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The Antifungal Activity of *Glycosmis calcicola* and *G. rupestris* Extracts

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Abstract

The antifungal activity of *Glycosmis calcicola* B.C. Stone and *G. rupestris* extracts was evaluated using poison food and spore germination techniques. The $CHCl_3$ extract of *G. calcicola* has been shown to be most effective in inhibiting mycelial growth, sporulation, and spore germination on three fungal pathogens of chili. The antifungal compounds were identified as flindersine and desmethoxyanthophylline and their structures were determined by spectroscopic methods and comparison with reported data.

Keywords: Antifungal, chili pathogens, desmethoxyanthophylline, flindersine, *Glycosmis calcicola*, *G. rupestris*.

Introduction

Glycosmis is a genus of 40 species of trees and shrubs of the family Rutaceae. Members of the genus are aromatic and traditionally used for the treatment of fever, swollen spleen, and as a stimulant to digestion (Burkill, 1966). In continuation of our work on Rutaceae (Rahmani et al., 1998, 2003, 2004), we wish to report the antifungal activity *of G. calcicola* B.C. Stone and *G. rupestris* Ridley extracts collected from two locations in Peninsula Malaysia. The $CHCl_3$ and MeOH extracts of these plants were tested for antifungal activity against *Chaonephora cucurbitarium* (Berk & Rav.) Thaxter, *Colletotrichum capsici* (Syd.) Butler and Bisby, and *Colletotrichum gloeocosporioides* (Penz.) Sacc. (Sariah, 1994). These pathogens are common causal agents of chili diseases that affect the production and quality of fresh chilies. The compounds responsible for giving this activity were monitored on thin-layer chromatography (TLC) plates by spraying the plates with spore suspension of an indicator fungus prepared from *Aspergillus flavus* (Link ex Rf.) and *A. niger* van Teghem. The chloroform extract of *G. calcicola* collected from Langkawi Island was the most active, and the major contributors to this antifungal activity were due to the presence of pyranoquinolone alkaloids, flindersine (**1**) and desmethoxyanthophylline (**2**) (Fig. 1).

Materials and Methods

General experimental procedures

All melting points were measured on a Kofler hot-stage apparatus and are uncorrected. The IR spectra were recorded using KBr disks on Perkin Elmer Fourier-transform infrared (FTIR) spectrophotometer (model 1275X). The UV spectra were recorded on a Shimadzu UV 2100 spectrophotometer in MeOH. The ^1H NMR and ^{13}C NMR spectra were obtained on a Jeol JNM CRX 400 FT NMR spectrometer equipped with 5-mm ^1H and ^{13}C probes operating at 400 MHz and 100 MHz, respectively. Chemical shifts are shown in δ values (ppm) with tetramethylsilane as an internal reference. Mass spectra were recorded on a Jeol JMS-AX 505 mass spectrometer, and ionization was induced by electron impact at 70 eV.

Plant materials

The two samples of *G. calcicola* were collected from two separate limestone areas in Langkawi Island and Templer Park, Malaysia. The bark and leaf samples of *G. rupestris*

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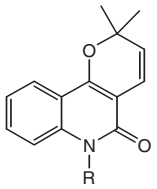


(1); R = H, Flindersine

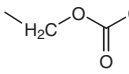
(2); R =  Desmethoxyanthophylline

Figure 1. Structures of flindersine (**1**) and desmethoxyanthophylline (**2**).

were collected from Langkawi Island. Voucher specimens of the plant materials were deposited at the Herbarium, Department of Biology, Universiti Putra Malaysia.

