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Antifungal compounds from turmeric and nutmeg with activity against plant pathogens

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ABSTRACT

The antifungal activity of twenty-two common spices was evaluated against plant pathogens using direct-bioautography coupled *Colletotrichum* bioassays. Turmeric, nutmeg, ginger, clove, oregano, cinnamon, anise, fennel, basil, black cumin, and black pepper showed antifungal activity against the plant pathogens *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides*. Among the active extracts, turmeric and nutmeg were the most active and were chosen for further investigation. The bioassay-guided fractionation led to the isolation of three compounds from turmeric (**1**–**3**) and three compounds from nutmeg (**4**–**6**). Their chemical structures were elucidated by spectroscopic analysis including HR-MS, 1D, and 2D NMR as curcumin (**1**), demethoxycurcumin (**2**) and bisdemethoxy-curcumin (**3**), *erythro*-(7R,8R)-$\Delta^{8'}$-4,7-dihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan (**4**), *erythro*-(7R,8R)-Δ8′-7-acetoxy-3,4,3′,5′-tetra-methoxy-8-*O*-4′-neolignan (**5**), and 5-hydroxy-eugenol (**6**). The isolated compounds were subsequently evaluated using a 96-well microbioassay against plant pathogens. At 30 μM, compounds **2** and **3** possessed the most antifungal activity against *Phomopsis obscurans* and *Phomopsis viticola*, respectively.

1. Introduction

Spices have been used since ancient times not only as flavoring agents, but also as folk medicines and food preservatives [1]. In addition, some spices are used to prolong the storage life of foods by preventing rancidity through their antioxidant activity or through antifungal or bactericidal activity [2]. Spices are generally recognized as safe (GRAS) due to their daily use over centuries in our food supply without any reports of deleterious effects [3].

Numerous studies have been published on the antifungal activity of plant extracts against different types of plant fungi. The methanol extracts of some commonly used Lamiaceae species were tested against four mycotoxigenic fungal species, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus*, and *Fusarium proliferatum*. Of which, *Origanum vulgare*, *Origanum minutiflorum*, and *Tilia spicata* extracts showed significant antifungal activity [4].

The in vitro antifungal activity of water extracts of seven spices from cardamom, chili, coriander, onion, garlic, ginger and galangal was evaluated against three Roselle (*Hibiscus sabdariffa*) pathogens, *Phoma exigua*, *Fusarium nygami* and *Rhizoctonia solani*. All the extracts at three concentrations (10, 20 and 30%) inhibited fungal mycelium growth with varying degrees of effectiveness as compared to the control. Garlic extract exhibited the most growth inhibition against *P. exigua*, *F. nygami* and *R. solani* especially at 20 and 30% concentrations [5].

Both the volatile oil and curcumin of *Curcuma longa* exhibited a significant inhibitory effect of aflatoxin AFB_1 and AFB_2 production by *A. flavus* [6]. Wilson et al. studied the antifungal activity against *Botrytis cinerea* for extracts from 345 plants and 49 essential oils, they found that allium and capsicum extracts as

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well as the essential oils of *Cymbopogon martini*, *Thymus zygis*, *Cinnamomum zeylanicum* and *Eugenia caryophyllata* exhibited the most antifungal activity [7]. An investigation on the antifungal effects of rosemary, cumin, sater (savory), basil and pickling herb hydrosols against *R. solani*, *Fusarium oxysporum*, *B. cinerea* and *Alternaria citri* was carried out and the result showed that the hydrosols of sater and pickling herb showed the most relevant fungicidal activity [8].

The antifungal effects of essential oils derived from twenty spices were investigated against *A. niger*, *Candida albicans*, *Candida blanki*, *Candida cylindracea*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, and *Saccharomyces cerevisiae* using the disc diffusion method [9]. Essential oil of cassia, allspice, clove, cumin, coriander, thyme, basil, anise, curry leaf, and asafetida inhibited all tested fungi, while oils from ginger, turmeric, and pomegranate were ineffective [9].

Strawberry anthracnose can be devastating since several plant parts may be infected in addition to the fruit. This fungal disease causes millions of dollars in crop loss each year [10]. *Phomopsis obscurans* causes a disease known as leaf blight of the cultivated strawberry and can infect foliage, runners, petioles, and fruits with a dark brown center surrounded by light-brown rings with purplish halos [11,12].

Phomopsis viticola causes phomopsis cane and leaf spot, which is an important disease of grapes worldwide, it affects most parts of the grapevine, such as leaves, rachis, flowers, and berries and up to 30% losses of the crop has been reported [13].

