A new furanocoumarin from the fruits of *Scaevola taccada* and antifungal activity against *Pythium insidiosum*

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**ABSTRACT**

A new coumarin, scataccanol (1) and 10 known compounds were isolated from the fruits of *Scaevola taccada* (Gaertn.) Roxb. All compounds were evaluated for antifungal activity against *Pythium insidiosum*. Compounds 5 and 7 showed strong antifungal activity with minimum inhibitory concentration values of 5 and 10 μg/mL, respectively. Structural determination of all compounds was accomplished by 1D and 2D-NMR, IR and MS.

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**1. Introduction**

*Scaevola taccada* (Gaertn.) Roxb. (synonym, *Scaevola taccada* var. sericea (Vahl) Merr.; Family Goodeniaceae), a flowering plant, is commonly found in beach scrubland around the Arabian Sea, the tropical Indian Ocean and tropical Islands of the Pacific. It is known as Naupaka Kahakai (Hawaiian), Magoo (Divehi), Merambong (Malay), Ngahu (Tongan) and Ruk Ta-lay (Thai). The flowers of this plant have a fan-like shape, and hence, a fan flower or half flower was given. In Thailand, the roots of *S. taccada* are used to cure food poisoning and the leaves are used as an anti-inflammatory (Ruangrungsi & Mangkhla 2004). This species contained coumarins, terpenoids and steroids (Wohlrabe & Hänsel 1977). In this study, the chemical constituents of the fruits of this plant have been investigated. All isolated compounds were evaluated for antifungal activity against *Pythium insidiosum*.
*P. insidiosum* is the etiological agent of pythiosis, a granulomatous disease characterised by cutaneous and subcutaneous lesion, corneal ulcer, keratitis and vascular disease. Conventional drugs are usually ineffective because of their cytoplasmic membrane lacking of ergosterol (Mendoza 2005). Radical excision of infected tissues or organs is the main option for the treatment of pythiosis. The significance of searching treatment from a novel microorganism or medicinal plants found in environmental area can be found from studying a natural process. The report of meroterpenes and furanocoumarin, which isolated from the leaves of culen (*Psoralea glandulosa*), has been reported for their antiphytopathogenicity against *Botrytis cinerea* and *Phytophthora cinnamomi* (Madrid Villegas et al. 2015). It has been reported the cytotoxicity of crude extract of an endophytic fungus against *Artemia salina* (Dame et al. 2016). The report of antifungal activity of phenazine-1-carboxylic acid (PCA) and 2-hydroxyphenazine (2-OH P) from *Pseudomonas chlororaphis* subsp. *aureofaciens* strain M71 against plant pathogenic fungi and oomycetes has been reported (Puopolo et al. 2013). Due to the need of effective treatments for pythiosis without using antifungal drugs by using the effective compounds, we aim to find the bioactive compounds against this fungus-like organism and our attention has been focused on the targeted medical plants such as *S. taccada*.

2. Results and discussion

EtOAc and MeOH extracts from the seeds of *S. taccada* were separated by column chromatography (CC) and preparative thin-layer chromatography (TLC), leading to a new compound, scataccanol (1). In addition, 10 known compounds, including five coumarins; *ent*-ammiatin (2) (Jiménez et al. 2000), nodachenetin (3) (Chiang et al. 2010), marmesin (4) (Li et al. 2014), xanthyletin (5) (Tatsimo et al. 2015) and umbelliferone (6) (Sriphana et al. 2013), a benzaldehyde derivative; 4-formylsyringol (7), two iridoids; 6-hydroxy-7-methyl-1-oxo-4-carbomethoxyoctahydrocyclopenta[c]pyran (8) and loganetin (9) (Yamamoto et al. 2002), a lignan; matairesinol (10) (Su & Wink 2015) and a cinnamoyl ester; 2-(4-hydroxyphenyl)-3-(3,4-dihydroxyphenyl)-2-propenoate (11) (Lee et al. 2005) were isolated (Figure 1). All isolates were elucidated by spectroscopic and spectrometric data, including 1D and 2D-NMR, IR, and MS, and by comparison with the literature values.

