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Antimicrobial activity of *Araucaria cunninghamii* Sweet and the chemical constituents of its twigs and leaves

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ABSTRACT

The present study was aimed at the identification of antimicrobial components from *Araucaria cunninghamii* with an activity-guided purification process. Eight compounds were obtained from the most active *n*-BuOH fraction and identified as the new compound 4-*n*-butoxyl-phenylpropanetriol (1), together with seven known compounds (2–8). These compounds were tested for antimicrobial activities against five bacteria and four plant pathogenic fungi. Within the series of compounds tested, compound 2 was the most active, particularly displaying moderate antibacterial activities against *Erwinia carotovora* and *Bacillus subtilis* with MICs 7.8 and 15.5 μg/ml. Moreover, this compound exhibited inhibitory activities against four plant pathogenic fungi: *Helminthosporium sativum*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. Niveum and *Fusarium oxysporum* f. sp. Cubense, with EC_{50} values of 42.3, 90.0, 62.7 and 100.2 μg/ml. To our knowledge, this is the first report that the *n*-BuOH fraction and compound 2 from *A. cunninghamii* showed inhibitory activity against plant pathogenic fungi.

1. Introduction

A substantial loss in food production due to plant diseases caused by fungi and bacteria is a major problem in both developing and developed countries (Fletcher et al., 2006). Additionally, Fungi and bacteria also have unfavorable effects on quality, safety and preservation of food. Synthetic chemicals are widely used in the control of plant diseases. However, they may leave toxic residues in treated products (Barnard et al., 1997). Therefore, many researchers have sought natural antimicrobials from natural sources (Kim et al., 1999; Kubo et al., 1995). Plants are a good source of antimicrobials since they have had to develop compounds to resist infections by fungi present in their environment. Thus, there has been growing interest in the possible use of extracts and compounds from plant as natural antimicrobials, which are less damaging to human health and the environment (Alviano and Alviano, 2009). The genus *Araucaria* (Araucariaceae) is composed of 18 species growing over the tropical zone, such as, the South America, Brazil and Australia (Chen et al., 1997). Many of these species are used widely in traditional medicine in China, and the resulting phytochemical investigations have, in turn, uncovered a variety of diterpenoids and biflavones from these species (Caputo et al., 1974; Ilyas et al., 1986). *Araucaria cunninghamii* Sweet is a tall, evergreen arbor and commonly grows to a height of over 20 m. In our previous paper, from the EtOAc extract of this species, three diterpenoids and three shikimic acid derivatives were isolated (Chen et al., 1997, 2011). However, there were no previous reports on the inhibitory activity of the chemical constituent from *A. cunninghamii* against plant pathogens. In the present paper, work was undertaken to deal with isolation and characterization of the new compound **1** and to hopefully obtain a better understanding of the potential bioactivity of chemical constituents *from A. cunninghamii* by bioassay-guided.

2. Results and discussion

2.1. General

The active *n*-BuOH fraction of *A. cunninghamii* extract was subjected to column chromatography over silica gel, reversed-phase C_{18} and Sephadex LH-20 to yield a new compound (**1**) and seven previously known compounds (**2**–**8**) (Fig. 1). All of the structures were elucidated on the comprehensive analysis of spectroscopic data.

2.2. Structure elucidation of the new compound

Compound **1** was obtained as a white amorphous powder. The $[M + NH_4]^+$ ion peak at *m/z* 258.1704 in the HRESIMS corresponded to a molecular formula $C_{13}H_{20}O_4$ with four degrees of unsaturation.

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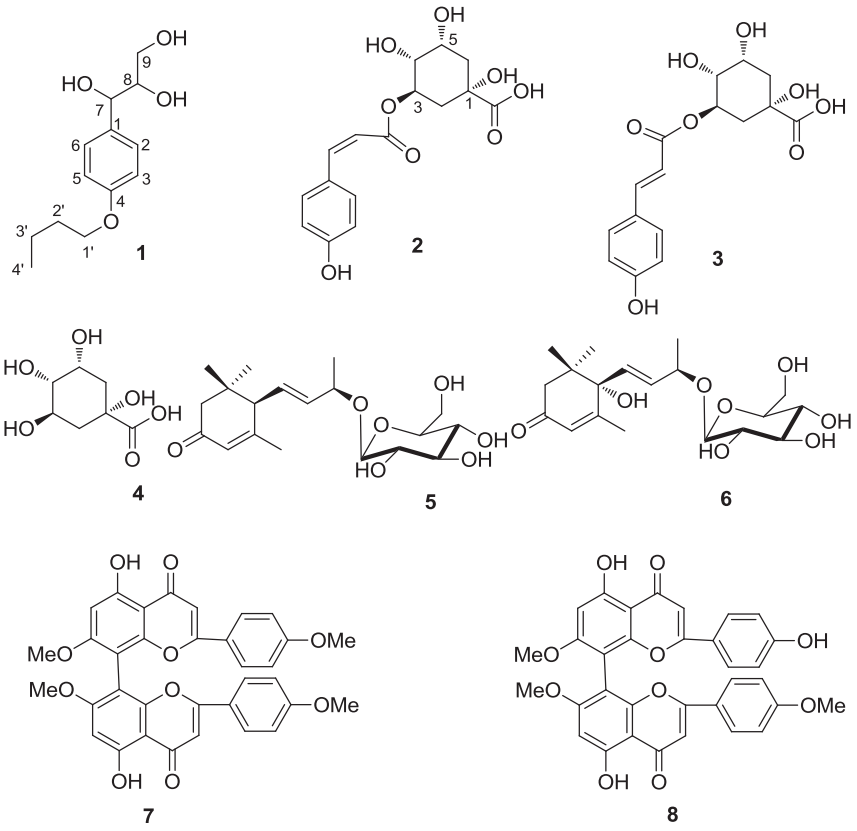

