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EXPERIMENTAL PAPER

Chemical constituents obtained from rhizomes of *Alpinia blepharocalyx* K. Schum. and their anti-inflammatory properties

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Summary

Introduction: The plants of *Alpinia* genus have been used in traditional medicine in Vietnam. They are perennial plants used for many decades to treat several ailments including pain, cold, stomach-ache or inflammation.

Objective: The aim of this study was to isolate new metabolites from the *Alpinia* genus and report the chemical constituents from the rhizomes of *A. blepharocalyx* K. Schum. and their anti-inflammatory activity.

Methods: The dried material was extracted using the maceration with methanol at a room temperature. The methanol extract was applied to column chromatograph to obtain purified compounds. The compounds were tested for their anti-inflammatory activity.

Results: Chemical constituents from rhizomes of *A. blepharocalyx* showed six compounds, including 4-hydroxy-2-methoxyphenoxy-*β*-D-{3"-O-[4-hydroxy-3'-methoxy (benzoate)]}-glucopyranoside (1), desmethoxyyangonin (2), trans-resveratrol (3), zerumbone (4), bisdemethoxycurcumin (5), and demethoxycurcumin (6). These compounds may have potentials as anti-inflammatory agents. Compounds 1–6 showed moderate inhibitory activities with IC_{50} values of 7.66–14.06 μM.

Conclusion: The rhizomes of *A. blepharocalyx* included bioactive compounds that could be used in food supplements as a natural anti-inflammatory agent.

Keywords: ***Alpinia blepharocalyx, anti-inflammatory, active compounds, phenolics, diarylheptanoids***

Słowa kluczowe: ***Alpinia blepharocalyx, właściwości przeciwzapalne, związki aktywne, flawonoidy, diaryloheptanoidy***

INTRODUCTION

The plants of genus *Alpinia* have been used in Asia for decades to treat several ailments, including pain, stomach-ache, inflammation, and cold [1, 2]. Their crudes and isolated compounds have been reported for antioxidant, anti-inflammatory, anticancer, antiproliferative activities as well as inhibition of enzymes [2-5]. The chemical constituents from *Alpinia* contain many groups of compounds, including: diarylheptanoids [6-8], terpenoids [9, 10], phenolics [11, 12], flavanones [13, 14], phenylpropanoids [15], and glycosidic ester [16]. A number of diarylheptanoids and phenolic compounds have been isolated from *A. blepharocalyx*, which are known to exhibit NO inhibitory, antiproliferative and cytotoxicity activities *in vitro* [17-22]. Herein, we studied the components from the rhizomes of *A. blepharocalyx* K. Schum. and their anti-inflammatory properties.

MATERIALS AND METHODS

General experimental procedures

The molecular formulas were identified by ESI and HR-ESI-MS (Agilent 1200 LC-MSD). The structure of compounds was shown by 1D and 2D NMR spectra (TMS as the internal standard, Bruker AV-III 500 NMR spectrometer). The Prep-HPLC was conducted on the Agilent 218 (Shim-pack XR-ODS II column). Column chromatography was used silica gel (Merck, 40–60 μm), RP-18, TLC, Sephadex LH-20 (Sigma Aldrich). The fractions were obtained by TLC, and the spots on TLC were indicated under UV light (254 and 365 nm) and by heating silica gel plates sprayed with H_2SO_4.

Plant material

Rhizomes of *Alpinia blepharocalyx* were harvested in Quephong, Nghean province, Vietnam in September 2018 by Dr. Binh N. Q., Vietnam National Museum of Nature, VAST, Vietnam. The rhizomes of *A. blepharocalyx* (sample code AB092018VN) were stored at the School of Chemistry, Biology and Environment, Vinh University.

Extraction and isolation

The rhizomes of *A. blepharocalyx* were dried in shade for 48 h. The dried material (8.5 kg) was extracted by maceration (3 × 20 l MeOH) at a room temperature. The extract was recovered by vacuum evaporator at 40–50°C in order to obtain a crude methanol extract (820 g). The crude methanol extract was dissolved in water. The solvent was re-extracted by liquid-liquid extraction method with hexane, chloroform, butanol to obtain three extracts, including hexane (ABRH, 20 g), chloroform (ABRC, 245 g), butanol (ABRB, 161 g) and water (ABRW, 126 g).