Compound 1 was obtained as a white solid (MeOH). A molecular formula of C_{14}H_{12}O_{4} was determined from a quasi-molecular ion peak at m/z 267.0629 [M + Na]^+ in the HRESIMS spectra and the 13C NMR data, corresponding to nine indices of hydrogen deficiency. In the IR spectrum, absorption bands at 3469 and 1700 cm\(^{-1}\) indicated the presence of a hydroxy and a carbonyl group, respectively. The 13C NMR and DEPT spectra showed 14 carbon signals, including three methylene (two aliphatic and one olefinic), five methine (one aliphatic, two aromatic and two olefinic), five quaternary (four aromatic and one olefinic) and one carbonyl carbons (Table 1). The 1H NMR data showed two doublet signals at δ 7.60 (J = 9.2) and 6.22 (J = 9.2), which were assigned as H-4 and H-3, respectively. These protons were connected to carbons at δ 143.7 and 112.6 in the HMQC spectrum, which suggested an α,β-unsaturated lactone system. In the HMBC spectrum, the proton H-4 (δ 7.60) correlated with C-2 (δ 161.5), C-9 (δ 156.0) and C-10 (δ 113.1) and H-3 (δ 6.22) with C-2 and C-10. The signals at δ_1/δ_C 7.23/123.6 and 6.76/98.3 were assigned as H-5 and H-8, respectively. The protons at δ 3.45 (dd, J = 15.2, 8.8, H-3’a) and at δ 3.20 (dd, J = 15.2, 8.8, H-3’b) showed correlation with methylene carbon at δ 34.2 (C-3’) in the HMQC spectrum. In the COSY experiment, the triplet
signal at $\delta_H 5.46$ ($J = 8.8$ Hz, H-2') correlated with protons at H-3'. This proton (H-2') correlated with the oxygenated carbon ($\delta$ 85.4) in the HMQC spectrum. Two singlet signals at $\delta$ 5.29 and 5.28 correlated with a methylene carbon at $\delta$ 113.1 in the HMQC experiment indicating the terminal double bond. In the HMBC spectrum, the correlations of H-5 ($\delta$ 7.23) with C-4 ($\delta$ 143.7), C-7 ($\delta$ 163.1), C-9 ($\delta$ 156.0), and C-3' ($\delta$ 34.2) were observed. In this spectrum, correlations of H-3' with C-5 ($\delta$ 123.6), C-7 ($\delta$ 163.1), C-2' ($\delta$ 85.4) and C-1'' ($\delta$ 146.8) were also displayed. The methylene proton at $\delta_H 4.27$ (br s, H-3'') was attached to the oxygenated carbon at $\delta 63.2$ in the HMQC spectrum. This proton showed correlations with C-2' ($\delta$ 85.4) and C-1'' ($\delta$ 146.8), which confirmed the allylic alcohol moiety. It was found that the specific rotation of this compound was $\left[\alpha\right]_D^{24} +37.2$ (c 1.0). Then, the absolute stereochemistry at C-2 was assumed as $2S$ by the comparison of the specific rotation with (S)-marmesin ($4, \left[\alpha\right]_D^{24} +28$ (c 1.1); Jiménez et al. 2000). In addition, compound 2 from this plant was assigned as

**Figure 1.** The structures of compounds 1–11.

**Table 1.** $^1$H and $^{13}$C NMR data (400 MHz, CDCl$_3$) for scataccanol (1).

| Position | $\delta_C$, type$^a$ | $\delta_H$ ($J$ in Hz) |
|----------|---------------------|---------------------|
| 2        | 161.5 C             |                     |
| 3        | 112.6 CH            | 6.22 d (9.6)        |
| 4        | 143.7 CH            | 7.60 d (9.6)        |
| 5        | 123.6 CH            | 7.23 s              |
| 6        | 124.5 C             |                     |
| 7        | 163.1 C             |                     |
| 8        | 98.3 CH             | 6.76 s              |
| 9        | 156.0 C             |                     |
| 10       | 113.1 C             |                     |
| 2'       | 85.4 CH             | 5.46 t (8.8)        |
| 3'       | 34.2 CH$_2$         | 3.45 dd (15.2, 8.8) |
|          |                     | 3.20 dd (15.2, 8.8) |
| 1''      | 146.8 C             | 5.29 s              |
| 2''      | 113.1 CH$_2$        | 5.28 s              |
| 3''      | 63.2 CH$_2$         | 4.27 br s           |
| OH       |                     | 1.69 br t (5.8)     |

$^a$Multiplicities were deduced from DEPT and HMQC experiments.
S- due to the specific rotation was \([\alpha]_D^{24} -16\) (c 1.0) while \((R)-\text{ammirin}\) showed \([\alpha]_D^{24} +7.6\) (c 1.08) (Yamaguchi et al. 2003). From these data, the structure of compound 1, named scataccanol, was established as shown.

