Mycocidal activity of crude extracts of marine-derived beneficial fungi against plant pathogenic fungi

T. Dethoup, D. Kumla and A. Kijjoa

ABSTRACT

The ethyl acetate (EtOAc) crude extracts of five marine-derived fungi, namely *Emericella nidulans* (KUFA 0104), *Hamigera avellanea* (KUFA 0106), *Neosartorya fischeri* (KUFA 0107), *N. pseudofischeri* (KUFA 0108) and *Talaromyces trachyspermus* (KUFA 0021), were evaluated for their *in vitro* antifungal activity against ten economically important plant pathogenic fungi. The crude extract of *Talaromyces trachyspermus* (KUFA 0021) exhibited the most effective mycelial growth inhibition in most of the plant pathogenic fungi whereas the rest of the crude extracts displayed relevant antifungal properties against the tested plant pathogenic fungi. The EtOAc crude extract of *T. trachyspermus* (KUFA 0021) was found to effectively inhibit the mycelial growth of *Alternaria brassicicola*, *Colletotrichum capsici*, *Helminthosporium maydis*, *Pythium aphanidermatum*, *Rhizoctonia solani* and *Sclerotium rolfsii* with IC_{50} values 100-186 ppm and IC_{90} values 205-807 ppm and displayed total inhibition of mycelial growth on all plant pathogenic fungi at the highest concentration tested (10,000 ppm). Interestingly, this extract was effective on the mycelial growth inhibition of *P. aphanidermatum* even at low concentration with have IC_{50} and IC_{90} values 100 and 205 ppm, respectively. Chemical investigation of the EtOAc crude extract of the culture of *T. trachyspermus* (KUFA 0021) resulted in the isolation of spiculisporic acid E, 3- acetylergosterol 5, 8- endoperoxide,ergosta- 4,6,8 (14), 22- tetraen- 3- one, glaucanic acid and glauconic acid. Spiculisporic acid E, glaucanic acid, glauconic acid and the combination of these compounds were tested for the antifungal activity and it was found that none of them was active against the tested plant pathogens.

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INTRODUCTION

The development of fungicide resistance in plant pathogen populations and the impact of fungicide residue on plant products on human health have aroused an interest of scientists to search for new alternative biofungicides from plants and microorganisms. On the other hand, fungi associated with marine invertebrates have been proved to be a rich source of secondary metabolites, many of which are known to have diverse biological activities, which can have potential for the development of new agrochemicals and medicines. In fact, several marine fungal species, as well as the products of their secondary metabolism, have been reported for their antibacterial and antifungal properties (Bugni and Ireland, 2004; Gomes *et al*., 2014; Li *et al*., 2012; Xiong *et al*., 2013; Xu*et al*., 2015).

Marine sponge-associated fungi can be an alternative source of fungicides to control plant pathogens since they have already been reported for their antagonistic activity against some plant pathogenic fungi (Devi *et al*., 2013; El-Kassas and Khairy, 2009; Gal-Hemed *et al*., 2011; Paz *et al*., 2010; Shen *et al.,* 2014; Wai *et al.,* 2009). For example, the marine fungi *Penicillium* strains DQ 25 and SC10, isolated from marine sponges, were found to inhibit the mycelial growth of

Alternaria solani (Wai *et al.*, 2009). In another study, the sponge-derived isolates of *Trichoderma* could effectively reduce *Rhizoctonia solani*, a causal agent of the damping-off disease, on bean and induced defense responses in cucumber seedling against *Pseudomonas syringae* pv. *Lachrimans* (Gal-Hemed *et al.*, 2011). *Myrothecium* sp. associated with the marine sponge *Axinella* sp. was also found to produce bioactive compounds against *Sclerotinia sclerotiorum* (Xie *et al.*, 2008).

