Phytochemistry Letters 28 (2018) 111–115

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

Identification and bioactivity evaluation of ingredients from the fruits of *Amomum tsaoko* Crevost et Lemaire

Hang Liu[a,1], Qingsong Yan[b,1], Deli Zou[b], Xialing Bu[b], Baojing Zhang[b], Xiaochi Ma[b], Aijing Leng[b], Houli Zhang[b], Dawei Li[b,*], Chao Wang[b,*]

[a] Department of Pharmacy, Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing 210008, PR China
[b] The First Affiliated Hospital of Dalian Medical University, College of Pharmacy, Dalian Medical University, Dalian 116011, China

ARTICLE INFO

Keywords:
Amomum tsaoko
Fatty acids
Antibacterial activity
Hypolipidemic
Antioxidant

ABSTRACT

Two new fatty acids, together with ten known compounds, were isolated from the fruits of *Amomum tsaoko*. Their structures were determined by extensive spectroscopic data analysis. The extracts and all the discovered compounds were tested for inhibition of *Klebsiella pneumoniae* (*K. pneumoniae*). The results showed that 95% ethanol and ethyl acetate extracts of the fruits of *A. tsaoko* had excellent inhibitory activities against *K. pneumonia*. Fatty acids **1**–**4**, **7**, **9**, and **11** showed significant *K. pneumoniae* inhibitory activity at a concentration of 50 μg/mL (inhibition rate > 99%). The lipase inhibitory activity and DPPH radical scavenging capacity of all compounds were also evaluated. Compounds **2** and **3** exhibited better inhibitory effects than did the positive control Orlistat at a concentration of 50 μg/mL; compound **11** exhibited excellent DPPH radical scavenging activity at a concentration of 100 μg/mL.

1. Introduction

Foods with bioactive ingredients provide valuable sources for the development of dietary supplements for preventing disease. *Amomum tsaoko* Crevost et Lemaire, which belongs to the Zingiberaceous family, is a perennial herbaceous plant that is widely distributed and cultivated in Yunnan, Guangxi, and Guangdong provinces of China (Li et al., 2011). Its dried fruit, named "Cao Guo" in Chinese, is a traditional Chinese spice with homogenous functions in food and medicine and with a strong fragrance and flavor (Feng et al., 2011). It is usually added to food as a flavoring agent in cooking in order to develop the food's distinct flavor (Yang et al., 2008). The dried fruits of the plant are also a traditional Chinese medicine, which has been used to treat indigestion, coughing and abdominal pain; in addition, it can produce a nice refreshing effect in the mouth, and tends to lower body weight (Feng et al., 2011). It is a commercially important spice in south-east Asian markets (Yang et al., 2010).

Recently, the fruits of *A. tsaoko* have attracted intensive attention as a functional food because of their various biological activities, such as antioxidant, antifungal, antitumor, and neuroprotection properties (Zhang et al., 2014; Moon et al., 2004; Zhang et al., 2015). Chemical studies of *A. tsaoko* revealed that these fruits contain monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, diarylheptanoids, flavones, and phenolic acids, etc. (Zhang et al., 2014; Moon et al., 2004; Zhang et al., 2015). It is also well known that the fruits of *A. tsaoko* contain a large amount of essential oils, including monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and others (Guo et al., 2017). 1,8-Cineole is the most important constituent in essential oils (45.24%) (Yang et al., 2010).

The fruits of *A. tsaoko* have been used for a long time as a spice and perfume, in addition to use as a medicine (Moon et al., 2004). There have been many reports focused on the correlation between the biological activities and chemical composition of essential oils from the fruits of *A. tsaoko* (Guo et al., 2017; Yang et al., 2010; Yang et al., 2008). The biological activity of plants or foods is usually generated by different phytochemical compositions. Fatty acids are important bioactive substances in natural products, but no reports refer to chemical constituents of fatty acids from the fruits of *A. tsaoko*.