Extraction and isolation

The ground, air-dried plant materials were extracted successively with $CHCI_3$ and MeOH to give dark, viscous solids. Part of the $CHCl_3$ extract (20 g) of *G. calcicola* (Templer Park) was fractionated on a column of silica gel eluted with hexane and increasing the amount of $CHCl_3$ to give 34 fractions of 250 ml each. Fractions 9–15 were combined and rechromatographed over silica gel column to furnish flindersine (**1**) (24 mg) as colorless needles, melting point (m.p.) 186–188 °C; UV λ_{max} nm (log ε): 239 (0.53), 253 (4.49), 321 (0.80), 332 (0.62), 348 (1.04); IR υ_{max} cm^{-1} (KBr): 3162, 3066, 1664, 1620, 1598, 1500, 1480, 1426, 1408, 1356, 1274, 1190, 866, 746; ^{1}H NMR δ (400 MHz, $CDCl_3$): 11.75 (br s, 1 H, N-H), 7.89 (dt, 2 Hz, 6 Hz, 1 H, H-8), 7.48 (dt, 2 Hz, 6 Hz, 1 H, H-7), 7.36 (d, 8 Hz, 1 H, H-5), 7.19 (m, 8 Hz, 1 H, H-6), 6.78 (d, 10 Hz, 1 H, H-0), 5.56 (d, 10 Hz, 1 H, H-11), 1.54 (s, 6 H, Me × 2); ^{13}C NMR ppm (100 MHz, $CDCl_3$): 162.6 (C-1), 157.2 (C-3), 137.9 (C-9), 130.8 (C-7), 126.2 (C-10), 122.5 (C-6), 122.1 (C-5), 117.2 (C-8), 115.9 (C-11), 115.3 (C-2), 105.7 (C-4), 79.0 (C-12), 28.3 (Me × 2); MS *m/e* (%): 227 (44), 212 (100), 198 (5), 183 (6), 166 (9), 154 (7), 146 (6), 120 (8), 106 (32), 99 (8). Further fractionation of the combined fractions 16–29 by column chromatography gave desmethoxyanthophylline (**2**) (15 mg) as white prisms, m.p. 104–105 °C; UV λ_{max} nm (log ε): 239 (0.33), 253 (5.00), 322 (1.20), 338 (1.58), 349 (1.84); IR υ_{max} cm-1 (KBr): 2970, 1738, 1664, 1500, 1464, 1370, 1356, 1222, 1148, 1018, 952, 850, 747; ^{1}H NMR δ (400 MHz, $CDCl_3$): 7.98 (dt, 1 Hz, 7 Hz, 1 H, H-8), 7.55 (dt, 1 Hz, 7 Hz, 1 H, H-7), 7.33 (d, 8 Hz, 1 H, H-5), 7.27 (dt, 1 Hz, 8 Hz, 1 H, H-6), 6.73 (d, 11 Hz, 1 H, H-10), 6.34 (s, 2 H, H-1′ × 2), 5.55 (d, 11 Hz, 1 H, H-11), 2.10 (s, 3 H, Me-3′), 1.53 (s, 6 H, Me × 2); MS *m/e* (%): 299 (21), 284 (61), 240 (18), 227 (6), 212 (100), 196 (8), 183 (9), 154 (7), 146 (6), 132 (44), 120 (25), 113 (11), 107 (8), 98 (9), 77 (39).

Antifungal study

Petri dishes containing the amended potato dextrose agar media were inoculated centrally with 5-mm disks of culture test fungus and incubated at room temperature. The fungus growth was recorded, and percentage of mycelial growth inhibition was compared with control. Spore concentration was determined by using a Neubauer hemocytometer (Hirscoman EMTech Colour, USA), and percentage of sporulation was calculated based on control. For the inhibition of spore germination, the spore suspension was spread over amended agar and after 24 h of incubation at room temperature, the number of spores germinated were counted and compared with control. For the TLC bioassays, extract B and the two pure compounds were spotted on commercial TLC plates (silica gel $60F_{254}$, 0.50 mm × 20 cm × 20 cm), developed twice with $CHCl_3$ and allowed to dry overnight. After locating the UV absorbing spots, the chromatograms were sprayed separately with spore suspension of the indicator fungus, *A. flavus* or *A. niger*, in Homans and Fuchs' solution and incubated at room temperature for 24–48 h (Homans & Fuchs, 1970). Inhibition areas appeared white against a background of green or black spores of *A. flavus* or *A. niger*, respectively.