In our ongoing search for bioactive compounds from marine-derived fungi from Thai waters, we have isolated several fungal species that produced novel molecules with biological activities (Eamvijarn *et al.*, 2012, 2013; Gomes *et al.*, 2012, 2014; Prompanya *et al.*, 2014, 2015). Besides the antimicrobial activity against human pathogens, we have also found that several fungal species exhibited antagonistic affect against plant pathogens in our preliminary test, five of which effectively inhibited the mycelial growth of plant pathogenic fungi tested by dual culture. Consequently, we have evaluated the *in vitro* antifungal effect of the EtOAc crude extracts of these five marine-derived fungi against ten plant pathogenic fungi as well as investigating the bioactive compounds from the most active extract.

MATERIALS AND METHODS

Isolation and identification of marine-derived fungi

The samples of the marine sponges were collected, by SCUBA diving at a depth of 10 meters, from coral reefs in different locations in Thailand. The sample tissues were cut into pieces of 0.5 x 0.5 cm and placed on separate Petri-dishes containing half potato dextrose agar (PDA) and malt extract agar. All media contained 70% of seawater and streptomycin sulphate and were incubated at room temperature for 7 days. Hyphal tips were transferred onto PDA slant for further identification.

Taxonomic identification

The identification of selected marine-derived fungi was based on morphological characteristics as observed from the growth pattern, colour and texture. Colony characteristics were examined under a stereoscopic microscope and with the naked eye. Microscopic characteristics were thoroughly investigated by a light microscope of a slide preparation using sterile water and lactophenol as the mounting medium. The study of the ornamentation of the ascospores was conducted using the scanning electron microscopy (SEM: SEOL JSM 6400). The pure cultures were maintained at the Fungal Collection, Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

Preparation of the marine fungal extracts

Five species of the marine-derived fungi were selected including *Emericella nidulans* (KUFA 0101) and *Hamigera avellanea* (KUFA 0106) which were isolated from the marine sponge *Halichondria* sp.; *Neosartorya fischeri* (KUFA 0107) and *Neosartorya pseudofischeri* (KUFA 0108) which were isolated from the marine sponge *Haliclona* sp.; and *Talaromyces trachyspermus* (KUFA 0021) which was isolated from the marine sponge *Clathria reinwardtii*. Each of the selected marine-derived fungi was cultured in 500 mL Erlenmeyer flasks containing PDB 200 mL, and incubated on a rotary shaker at 150 rpm for 7 days. Twenty-five 1,000 mL Erlenmeyer flasks, each containing 300 g of cooked rice were autoclaved at 121°C for 15 minutes and then inoculated with approximately 20 mL of mycelial suspension of each of the selected marine-derived fungi. The inoculated flasks were then incubated at room temperature for 30-days, after which 500 mL of ethyl acetate was added to each flask and macerated for 7 days. Filtration with the filter paper (Whatman No.1) was done and the organic solutions were combined and evaporated under reduced pressure to obtain the EtOAc crude extracts of each fungus.

In vitro antifungal activity test of the EtOAc crude extracts

Dilution plate method was used for the evaluation of the *in vitro* antimycelial growth of plant pathogenic fungi. The final concentrations of 10, 100, 1,000 and 10,000

ppm of each extract were tested for their antifungal activity against ten plant pathogenic fungi, according to Boonsang *et al*. (2014). Each treatment was performed with five replications with complete randomized design.

Isolation of fungal secondary metabolites and their structure elucidation

Chemical study of the EtOAc crude extract of the culture of *T. trachyspermus* (KUFA 0021) was performed according to Kumla *et al*., (2014). The structures of the compounds were established by 1D (^{1}H and ^{13}C) and 2D (COSY, HSQC, HMBC) NMR spectral analysis and HRMS, as well as by comparison of their NMR data with those in the literatures.

Data analyses

Data obtained from the *in vitro* antifungal activity evaluation were subjected to the analysis of variance (ANOVA), IC_{50}/ IC_{90} values, and means were compared by Duncan's multiple range test ($P<0.05$) using the statistical program SPSS version 19 (IBM Corporation, Somers, NY).