According to the high mortality and fatality rates of this disease caused by \(P.\) \textit{insidiosum}, several experiments both \textit{in vivo} and \textit{in vitro} have been studied for the new alternative therapies especially from the medicinal plants products. The antifungal activity of essential oils from the \textit{Origanum vulgare}, \textit{Origanum majorana}, \textit{Mentha piperita} and \textit{Rosmarinus officinalis} has been reported for their minimum inhibitory concentration (MIC) to \(P.\) \textit{insidiosum} ranging from 0.05 to 1.75 mg/mL (Fonseca et al. 2015). All isolated compounds in this study were also evaluated and compounds 5 and 7 demonstrated strong activity by showing MIC values of 5 and 10 μg/mL, which give the inhibition of 100% of mycelium growth at the lower concentration, while the standard drugs, terbinafine, showed MIC values of eight (Table 2). This indicated that our compounds have a higher potential against the growth of \(P.\) \textit{insidiosum}. \textit{In vivo} investigations, the use of topical formulation of essential oils of \(O.\) \textit{vulgare} and \(M.\) \textit{piperita} both singly, associated and in combination with immunotherapy had little or no action on the evolution of the disease. However, our study did not perform \textit{in vivo} study, the formulation of compounds 5 and 7 might be test \textit{in vivo} in the near future.

### 3. Experimental

#### 3.1. General experimental procedures

A SANYO Gallenkamp (UK) melting point apparatus was used to determine melting points. A JASCO DIP-1000 digital polarimeter was used to measure the optical rotation. An Agilent 8453 UV-Visible spectrophotometer (Germany) was used to record the UV spectra. IR spectra were recorded as thin films using a Perkin Elmer Spectrum One FT-IR spectrophotometer (UK). The NMR spectra were recorded on a Varian Mercury plus spectrometer (UK) operating at 400 MHz (\(^1\text{H}\)) and at 100 MHz (\(^1\text{C}\)). The solvent residual peak was used for chemical shift referencing (\(\delta\) \(_H 3.31, \delta\) \(_C 49.0\) for methanol-\(d_4\) and \(\delta\) \(_H 7.26, \delta\) \(_C 77.2\) for CDCl\(_3\)). Mass spectra were obtained on a Micromass Q-TOF 2 hybrid quadrupole time-of-flight (Q-TOF) mass spectrometer with a Z-spray ES source (Micromass, UK). CC was carried out using silica gel 60 (100–200 mesh, Merck). TLC was performed on silica gel 60 F\(_{254}\) (Merck) precoated aluminium sheets. The compounds were visualised under UV light and by spraying with acidic anisaldehyde solution followed by heating. Gel filtration was carried out over Sephadex LH-20 (Pharmacia) suspended in MeOH. Distilled solvents were used throughout the separation process.
3.2. Plant material

The fruits of *S. taccada* were collected from Satun Province, Thailand, in December 2014. The plant was identified by Prof Dr Pranom Chantaranothai, Faculty of Science, Khon Kaen University, where a voucher specimen (KKU012014) is deposited.

3.3. Extraction and isolation

Air-dried fruits (2.0 kg) of *S. taccada* were ground and successively extracted at room temperature with EtOAc (3 × 3 L), and MeOH (3 × 3 L). After the evaporation of solvents, the EtOAc (35 g) and MeOH (104 g) extracts were obtained. The EtOAc extract was separated by silica gel flash column chromatography (FCC) and eluted with a gradient system of EtOAc and MeOH. Based on their TLC characteristics, the fractions that contained the same major compounds were combined to give six fractions, EF₁–EF₆. Further purification of fraction EF₃ by FCC using CH₂Cl₂ as eluent gave three subfractions, EF₃.₁–EF₃.₃. The purification of EF₃.₂ by FCC and development with 50% EtOAc–hexane afforded 5 (20.0 mg, 0.0022%) and 2 (27.8 mg, 0.0031%). Fraction EF₃.₃ was purified by silica gel FCC and eluted with an isocratic system of 5% acetone–hexane to give 6 (7.9 mg, 0.0008%). Fraction EF₄ was purified by FCC using pure CH₂Cl₂ as eluting solvent to give two subfractions, EF₄.₁ and EF₄.₂. The purification of these two subfractions by PLC, developed with 30% EtOAc–hexane and 40% EtOAc–hexane gave 7 (5.3 mg, 0.0006%) and 3 (7.5 mg, 0.0008%), respectively. Fraction EF₅ was subjected to gel filtration over Sephadex LH-20 (MeOH) to afford two subfractions, EF₅.₁ and EF₅.₂. Subfraction EF₅.₁ was purified by Sephadex LH-20 (MeOH) to obtain three subfractions, EF₅.₁.₁–EF₅.₁.₃. Further purification of EF₅.₁.₁ and EF₅.₁.₃ by PLC developed with 30% acetone–hexane and 5% MeOH–CH₂Cl₂ yielded 8 (17.5 mg, 0.0019%) and 1 (8.9 mg, 0.0009%), respectively. The purification of EF₅.₂ by PLC (30% acetone–hexane) afforded 9 (7.4 mg, 0.0008%).