Results and Discussion

The three plant samples were collected from different locations. Two of the samples were the same species (*G. calcicola*): one was collected from Langkawi Island and the other from Templer Park. The third sample (*G. rupestris*) was collected from Langkawi Island and divided into two parts, the leaves and the bark. Each of these plant samples were extracted separately and successively with $CHCl_3$ followed by MeOH to give eight extracts as dark viscous solids.

Different extracts of *G. calcicola* and *G. rupestris* have a significant effect on the growth of the fungal pathogens of chili. All the $CHCl_3$ and MeOH extracts of *G. calcicola* showed higher activity on mycelial growth compared to *G. rupestris* extracts. However, the $CHCl_3$ extract (extract B) showed maximum inhibitory effect on mycelial growth of the three test fungi and also the most effective in inhibiting more than 80% the sporulation of *Colletotrichum capsici* and *Colletotrichum gloeocosporioides* (Table 1). No sporulation was observed when the extracts were tested against *Chaonephora cucurbitarium*. With regard to the spore germination assay, again, extract B was the most effective in inhibiting more than 64% of the spore germination of the three test fungi. Thus, further isolation and bioassays were performed on this extract to determine the constituents responsible for giving the strong and effective activity.

Flindersine (**1**) and desmethoxyanthophylline (**2**) were isolated from $CHCl_3$ extract (extract B) of *G. calcicola* by silica gel column chromatography. Compound **1** was obtained as colorless needles, m.p. 186–188 °C, and the MS gave molecular ion peak at *m/e* 227 consistent with molecular formula

Table 1. Effect of *G. calcicola* and *G. rupestris* extracts on spore germination and mycelial growth of chili pathogens.

	% Inhibition[b]								
	Chaonephora cucurbitarium			*Colletotrichum capsici*			*Colletotrichum gloeocosporioides*		
Extracts no.[a]	Spore germination	Mycelial growth	Sporulation	Spore germination	Mycelial growth	Sporulation	Spore germination	Mycelial growth	Sporulation
A	35.41	20.83	0	26.69	22.37	62.45	40.68	33.48	84.15
B	68.43	40.14	0	74.17	39.49	88.56	64.34	40.09	97.85
C	20.80	34.85	0	74.41	12.09	22.04	57.63	12.07	38.80
D	35.47	38.71	0	60.90	14.64	45.24	55.52	22.90	62.48
E	59.78	0	0	39.26	17.49	78.20	48.13	7.06	0
F	27.17	0	0	58.68	6.87	67.77	60.02	0	0
G	25.86	23.91	0	48.98	32.03	97.60	43.95	13.95	0
H	31.52	0	0	28.51	4.61	37.82	30.67	11.47	0

[a] A and C: $CHCI_3$ and MeOH extracts of *G. calcicola* from Langkawi, respectively. B and D: $CHCI_3$ and MeOH extracts of *G. calcicola* from Templer Park, respectively. E and G: $CHCI_3$ and MeOH extracts of *G. rupestris*, respectively (leaves). F and H: $CHCI_3$ and MeOH extracts of *G. rupestris*, respectively (bark).
[b] Each value represents the average of four replicates.

$C_{14}H_{13}NO_2$ with nine degrees of unsaturation. The UV spectrum showed absorptions at 239, 253, 321, 332, and 348 nm, while the IR spectrum exhibited absorptions due to amide carbonyl (1664 cm^{-1}), aromatic residue (1620, 1598, 1500 cm^{-1}), and a 1,2-disubstituted benzene ring at 746 cm^{-1}. These spectral behaviors are characteristic of a 2-quinolone skeleton (Bhattacharyya & Chowdhury, 1985). The integration of the ^1H NMR spectrum confirms the presence of 13 protons with the appearance of AB doublets at δ 5.56 and 6.78 (*J* 10 Hz) together with a 6-protons singlet at δ 1.54, which indicates the presence of a dimethylpyran ring system (Furukawa et al., 1986). The low-field broad resonance at δ 11.75 was attributed to the N-H group. The ^{13}C NMR spectrum confirmed the presence of 13 carbon atoms, and the occurrence of a low field signal at 162.6 ppm further support the existence of amide carbonyl. This compound was preciously isolated from *Haplophylum suereolens* (DC) G. Don (Ulubelen, 1984).