RESULTS AND DISCUSSIONS

***In vitro* antimycelial growth activity** The EtOAc crude extracts of *Emericella nidulans* (KUFA 0101), *Hamigera avellanea* (KUFA 0106), *Neosartorya fischeri* (KUFA 0107), *Neosartorya pseudofischeri* (KUFA 0108), and *Talaromyces trachyspermus* (KUFA 0021) were tested for their antifungal activity against ten plant pathogenic fungi, namely *Rhizoctonia solani*, *Sclerotium rolfsii*, *Colletotrichum capsici*, *C. gloeosporioides*, *Lasiodiplodia theobromae*, *Alternaria brassicicola*, *Fusarium oxysporum, Helminthosporium maydis*, *Pythium aphanidermatum* and *Phytophthora palmivora*. The effectiveness of the antifungal activity of the selected marine-derived fungi was assessed based on the percentage of mycelial growth inhibition.

Antifungal activity evaluation of the crude extract of *Emericella nidulans*

Antifungal activity evaluation of *E. nidulans* (KUFA 0101) crude extract against the selected plant pathogenic fungi revealed a relevant antagonistic effect at the highest concentration tested. At the concentration of 10,000 ppm, *E. nidulans* (KUFA 0101) EtOAc crude extract caused a complete inhibition on *Ph. palmivora* and *P. aphanidermatum* mycelial growth (IC_{50} and IC_{90} values 2,921 ppm and 6,706 ppm, respectively) for both plant pathogens, as well as a moderate effect against *R. solani* and *S. rolfsii*, leading to 57.4 % of mycelial growth inhibition with IC_{50} values 5,850-8,681 ppm (Table 1).

Table 1. Growth inhibitory effect of *Emericella nidulans* (KUFA 0101) EtOAc crude extract on the mycelial growth of ten plant pathogenic fungi.

Plant pathogenic fungi	**% mycelial growth inhibition at concentrations (ppm)**				**IC_{50} (ppm)**	**IC_{90} (ppm)**
	10	**100**	**1,000**	**10,000**		
Rhizoctonia solani	0^{m}	0^{m}	0^{m}	57.40^{b}	8,681.55	22,618.72
Sclerotium rolfsii	0^{m}	0^{m}	24.44^{f}	57.40^{b}	5,850.04	73,534.68
Colletotrichum capsici	11.48^{l}	12.59^{k}	24.81^{f}	32.00^{e}	525,645.49	$>10^{10}$
Colletotrichum gloeosporioides	0^{m}	0^{m}	11.11^{l}	32.22^{e}	26,694.15	560,711.17
Lasiodiplodia theobromae	0^{m}	0^{m}	19.63^{h}	31.48^{e}	27,561.73	$>10^{6}$
Alternaria brassicicola	11.85^{kl}	14.07^{j}	21.11^{g}	44.44^{c}	52,539.79	$>10^{8}$
Fusarium oxysporum	0^{m}	0^{m}	0^{m}	17.40^{i}	32,699.85	169,665.68
Helminthosporium maydis	0^{m}	0^{m}	0^{m}	36.66^{d}	15,163.21	69,613.72
Pythium aphanidermatum	0^{m}	0^{m}	0^{m}	100.00^{a}	2,921.69	6,706.22
Phytophthora palmivora	0^{m}	0^{m}	0^{m}	100.00^{a}	2,921.69	6,706.22

Means followed by the same letter do not significantly different at $P<0.05$, when analyzed using Duncan's multiple range test of One-Way ANOVA. IC_{50} and IC_{90} (ppm) are concentrations of the extract inhibiting mycelial growth by 50% and 90%, respectively.