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium of worldwide importance because it causes pneumonia associated with high morbidity and mortality (Vieira et al., 2016; Mizgerd, 2006; Armstrong et al., 1999). It is found widely in the

* Corresponding authors.
E-mail addresses: lidw87@163.com (D. Li), wach_edu@sina.com (C. Wang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.phytol.2018.10.007
Received 6 August 2018; Received in revised form 22 September 2018; Accepted 4 October 2018

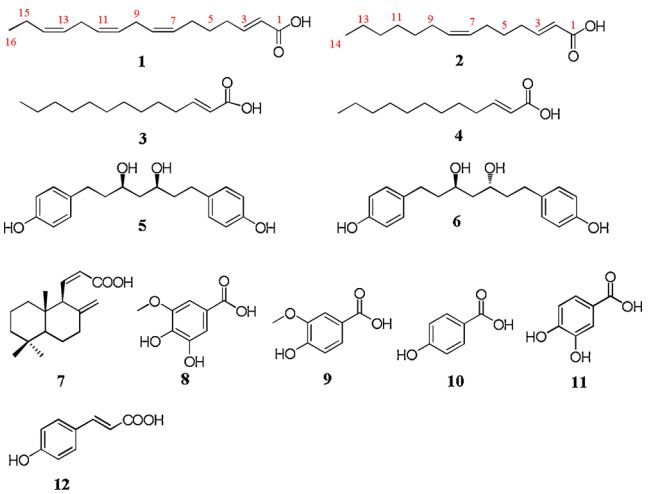

Fig. 1. Chemical structures of isolated compounds **1–12.**

environment and also in the intestinal tracts of mammals (Krusong et al., 2015). Because many micro-organisms are resistant to antibiotics, more researchers have been interested in extracting biologically active compounds from plant species in order to eliminate pathogenic micro-organisms (Essawi and Srour, 2000; Yang et al., 2008). *K. pneumoniae* infection is recognized as a major health threat due to the increasing antibiotic-resistance limiting efficient therapies (Vieira et al., 2016). In the present study, we describe the identification of the chemical components of the fruits of *A. tsaoko* and evaluate their *K. pneumoniae* inhibitory activity for the prevention of pneumonia.

In this study, the inhibitory effects of crude extracts and four fractions of fruits of *A. tsaoko* against *K. pneumoniae* were investigated in order to confirm the bioactive compounds via bioactivity-guided separation. Thus, two new fatty acids (**1** and **2**), together with ten known compounds including two fatty acids (**3** and **4**), two diarylheptanoids (**5** and **6**), one diterpenoid (**7**), and five phenolic acids (**8—12**), were isolated from 95% ethanol extracts of the fruits of *A. tsaoko* by several chromatographic technologies (Fig. 1). The inhibitory effects of all compounds against *K. pneumoniae* and lipase activity were first evaluated. Additionally, DPPH radical scavenging capacity of all compounds was also evaluated. The current data suggest that fatty acids are important bioactive substances that can be developed as antibacterial and hypolipidemic agents in future translational studies.

2. Results and discussion

2.1. Structure determination

Compound **1** was obtained as a colorless oil. The molecular formula was determined as $C_{16}H_{24}O_2$ by HRESIMS at *m/z* 247.1706 $[M-H]^-$ (calcd for $C_{16}H_{23}O_2$, 247.1704), requiring 5 ° of unsaturation. The ^1H NMR spectrum of **1** revealed the presence of eight olefin protons [δ_H 5.80 (1H, d, J = 15.6 Hz, H-2), 6.93 (1H, m, H-3), and 5.28–5.39 (6H, m)], six methylene groups [δ_H 2.80 (4H, m), 2.08 (2H, m, H-15), 2.23 (2H, m, H-4), 1.54 (2H, m, H-5), and 2.13 (2H, m, H-6)], and one methyl at 0.96 (3H, t, J = 7.8 Hz, H-16). The ^{13}C NMR and HSQC spectra confirmed the presence of the above moieties and exhibited one carbonyl carbon (δ_C 170.4) (Table 1). Five degrees of unsaturation were fully accounted for by one carbonyl group and four olefins. These spectral data indicated the tetra-unsaturated fatty acid skeleton in **1**.

In the HMBC experiment, correlations of H-2 and H-3/δ_C 170.4 indicated the presence of a *trans* *α,β*-unsaturated carboxylic acid moiety. The ^1H NMR spectrum showed a six-proton olefinic multiplet at δ_H 5.28–5.39, which was coupled with a four-proton multiplet located at δ_H 2.80 due to two bis-allylic methylenes, indicating the presence of a skipped triene substructure. These assignments were further elucidated by 2D NMR data (Fig. 2). In the ^1H–^1H COSY spectrum, correlations for H-15/δ_H 5.39 and H-16 suggested that an ethyl group was linked at the end of the triene substructure. ^1H-^1H COSY correlations between H-4/H-5/H-6 and a series of HMBC correlations (Fig. 2) from H-2 to C-4; from H-6 to C-4 and C-7; from H-7 to C-5, taking the chemical shifts of these proton and carbon resonances into consideration, confirmed that C-4 and C-6 were attached to C-3 and C-7, respectively.