In this study, the antifungal activity of 22 common spices using direct bio-autography, coupled to a *Colletotrichum* species was performed to evaluate their potential utility in managing these plant pathogens. Turmeric, nutmeg, ginger, clove, oregano, cinnamon, anise, fennel, black pepper, basil and black cumin showed antifungal activity against three *Colletotrichum* species. Turmeric and nutmeg extracts were chosen for bioassay-guided fractionation, because they were the two most active spices among the tested spices. Three curcuminoids (**1**–**3**) were isolated from turmeric and two 8-*O*-4′-neolignans (**4**, **5**) along with 5-hydroxyeugenol (**6**) were isolated from nutmeg. The isolated compounds were subsequently evaluated using a 96-well micro-dilution broth assay against plant pathogens.

2. Materials and methods

2.1. General experimental procedures

UV spectra were obtained in MeOH using a Varian Cary 50 Bio UV–visible spectrophotometer and IR spectra were recorded using a Brucker Tensor 27 spectrophotometer. 1D and 2D NMR spectra were obtained on a Varian AS 400 spectrometer. High resolution electrospray ionization mass spectroscopy (HRESIMS) was recorded on a Brucker Bioapex FTMS in ESI mode. Classical TLC analysis was performed on silica gel 60 F_{254} 20 × 20 cm on an aluminium sheet (EMD). Detection was carried out under UV light (254 nm, 366 nm) and visualization made with vanillin–H_2SO_4 (1 g vanillin in 100 mL of 20% H_2SO_4 in EtOH) reagent followed by heating. Column chromatographic separations were performed on Si gel (Merck, 70–230 Mesh, and 63–200 μm), Sephadex (LH-20, Aldrich) and Strata Si-SPE (Phenomenex). Purity of the isolated compounds was confirmed by HPLC (Waters, PDA detector, C18 analytical Luna Phenomenex columns). The absorbance of the well plates was recorded on a Packard Spectra Count Microplate photometer (Downers Grove, IL).

2.2. Plant materials and chemicals

Spices were purchased as powders from McCormick & CO., Hunt Valley, MD, USA. ACS-grade solvents, methanol, dichloromethane (DCM), acetone, petroleum ether, ethyl acetate (EtOAc), and *n*-hexane were purchased from Fisher Scientific. Fungicide standards benomyl, cyprodinil, azoxystrobin, and captan were purchased from (Chem Service Inc., West Chester, PA).

2.3. Extraction

Each powdered material (30 g each) was extracted with MeOH (200 mL × 3) at room temperature for three days. Extracts were filtered and evaporated until dryness under reduced pressure.

2.4. Biossay guided isolation of compounds *1*–*3* from turmeric

Dried turmeric methanol extract (8.0 g) was fractionated on Si gel VLC (200 g, 10 × 30 cm) using EtOAc/*n*-hexane [25:70, 50:50, 75:25, 100:0 (500 mL each fraction)], followed by MeOH/EtOAc [50:50, 100:0 (1 L each)]. All the fractions were concentrated in vacuo and submitted for antifungal evaluation, fraction C (75% EtOAc/*n*-hexane) was the most active fraction against *Colletotrichum* species. Fraction C (2.8 g) was subsequently subjected to Si gel flash column chromatography (80 g, 2 × 60 cm) eluted with MeOH/DCM (0:100 to 15:85) to afford 8 subfractions (C-1 to C-8). The active fraction, C-4 (233 mg) was further purified by sephadex LH-20 using MeOH as an eluent followed by Si SPE column (10 g), eluted with isocratic 30% EtOAc in petroleum ether to yield 3 pure curcumenoids; curcumin (**1**, 40.0 mg), demethoxyated curcumin (**2**, 17.6 mg) and bisdemethoxy curcumin (**3**, 86.5 mg).

2.5. Bioassay guided isolation of compounds *4*–*6* from Nutmeg

Nutmeg methanol extract (5.0 g) was fractionated on Si VLC using the same method applied for turmeric, to yield 9 fractions (A-I). Fraction D [(75% EtOAc/*n*-hexane)] displayed antifungal activity against *Colletotrichum* species. Fraction D (644 g) was chromatographed on Si SPE (20 g) eluted by 10% EtOAc/*n*-hexane to afford 10 subfractions (D1–D10). Fraction D2 (430 mg) was subjected to amino SPE column (10 g) with a mobile phase of isopropanol/DCM (0:100 to 100:20), giving 9 fractions (D2a–D2i). Compound **4** (*erythro*-(7*R*,8*R*)-$\Delta^{8'}$-4,7-dihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan, 155 mg) was isolated from fraction D2d by washing with cold methanol. Fraction D2a (45 mg) was further purified on C8 SPE column eluted with 65% MeOH/H_2O to provide **5** (*erythro*-(7R,8R)-$\Delta^{8'}$-7-acetoxy-3,4,3′,5′-tetra-methoxy-8-*O*-4′-neolignan, 11.5 mg). Fraction D2h (37.9) was subjected to C18 SPE column using 75% MeOH/H_2O as an eluent to yield compound **6** (5-hydroxyeugenol 16.6 mg).