Antifungal activity evaluation of the crude extract of *Hamigera avellanea*

At the highest concentration tested (10,000 ppm), *H. avellanea* (KUFA 0106) crude extract displayed relevant antifungal activity against most of the tested plant pathogenic fungi, causing a complete mycelial growth inhibition of *R. solani*, *S. rolfsii*, *C.*

gloeosporioides, *L. theobromae* and *P. aphanidermatum*, with IC_{50} values 371-2,921 ppm as well as a moderate effect against the remaining plant pathogenic fungi, causing more than 50% of mycelial growth inhibition and IC_{50} values ranging from 1,331-4,490 ppm. Additionally, at the concentration of 1,000 ppm, *H. avellanea* (KUFA 0106) crude extract displayed also a moderate inhibitory effect on the mycelial growth of both Agonomycetes *R. Solani* and *S. rolfsii*, causing 66.66% and 50.74% of mycelial growth inhibition respectively (Table 2).

Table 2. Growth inhibitory effect of *Hamigera avellanea* (KUFA 0106) EtOAc crude extract on the mycelial growth of ten plant pathogenic fungi.

Plant pathogenic fungi	% mycelial growth inhibition at concentrations (ppm)				IC_{50} (ppm)	IC_{90} (ppm)
	10	100	1,000	10,000		
Rhizoctonia solani	0^{p}	25.92^{j}	66.66^{c}	100.00^{a}	371.17	2766.03
Sclerotium rolfsii	0^{p}	29.63^{i}	50.74^{f}	100.00^{a}	481.09	4,867.83
Colletotrichum capsici	16.66^{n}	28.14^{i}	39.25^{h}	72.59^{b}	1,331.31	451,301.51
Colletotrichum gloeosporioides	22.22^{l}	28.14^{i}	30.04^{i}	100.00^{a}	489.90	34,951.35
Lasiodiplodia theobromae	0^{p}	0^{p}	0^{p}	100.00^{a}	2,921.69	6,706.22
Alternaria brassicicola	19.25^{m}	25.55^{jk}	43.33^{g}	66.66^{c}	1,663.81	$>10^{6}$
Fusarium oxysporum	0^{p}	14.07^{o}	38.88^{h}	66.66^{c}	2,562.25	80,164.88
Helminthosporium maydis	0^{p}	16.66^{m}	23.70^{kl}	62.96^{d}	4,427.61	194,916.82
Pythium aphanidermatum	0^{p}	0^{p}	0^{p}	100.00^{a}	2,921.69	6,706.22
Phytophthora palmivora	0^{p}	0^{p}	40.47^{h}	55.55^{e}	4,490.80	85,727.71

Means followed by the same letter do not significantly different at $P<0.05$, when analyzed using Duncan's multiple range test of One-Way ANOVA. IC_{50} and IC_{90} (ppm) are concentrations of the extract inhibiting mycelial growth by 50% and 90%, respectively.

Antifungal activity evaluation of the crude extract of *Neosartorya fischeri*

Antifungal activity screening revealed that at the highest concentration tested, *N. fischeri* (KUFA 0107) EtOAc crude extract caused complete mycelial growth inhibition in all plant pathogens, except for *L. theobromae* with IC_{50} values of 484-2,921 ppm and IC_{90} values 2,205-6,817 ppm. Additionally, at the concentration of 1,000 ppm, *N. fischeri* (KUFA 0107) crude extract displayed a moderate antifungal activity against *S. rolfsii* and *P. aphanidermatum*, leading to 62.96% and 51.11% of mycelial growth inhibition respectively (Table 3).

Table 3. Growth inhibitory effect of *Neosartorya fischeri* (KUFA 0107) EtOAc crude extract on the mycelial growth of ten plant pathogenic fungi.