The MeOH extract was separated by FCC and eluted with a gradient system of EtOAc and MeOH to yield three fractions, MF₁–MF₃. Fraction MF₂ was further purified by FCC and eluted with 50% EtOAc–hexane to give three subfractions, MF₂.₁–MF₂.₃. Subfraction MF₂.₁ was tested on PLC using 30% EtOAc–hexane as the developing solvent to give 10 (9.7 mg, 0.0011%). Further purification of MF₂.₂ by PLC (30% EtOAc–hexane) afforded 4 (11.9 mg, 0.0013%). Subfraction MF₂.₃ was subjected to gel filtration over Sephadex LH-20 (MeOH) to afford 11 (7.6 mg, 0.0008%).

3.3.1. Scataccanol (1)

White solid (MeOH); m.p. 140–143 °C; [α]D₂⁰ +37.2 (c 0.1, CHCl₃); UV (CHCl₃) λ_max (log ε) 300 (3.01), 336 (3.41) nm; IR (neat) ν_max 3469 (OH), 2923, 1700 (C=O), 1622, 1569, 1262, 1127, 821 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.60 (1H, d, J = 9.6, H-4), 7.23 (1H, s, H-5), 6.76 (1H, s, H-8), 6.22 (1H, d, J = 9.6, H-3), 5.29 (1H, s, H-2″a), 5.28 (1H, s, H-2″b), 5.46 (1H, d, J = 8.8, H-2″), 4.27 (2H, br s, H-3″″), 3.45 (1H, dd, J = 15.2, 8.8, H-3′a), 3.20 (1H, dd, J = 15.2, 8.8, H-3′b), 1.69 (1H, br t, J = 5.8, OH) and ¹³C NMR (100 MHz) δ 163.1 (C-7), 161.5 (C-2), 156.0 (C-9), 146.8 (C-1″″), 143.7 (C-4), 112.6 (C-3), 123.6 (C-5), 124.5 (C-6), 113.1 (C-10), 113.1 (C-2″″), 98.3 (C-8), 85.4 (C-2′), 63.2 (C-3″″), 34.2 (C-3″); HRESIMS m/z 267.0629 [M + Na]+ (Calcd for C₁₄H₁₂O₄Na, 267.0633).
3.4. Antifungal activity; disc diffusion assay

3.4.1. Fungal isolates

*P. insidiosum* strain SIMI6666, a clinical isolated from a cornea pus of patient with ocular pythiosis who live in Kampang Phet province, Thailand (Thongsri et al. 2013). The identities of this isolated was confirmed by a polymerase chain reaction based assay and its physiological characteristic of the reproductive structure by zoosporogenesis technique. The fungal strain was subculture on Sabouraud dextrose agar (SDA) (Oxoid, UK) slant and stored at 25 °C.

3.4.2. Susceptibility testing

The susceptibility of the *P. insidiosum* strains to the tested compounds was tested by disc diffusion assay and applied following the CLSI M-51-P. The entire surface of each 100 mm-diameter non-supplemented Sabouraud Dextrose Agar (Oxoid, UK) plate was inoculated with the hyphal block of 1 × 1 cm of *P.insidiosum*. The tested compounds were dissolved at a concentration of 10 μg/mL and performed 2-fold serial dilution range from 0.0780 to 10 μg/mL. Then, 20 μL of the tested compounds were impregnated on sterilised discs (6.0 mm) (Whatman, England) and placed on a SDA plate (Oxoid, UK). Terbinafine (20 mg/100 μL; 20 μL/disc) (Sigma-Aldrich, USA) and a disc with EtOAc or methanol only were used as control discs. Plates were kept at room temperature for 2 h in a laminar flow cabinet and incubated at 25 °C for 3, 6 and 9 days. The MIC of each compound was determined by visual observation and represents the inhibition of 100% of mycelium growth of *P. insidiosum*.