Fig. 1. The structures of compounds **1**–**8**.

The ^{1}H NMR spectrum showed the presence of two sets of *ortho*-coupled protons assigned to a 1,4-disubstituted phenyl group [δ_H 7.18 (2H, *d*, *J* = 8.4 Hz, H-2, 6) and 6.78 (2H, *d*, *J* = 8.4 Hz, H-3, 5)]. The ^{13}C NMR spectrum displayed a total of 13 carbon resonances which were assigned to one methyl (δ_C 14.2), four methylenes (four sp^3 hybridized), six methines (two oxygenated and four aromatic) and two quaternary carbon atoms. Partial structures **a** (C-7–C-9), **b** (C-1′–C-4′), **c** (C-2–C-3) and **d** (C-5–C-6), shown in bold lines in Fig. 2 were deduced from a detailed analysis of the ^{1}H–^{1}H COSY spectrum. The HMBC cross-peaks between H-7/C-2(6), H-7/C-1, H-2(6)/C-1 and H-2(6)/C-7 indicated that the C-1 connected the three units **a**, **c** and **d** together. The correlations from H-1′ and H-3(5) to C-4, suggested that the C-4 connected the three units **b**, **c** and **d** together. The gross structure of **1** was thus elucidated to be as shown in Fig. 2. There are two chiral centers in the side chain of compound **1** and it is difficult to determine its absolute configuration (Chang et al., 1999). The side chain, 1,2-dihydroxyethyl moiety, in **1** is flexible and attached to the rest of the molecule through a quaternary carbon, which prevents the use of NMR techniques. Furthermore, the UV–vis spectral region of acyclic 1,2-diols below about 190 nm prevents the use of chiroptical methods (Mason, 1982) for the direct analysis of their absolute configuration, unless a chemical derivatization is carried out on the chiral substrate by addition of a suitable chromophoric group. Unfortunately, no further chemical reaction could be performed due to the low yield of this compound. Hence, the structure of compound **1** was elucidated as 4-*n*-butoxyl-phenyl-propanetriol. Compound **1** was detected in the ethanol extract of *A. cunninghamii* by HPLC investigation, the new compound is probably a metabolite of the plant. It is rare that the *n*-butoxyl group is substituted on the benzene moiety of propanetriol derivatives in natural products.

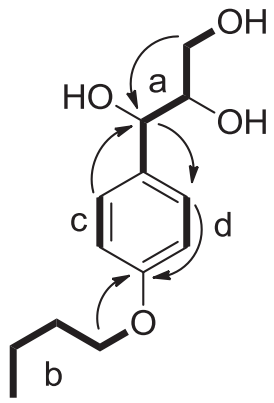

Fig. 2. Important ^{1}H–^{1}H COSY(−) and HMBC (H → C)correlations of compound **1**.

2.3. Structure elucidation of the known compounds

In addition to a new propanetriol derivative 4-*n*-butoxyl-phenylpropanetriol (**1**), seven known compounds were isolated and their structures were identified as 5-*p*-cis-coumaroylquinic acid (**2**) (Jurgenliernk and Nahrstedt, 2002), 5-*p*-trans-coumaroylquinic acid (**3**) (Schaller and Von Elbe, 1970), quinic acid (**4**) (Steck, 1967), (6*R*,9*S*)-3-oxo-α-ionol-9-O-β-D-glucopyranoside (**5**) (Pabst et al., 1992), (6*S*,9*S*)-roseoside (**6**) (Yoshinari et al., 1989), 5,5″-dihydroxy-7,7″,4′,4‴-tetramethoxy biflavone (**7**) (Rahman and Bhatnagar, 1968) and 4′,7,7″-trimethoxy cupressuflavone (**8**) (Khan et al., 1971), by spectral analyses and comparison with literature values.

2.4. Antifungal activity

Initially, the inhibitory activity of 95% ethanol crude extract of the twigs and leaves of *A. cunninghamii* against *Helminthosporium*