Plant pathogenic fungi	% mycelial growth inhibition at concentrations (ppm)				IC_{50} (ppm)	IC_{90} (ppm)
	10	100	1,000	10,000		
Rhizoctonia solani	0^{j}	15.55^{h}	41.48^{d}	100.00^{a}	793.94	5,823.98
Sclerotium rolfsii	0^{j}	17.40^{g}	62.96^{b}	100.00^{a}	484.99	3,097.61
Colletotrichum capsici	0^{j}	0^{j}	18.51^{g}	100.00^{a}	1,832.96	4,144.03
Colletotrichum gloeosporioides	0^{j}	0^{j}	17.40^{g}	100.00^{a}	1,881.43	4,248.92
Lasiodiplodia theobromae	0^{j}	0^{j}	0^{j}	0^{j}	0.00	0.00
Alternaria brassicicola	0^{j}	11.11^{i}	34.81^{e}	100.00^{a}	947.69	5,922.18
Fusarium oxysporum	0^{j}	11.85^{i}	32.59^{f}	100.00^{a}	1,038.60	6,817.45
Helminthosporium maydis	0^{j}	0^{j}	0^{j}	100.00^{a}	2,921.69	6,706.22
Pythium aphanidermatum	0^{j}	0^{j}	51.11^{c}	100.00^{a}	984.00	2,205.87
Phytophthora palmivora	0^{j}	0^{j}	0^{j}	100.00^{a}	2,921.69	6,706.22

Means followed by the same letter do not significantly different at $P<0.05$, when analyzed using Duncan's multiple range testof One-Way ANOVA. IC_{50} and IC_{90} (ppm) are concentrations of the extract inhibiting mycelial growth by 50% and 90%, respectively.

Antifungal activity evaluation of the crude extract of *Neosartorya pseudofischeri*

The results from the antagonistic effect evaluation of *N. pseudofischeri* (KUFA 0108) EtOAc crude extract against plant pathogenic fungi revealed a moderate to strong effect against all the plant pathogenic fungi at the highest concentration tested (10,000 ppm). This extract displayed a strong antifungal activity against *Colletotrichum* spp., *L.*

theobromae and *H. maydis*, leading to more than 70% of mycelial growth inhibition and with IC_{50} values 1,156-1,776 ppm, as well as a complete mycelial growth inhibition of *P. aphanidermatum* (Table 4). For the remaining plant pathogens, *N. pseudofischeri* (KUFA 0108) EtOAc crude extract revealed a moderate antifungal activity, causing more than 50% of mycelial growth inhibition with IC_{50} values 2,921-9,281 ppm.

Table 4. Growth inhibitory effect of *Neosartorya pseudofischeri* (KUFA 0108) EtOAc crude extract on the mycelial growth of ten plant pathogenic fungi.

Plant pathogenic fungi	% mycelial growth inhibition at concentrations (ppm)				IC_{50} (ppm)	IC_{90} (ppm)
	10	100	1,000	10,000		
Rhizoctonia solani	0[s]	0[s]	47.03[k]	61.11[h]	3,163.19	52,464.81
Sclerotium rolfsii	0[s]	0[s]	0[s]	64.44[g]	7,603.17	19,407.29
Colletotrichum capsici	0[s]	22.22[p]	37.22[n]	73.70[f]	1,776.38	59,295.87
Colletotrichum gloeosporioides	0[s]	9.63[r]	23.33[p]	92.59[b]	1,666.87	13,463.83
Lasiodiplodia theobromae	0[s]	12.59[q]	43.70[l]	87.22[c]	1,185.96	14,071.75
Alternaria brassicicola	38.14[n]	40.37[m]	49.44[j]	77.77[e]	238.33	>10^6
Fusarium oxysporum	0[s]	0[s]	11.85[q]	50.74[i]	9,281.37	88,764.63
Helminthosporium maydis	0[s]	25.00[o]	42.77[l]	80.37[d]	1,156.95	30,149.68
Pythium aphanidermatum	0[s]	0[s]	0[s]	100.00[a]	2,921.69	6,706.22
Phytophthora palmivora	0[s]	0[s]	0[s]	77.03[e]	5,873.37	14,572.80

Means followed by the same letter do not significantly different at $P<0.05$, when analyzed using Duncan's multiple range test of One-Way ANOVA. IC_{50} and IC_{90} (ppm) are concentrations of the extract inhibiting mycelial growth by 50% and 90%, respectively.