4. Conclusion

Chemical investigation of the EtOAc and MeOH extracts from the fruits of *S. taccada* led to the isolation of 11 compounds. A new furanocoumarin, scataccanol (1) and 10 known compounds were discovered. Antifungal activity against *P. insidiosum* was testing by disc diffusion assay. Compounds 5 and 7 displayed antifungal activity with MIC values of 5 and 10 μg/mL, respectively, while the other compounds showed inactive against *P. insidiosum*.

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Disclosure statement

The authors declare no conflict of interest.

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References

Chiang CC, Cheng MJ, Peng CF, Huang HY, Chen IS. 2010. A novel dimeric coumarin analog and antimycobacterial constituents from Fatoua pilosa. Chem Biodiversity. 7:1728–1736.

Dame ZT, Silima B, Gryzenhout M, van Ree T. 2016. Bioactive compounds from the endophytic fungus Fusarium proliferatum. Nat Prod Res. 30:1301–1304.

Fonseca AOS, Pereira DIB, Jacob RG, Maia Filho FS, Oliveira DH, Maroneze BP, Valente JSS, Osório LG, Botton SA, Meireles MCA. 2015. In vitro susceptibility of Brazilian Pythium insidiosum isolates to essential oils of some Lamiaceae family species. Mycopathologia. 179:253–258.

Jiménez B, Grande MC, Anaya J, Torres P, Grande M. 2000. Coumarins from Ferulago capellaris and F. brachyloba. Phytochemistry. 53:1025–1031.

Lee YT, Don MJ, Liao CH, Chiou HW, Chen CF, Ho IK. 2005. Effects of phenolic acid esters and amides on stimulus-induced reactive oxygen species production in human neutrophils. Clin Chim Acta. 352:135–141.

Li Y, Zhang M, Luo M, Wu X, Xiao Q, Hua J, Li X. 2014. Two new linear furanocoumarins from Angelica apaensis. Heterocycles. 89:465–471.

Madrid Villegas A, Diaz Peralta K, Gonzalez Tapia C, Catalán Marín K, Espinoza Catalán L. 2015. Antiphytopathogenic activity of Psoralea glandulosa (Fabaceae) against Botrytis cinerea and Phytophthora cinnamomi. Nat Prod Res. 29:586–588.

Mendoza L. 2005. Pythium insidiosum. In: Merz WG, Hay, RJ, Topley and Wilson’s microbiology and microbial infections. Medical Mycology. 10th ed. London, UK: ASM Press; p. 617–630.

Puopolo G, Masi M, Raio A, Andolfi A, Zoina A, Cimmino A, Evidente A. 2013. Insights on the susceptibility of plant pathogenic fungi to phenazine-1-carboxylic acid and its chemical derivatives. Nat Prod Res. 27:956–966.

Su S, Wink M. 2015. Natural lignans from Arctium lappa as antiaging agents in Caenorhabditis elegans. Phytochemistry. 117:340–350.

Tatsimo SJN, Tamokou JDD, Lamshöft M, Mouafu FT, Lannang AM, Sarkar P, Bag PK, Spiteller M. 2015. LC-MS guided isolation of antibacterial and cytotoxic constituents from Clausena anisata. Med Chem Res. 24:1468–1479.

Thongsri Y, Wonglakorn L, Chaiprasert A, Svobodova L, Hamal P, Prariyachatigul C. 2013. Evaluation for the clinical diagnosis of Pythium insidiosum using a single-tube nested PCR. Mycopathologia. 176:369–376.

Wohlrabe K, Hänsel R. 1977. Coumarins from Scaevola frutescens. Archiv der Pharmazie. 310:972–974.

Yamaguchi S, Muro S, Kobayashi M, Miyazawa M, Hirai Y. 2003. Absolute structures of some naturally occurring isopropenylidihydrobenzofurans, remirol, remiridol, angelomalin, and isoangelomalin. J Org Chem. 68:6274–6278.

Yamamoto H, Sha M, Kitamura Y, Yamaguchi M, Katano N, Inoue K. 2002. Iridoid biosynthesis: 7-deoxyloganetic acid 1-O-glucosyltransferase in cultured Lonicera japonica cells. Plant Biotechnol. 19:295–301.