Desmethoxyanthophylline (**2**) was obtained as white prisms with a m.p. 104–105 °C. The UV and IR spectra gave similar absorptions as in **1** but with an extra strong carbonyl absorption at 1738 cm^{-1}. The ^1H NMR of **2** is similar to **1** with the addition of a 3-protons singlet at δ 2.10 attributed to the acetyl group and a downfield resonance for a methylene proton at δ 6.34 and the disappearance of the low field signal at δ 11.75. The MS spectrum gave the molecular ion peak at *m/e* 299, which established the molecular formula as $C_{17}H_{17}NO_4$. Fragment ion at *m/e* 227 indicates the flindersine part of the molecule ($[M\text{-}C_3H_5O_2]^+$), and the base peak at *m/e* 212 ($[M\text{-}C_3H_5O_2\text{-}CH_3]^+$) is consistent with the flindersine skeleton. These spectral data provide evidence to suggest that **2** is a new derivative of **1** with the replacement of the amidic proton by a–CH_2-$OCOCH_3$ group and identified as desmethoxyanthophylline (**2**).

The $CHCl_3$ extract and the two compounds were spotted on TLC plates, developed in $CHC1_3$, sprayed with spore suspensions *of A. flavus* and *A. niger* in Homans and Fuchs' nutrient solution, and incubated for 24–48 h. The extract B produced three inhibition zones with R_f values of 0.14, 0.32, and 0.53 in which the most polar spot demonstrated the greatest activity. The inhibition zones with R_f of 0.14 and 0.53 corresponded to flindersine (**1**) and desmethoxyanthophylline (**2**), respectively. The third antifungal constituent with R_f 0.32 has yet to be isolated and identified, but we believe it to possess the same basic pyranoquinolone alkaloid skeleton.

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References

Bhattacharyya P, Chowdhury BK (1985): Glycone: a quinolone alkaloid from *Glycosmis pentaphylla. Phytochemistry 24*: 634.

Burkill IH (1935): A dictionary of the economic products of the Malay Peninsula. In: *Crown Agents for the Colonies*. London. Ministry of Agriculture and Cooperatives, Kuala Lumpur (reprinted 1966).

Furukawa H, Ito C, Yogo M, Wu TS (1986): Structures of murrayastine, murrayaline and pyrayafoline: Three new

carbazole alkaloids from *Murraya euchrestifolis*. *Chem Pharm Bull 34*: 2672–2675.

Homans AL, Fuchs A (1970): Direct bioautography on thin-layer chromatography as a method for detecting fungitoxic substances. *J Chromatogr 51*: 327–329.

Rahmani M, Chew YL, Sukari MA, Ismail HBM, Meon S, Aimi N (1998): 7-Methoxyglycomaurin: A new carbazole alkaloid from *Glycosmis rupestris*. *Planta Med 64*: 780.

Rahmani M, Kwan WL, Ismail HBM, Taufiq-Yap YH, Sukari MA, Ali AM, Kulip J (2004): A new flavonoid and sulphur containing amides from *Glycosmis chlorosperma*. *Nat Prod Res 18*: 85–88.

Rahmani M, Susidarti RA, Ismail HBM, Sukari MA, Taufiq-Yap YH, Ee GCL, Ali AM, Kulip J, Waterman PG (2003): Coumarins from Malaysian *Micromelum minutum. Phytochemistry 64*: 873–877.

Sariah M (1994): Incidence of *Colletotrichum* spp. on chilli in Malaysia and pathogenicity of *C. gloeosporiodes*. In: *Crop and Pathogen Biology and Control*. SEAMO BIOTROP Publ., Indonesia 54, p. 103.

Ulubelen A (1984): Alkaloids from *Haplophyllum suareolens, Phytochemistry 9*: 2123–2124.