Antifungal activity evaluation of the crude extracts of *Talaromyces trachyspermus*

Antifungal activity screening of *T. trachyspermus* (KUFA 0021) EtOAc crude extract revealed a complete mycelial growth inhibition in all plant pathogenic fungi, at the concentration of 10,000 ppm. Inhibition on the mycelial growth remained effective even at the concentration of 1,000 ppm, causing a complete mycelial growth inhibition of *R. solani*, *S. rolfsii*, *C. capsici*, *A. brassicicola*, *H. maydis* and *P. aphanidermatum*, with IC_{50} values 100-186 ppm and IC_{90} values 205-807 ppm. Also, at the concentration of 1,000 ppm, *T. trachyspermus* (KUFA 0021) EtOAc crude extract displayed a moderate to strong antifungal activity against *C. gloeosporioides*, *L. theobromae* and *F. oxysporum*, with mycelial growth inhibition values ranging from 68.51 to 84.07% and have IC_{50} values 329-650 ppm and IC_{90} values 1,138-2,560 ppm (Table 5).

Table 5. Growth inhibitory effect of *Talaromyces trachyspermus* (KUFA 0021) EtOAc crude extract on the mycelial growth of ten plant pathogenic fungi.

Plant pathogenic fungi	% mycelial growth inhibition at concentrations (ppm)				IC_{50} (ppm)	IC_{90} (ppm)
	10	100	1,000	10,000		
Rhizoctonia solani	0[n]	30.00[h]	100.00[a]	100.00[a]	133.04	267.47
Sclerotium rolfsii	0[n]	45.92[f]	100.00[a]	100.00[a]	106.05	216.37
Colletotrichum capsici	0[n]	13.33[l]	100.00[a]	100.00[a]	186.43	371.70
Colletotrichum gloeosporioides	0[n]	0[n]	75.92[c]	100.00[a]	650.18	1,380.78
Lasiodiplodia theobromae	0[n]	0[n]	84.07[b]	100.00[a]	546.92	1,138.15
Alternaria brassicicola	15.53[k]	27.77[i]	100.00[a]	100.00[a]	104.16	807.94
Fusarium oxysporum	0[n]	29.63[h]	68.51[d]	100.00[a]	329.17	2,560.95
Helminthosporium maydis	6.66[m]	21.48[j]	100.00[a]	100.00[a]	147.45	723.65
Pythium aphanidermatum	0[n]	50.00[e]	100.00[a]	100.00[a]	100.22	205.09
Phytophthora palmivora	0[n]	0[n]	31.66[g]	100.00[a]	1,377.07	3,106.34

Means followed by the same letter do not significantly different at $P<0.05$, when analyzed using Duncan's multiple range test of One-Way ANOVA. IC_{50} and IC_{90} (ppm) are concentrations of the extract inhibiting mycelial growth by 50% and 90%, respectively.

Analysis of the results demonstrated clearly that the EtOAc crude extract of *T. trachyspermus* (KUFA 0021) was the most active extract, leading to a complete mycelial growth inhibition of all the plant pathogenic

fungi at highest concentration tested and has IC_{50} values 100-1,377 ppm and IC_{90} values 205-3,106 ppm. Additionally, this extract also displayed a moderate to strong antifungal activity, at the concentration of 1,000 ppm, against the majority of the selected plant pathogens. Since the EtOAc crude extract of *T. trachyspermus* (KUFA 0021) was found to be the most active, it was further investigated for its chemical constituents.

