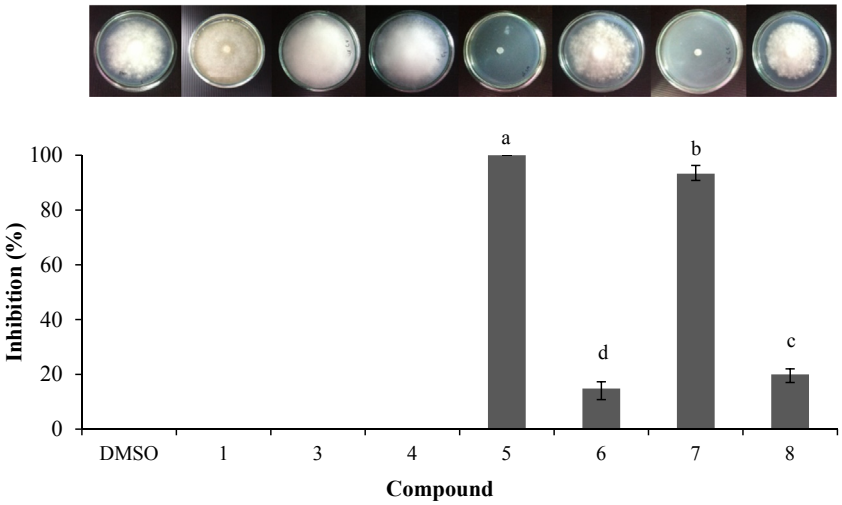

Fig. 3. Antifungal activity of isolated compounds against *P. parasitica* at 1000 μg/mL concentration for hexacosanol (**1**), β-sitosterol (**3**), melodorinone B (**4**), benzoic acid (**5**), chrysin (**6**), melodorinol (**7**) and melodorinone A (**8**). (Error bars show mean ± SD; different lower case letters for each column indicate a significant difference at $p < 0.05$).

mycelial growth inhibition, whereas 1-hexacosanol (**1**), *β*-sitosterol (**3**) and melodorinone B (**4**) did not show antifungal activity. These compounds were further tested at various concentrations against four fungi and their IC_{50} values were compared (Table 2). Benzoic acid (**5**) was found to possess the strongest inhibition compared to the other compounds. With *P. parasitica* as the sensitive strain, benzoic acid (**5**) displayed a lower IC_{50} than melodorinol (**7**) at concentrations of 108 μg/mL and 130 μg/mL, respectively. However, benzoic acid (**5**) and melodorinol (**7**) showed IC_{50} greater than 1000 μg/mL against *C. gloeosporioides* and *F. oxysporum*, respectively. For the other fungi, benzoic acid (**5**) against *A. porri* and *F. oxysporum* had IC_{50} of 325 μg/mL and 450 μg/mL, respectively,

Table 2
Antifungal activity of benzoic acid (**5**) and melodorinol (**7**) and the half maximal inhibitory concentration (IC_{50}).

Compound	Fungi	Inhibition (%)[a]					**IC_{50}**
		10[b]	100	250	500	1000	
Benzoic acid (**5**)	*A. porri*	7.1 ± 0.6	8.2 ± 1.5	25.1 ± 1.2	100.0 ± 0.0	100.0 ± 0.0	325
	C. gloeosporioides	0.0 ± 0.0	0.0 ± 0.0	5.6 ± 0.0	10.7 ± 0.3	13.7 ± 0.3	>1000
	F. oxysporum	9.3 ± 2.9	19.6 ± 1.2	40.4 ± 1.5	51.1 ± 1.7	100.0 ± 0.0	450
	P. parasitica	6.3 ± 0.6	46.0 ± 1.0	90.6 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	107.5
Melodorinol (**7**)	*A. porri*	16.1 ± 0.0	31.8 ± 0.0	42.3 ± 1.0	58.1 ± 0.0	68.5 ± 1.0	350
	C. gloeosporioides	8.0 ± 0.6	23.9 ± 0.6	26.7 ± 1.2	33.3 ± 1.2	52.4 ± 0.6	900
	F. oxysporum	17.8 ± 1.5	21.9 ± 0.6	28.1 ± 0.6	34.1 ± 2.5	44.3 ± 1.7	>1000
	P. parasitica	11.1 ± 1.2	45.2 ± 1.0	65.9 ± 0.3	76.7 ± 1.0	93.3 ± 0.6	130

[a] Mean ± SD.
[b] Concentration (μg/mL).

while melodorinol (**7**) inhibited *A. porri* and *C. gloeosporioides* with IC_{50} of 350 μg/mL and 900 μg/mL, respectively. Regarding structural activity relationships, benzoic acid derivatives such as melodorinone A (**8**), melodorinone B (**4**) and melodorinol (**7**) revealed less antifungal activity than benzoic acid (**5**). Therefore, the core structure of benzoic acid was essential for antifungal activity. As natural plant defenses, benzoic acids are synthesized directly by the shikimate pathway. Moreover, these compounds acted as a precursor to protect plants from biotic and abiotic stress (Wildermuth, 2006). Furthermore, benzoic acid is used as a food preservative and as an antimicrobial in pharmaceutical and cosmetic production and for its antimicrobial activities (Hazan et al., 2004; Borawska et al., 2008). Melodorinol (**7**) has shown cytotoxicity to a human cell line and larvicidal activities (Lu et al., 1997; Kihampa et al., 2009; Hongnak et al., 2015). The current study is the first report of melodorinol (**7**) with regard to plant pathogenic fungi. Therefore, these compounds could be further developed for the control of phytopathogenic fungi in sustainable agricultural management.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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