Table 1
NMR spectroscopic data of compounds **1** and **2** [a].

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		170.4		171.6
2	5.80 d (15.6)	123.1	5.80 d (15.6)	121.0
3	6.93 m	150.4	7.06 m	152.0
4	2.23 m	32.6	2.23 m	31.9
5	1.54 m	29.2	1.53 m	28.0
6	2.13 m	27.6	2.06 m	26.7
7	5.39 m	130.3	5.32 m	128.7
8	5.34 m	129.3	5.40 m	131.1
9	2.80 m	26.5	2.00 m	27.4
10	5.38 m	129.6	1.31 m	29.8
11	5.32 m	129.0	1.29 m	29.1
12	2.80 m	26.4	1.27 m	31.9
13	5.28 m	128.2	1.27 m	22.8
14	5.39 m	132.8	0.88 t (7.2)	14.2
15	2.08 m	21.5		
16	0.96 t (7.8)	14.7		

[a] Data (δ) were measured at 600 MHz for ^1H and at 150 MHz for ^{13}C in CD_3OD for **1** and $CDCl_3$ for **2**.

The geometry of the four double bonds was assigned on the basis of the ^{13}C chemical shifts of the bis-allylic methylene carbons. The chemical shift values of the two bis-allylic methylene carbons (δ_C 26.4 and 26.5) assigned by the HETCOR experiment were in the range characteristic of a methylene carbon between two cis-substituted olefins (Alamsjah et al., 2005; Batchelor et al., 1974; Tulloch, and Mazurek, 1976; Rakoff, and Emken, 1983). Moreover, the undeca-7,10,13-triene group of **1** spectroscopic data is similar to the same moiety of *α*-linolenic acid (Alamsjah et al., 2005) and the carbon resonances were shifted by $\Delta\delta_C \leq \pm$ 2.0 ppm, which also supported that $\Delta^{7,8}$, $\Delta^{10,11}$, and Δ13,14 double bonds had *cis*-geometry. Therefore, compound **1** was determined to be (2*E*,7*Z*,10*Z*,13*Z*)-hexadeca-2,7,10,13-tetraenoic acid.

Compound **2**, a colorless oil, gave a molecular formula of $C_{14}H_{24}O_2$ as determined by HRESIMS and NMR data (Table 1), which showed 24 less mass units than **1**. By comparison of NMR spectra with those of compound **1**, it was evident that compound **2** was a homolog of compound **1**. The NMR spectrum of **2** signals at δ_H 5.80 (d, J = 15.6 Hz, H-2) and 7.06 (m, H-3) and δ_C 171.6 (Table 1) indicated the presence of a *trans* *α,β*-unsaturated carboxylic acid group, and signals at δ_H 5.32 (m, H-7) and 5.40 (m, H-8) revealed the presence of a double bond unit. The geometry of the double bond was deduced to be *Z* based on coupling constant values and by comparison with (5*Z*,9*Z*)-5,9-hexadecadienoic acid (Carballeira et al., 1999). Furthermore, the location of a $\Delta^{7,8}$ double bond was corroborated by the HMBC correlations from H-7 to C-9 and from H-8 to C-6 and 10 (Fig. 2). Therefore, compound **2** was determined as (2*E*,7*Z*)-tetradeca-2,7-dienoic acid.

Ten known compounds (Fig. 1) were characterized by their corresponding NMR spectra. The obtained data were compared with the data reported in the literature. The known compounds were identified as (*E*)-tetradec-2-enoic acid (**3**) (Hayama et al., 2016), (*E*)-dodec-2-enoic acid (**4**) (Sonneck et al., 2015), (+)-hannokinol (**5**) (Yang et al., 2017), meso-hannokinol (**6**) (Yang et al., 2017), coronadiene (**7**) (Nakamura et al., 2008), 3-*O*-methylgallic acid (**8**) (Liao et al., 2014), vanillic acid (**9**) (Yaguchi et al., 1988), p-hydroxybenzoic acid (**10**) (Zhou et al.,