***Talaromyces trachyspermus*- secondary metabolites**

The EtOAc crude extract of the culture of this fungus furnished, besides glaucanic (**1a**), glauconic acids (**1b**), a new spiculisporic acid derivative which we have named spiculisporic acid E (**4**), a new natural product 3-acetylergosterol 5, 8-endoperoxide (**3**) as well as ergosta-4, 6, 8 (14), 22-tetraen-3-one (**2**) (Fig 1) (Kumla *et al.*, 2014). Since spiculisporic acid E, glaucanic acid and glauconic acid were isolated in sufficient quantities, they were evaluated, in pure form and in combination with each other, for their *in vitro* antifungal activity against ten plant pathogenic fungi by the dilution plate method. However, none of them was found to be active at the highest concentration tested.

As previously referred to, at the concentration of 10,000 ppm, *E. nidulans* EtOAc crude extract displayed a strong antifungal activity against some plant pathogenic fungi. This is not surprising since Sibounnavong *et al.*, (2009) have already reported the antifungal

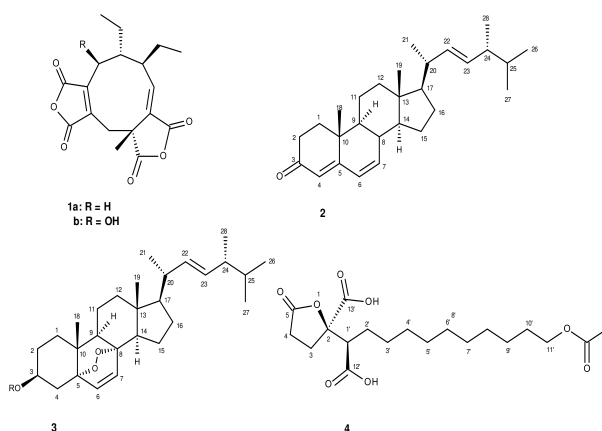

Fig 1. Secondary metabolites isolated from the culture of the marine-derived *Talaromyces trachyspermus* KUFA 0021 (Kumla *et al.*, 2014).

activity of *E. nidulans* crude extract against *Fusarium* wilt pathogen. Additionally, some secondary metabolites with antifungal properties have also been reported from *E. nidulans*, which may partially explain the activity of its crude extract against plant pathogenic fungi. Moreover, the hexane and EtOAc crude extracts of *E. nidulans* isolate EN01 were reported to yield six compounds including epishamixanthone, shamixanthone, emericellin, ergosta-6, 22-diene-3-ol-5, 8-epidioxy- (3β-5α, 22E), sterigmatocystin and demethyl sterigmatocystin, some of them displaying antifungal activity against plant pathogenic fungi (Moosophoon *et al.*, 2006).

On the other hand, the chemical investigation of *H. avellanea* crude extract resulted in the isolation of (*Z,Z*)-*N,N′*-[1-[(4-hydroxyphenyl)-methylene]-2- [(4-methoxyphenyl) -methylene] -1,2 -ethanediyl] -*bis*-formamide, which exhibited marginal activity against a variety of pathogenic fungi (*Pyricularia oryzae* and *Venturia inaequalis*) and bacteria (Breinholt *et al.*, 1996; Adbel Rahim, 2011).

The EtOAc crude extract of *N. fischeri* was found to exhibit stronger growth inhibitory activity than that of *N. pseudofischeri* (KUFA 0108) against the mycelial growth of phytopathogenic fungi, causing a complete mycelial growth inhibition of all tested plant pathogenic fungi except for *L. theobromae* at the highest concentration tested, as well as strong to moderate antifungal effect against the majority of the plant pathogenic fungi, at the concentration of 1,000 ppm. Interestingly, Eamvijarn *et al.* (2012, 2013) have reported the secondary metabolites from the cultures of *N. fischeri* strain KUFC 6344 and *N. pseudofischeri* strain KUFC 6422, collected from soil samples: however, their antifungal activity was not evaluated.

The results obtained demonstrated that the EtOAc crude extract of *T. trachyspermus* (KUFA 0021) was the most active, causing a complete mycelial growth inhibition of all the plant pathogenic fungi at 10,000 ppm. Additionally, this extract also displayed moderate to strong antifungal activity, at the concentration of 1,000 ppm, against the

majority of the selected plant pathogens. Although, Sreeta *et al.* (2015) have recently reported the antifungal activity of the crude extracts of the plant endophytic strain of *T. trachyspermus* against *R. solani,* its effect was moderate when compared to that of our marine-derived strain KUFA 0021, which caused 50% of mycelial growth inhibition.

It is interesting to note that despite the reports on the isolation of secondary metabolites from *T. trachyspermus* such as trachyspic acid, decylcitric acid and spiculisporic acid, so far there are no reports on the antifungal activity of metabolites isolated from the *Talaromyces* genus. Therefore, taking into account that the EtOAc crude extract of marine-derived *T. trachyspermus* was the most active against the plant pathogenic fungi of the economically important crops, it is important to investigate the secondary metabolites responsible for this activity as well as their mechanism of action and the synergistic effect of these secondary metabolites. Further studies on the isolation of the secondary metabolites of the active crude extracts of other marine-derived fungi as well as the evaluation of their effect on the mycelial growth of these plant pathogenic fungi are also needed and will be performed in the future.

Due to their relevant antagonistic activity, the five fungal species were selected to evaluate for the antifungal activity of their EtOAc crude extracts. Despite a strong antifungal activity of *N. fischeri* and *H. avellanea* EtOAc crude extracts, causing the inhibition of mycelial growth of some plant pathogenic fungi, even at lower concentration (1,000 ppm), *T. trachyspermus* (KUFA 0021) EtOAc crude extract was identified as the most active extract. *T. trachyspermus* EtOAc crude extract caused a complete mycelial growth inhibition in all the tested plant pathogenic fungi at the highest concentration tested (10,000 ppm), with IC_{50} values 100-1,377 ppm and IC_{90} values 205-3,106 ppm. The antifungal activity remained at a lower concentration (1,000 ppm), leading to the complete growth inhibition in half of the pathogens isolates. Even at 100 ppm, *T. trachyspermus* crude extract displayed a moderate effect against *P. aphanidermatum.*

Due to a relevant antifungal activity of the EtOAc crude extract *T. trachyspermus*, its chemical constituents were investigated. Isolates were spiculisporic acid E, 3-acetylergosterol 5, 8-endoperoxide, glaucanic acid, glauconic acid and ergosta-4, 6, 8 (14), 22-tetraen-3-one. Spiculisporic acid E, glaucanic acid, glauconic acid, in pure form and in combination with each other, were also evaluated for their antifungal activity against ten plant pathogenic fungi, and neither of the compounds was active. Despite the lack of activity of the isolated compounds, *T. trachyspermus* crude extract was identified as a potential source of metabolites with antifungal activity against plant pathogenic fungi, as demonstrated by the results in this study.

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T. Dethoup[a]*, D. Kumla[a,b] and A. Kijjoa[b, c]

[a]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

[b]*ICBAS*-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal

[c]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

*Corresponding author

E-mail: agrtdd@ku.ac.th.

URGENT INFORMATION TO AUTHORS

All the authors and co- authors are hereby informed that all the research papers received by us up to 30th November 2015 have been considered by the experts. The papers found suitable for publication in "**Journal of Biopesticides**" have been published. No paper is left pending with us for year 2015. The papers received November 2015 before and are not published are found unsuitable as per the theme and the review reports of the Journal. Therefore those authors, whose papers have not been published till the 2015 issue, can send their papers to other suitable journals. We do not entertain any correspondence regarding rejection of the papers or return of original manuscript. All manuscripts submitted to us whether approved or rejected, are the property of the Journal.

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