The chloroform extract (ABRC) was applied to column chromatography (CC) (500 g silica gel, 150 cm × 10 cm) with a gradient of mixture n-hexane/acetone (100:1 to 0:1, v:v) to collect eight main fractions (Frs. ABRC1-ABRC8). Fraction ABRC2 (8.8 g) was isolated by CC (150g silica gel, 80 × 1.5 cm) eluting with mixture n-hexane:acetone step gradient system (6:1, v:v) to yield compound **4** (35 mg). Moreover, fraction ABRC5 (11.8 g) was eluted with mixture chlorofom:methanol (15:1, v:v) to obtain compound **2** (19 mg). Fraction ABRC8 (33 g) was subjected to CC (150g silica gel, 80 × 1.5 cm) eluting with mixture hexane:ethyl acetate (15:1, v:v) to give six subfractions (Frs. ABRC 8.1 – ABRC 8.6). The subfraction ABRC 8.3 (6.8 g) was rechromatographed on CC (150 g silica

gel, chloroform-methanol, 10:1 to 2:1) to yield compound **3** (8.7 mg).

The butanol extract (ABRB) was isolated by CC (300 g silica gel, 150 cm × 10 cm), eluted with a stepwise gradient of chloroform:methanol (100:1 to 0:1) to give seven main fractions (Frs. ABRB1-ABRB7). Fraction ABRB2 (2.1 g), eluting with chloroform:methanol (9:1, v:v), was purified by RP-18 to yeild compound **1** (55 mg). Fraction ABRB4 (3.6 g) was rechromatographed on CC (100 g silica gel, 80 × 1.5 cm), eluting with chloroform:methanol (12:1, v:v) to collect five subfractions (Frs. ABRB4.1–ABRB4.5). The subfraction ABRB4.3 (1.7 g) was repeatedly chromatographed on CC (50 g silica gel, 80 × 1.5 cm), eluting with ethyl acetate/methanol (18:1, 15:1, v:v) to afford compound **5** (9.5 mg) and **6** (15.8 mg).

Anti-inflammatory properties

The assays of the inhibitory effect on the NO production were determined as described previously [23].

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSIONS

Compound **1** was obtained in a form of white powder. Its molecular formula was established from peak at *m/z* 451.12 $[M–H]^-$ (calcd. 451.1245, $C_{21}H_{24}O_{11}$). The ^{1}H NMR and ^{13}C NMR of **1** showed two trisubstituted benzene ring protons at δ_H 6.27–7.51 and 12 aromatic carbons, two phenol hydroxyl groups at δ_H 9.09 and 9.86, two methoxy groups at δ_H 3.27, 3.81 and δ_C 55.7, 56.1. In addition to the anomeric doublet proton of β-D-glucose at δ_H 4.81 (*d*, *J*=8.0 Hz), other sugar protons at δ_H 3.32–4.96, and three sugar hydroxyl groups at δ_H 5.29 (*d*, *J*=5.5 Hz), 5.16 (*m*), 4.60 (*t*, *J*=6.0 Hz). Moreover, the ^{13}C NMR of compound **1** showed a carbonyl group (δ_C164.7), two methoxy groups, and six glucosyl carbons. This analysis spectra of compound **1**, which suggested that it is 4-hydroxy-2-methoxyphenoxy-β-D-{3"-O-[4'-hydroxy-3'-methoxy (benzoate)]}-glucopyranoside. The data spectral of compound **1** has been also compared with literature (fig. 1) [23].

Compound **2** was isolated as white crystals. The ^{1}H NMR spectrum of **2** exhibited a methoxy group at δ_H 3.76 (*s*), 5 aromatic protons appearing at δ 7.34–7.51, 4 olefinic protons signals at δ_H 7.49 (*m*), 6.58 (*d*, *J*=21.0 Hz), 5.94 (*d*, *J*=2.0 Hz), and 5.49 (*d*, *J*=2.5 Hz). The ^{13}C NMR spectrum of **2** showed signals of 14 carbons, including six aromatic carbons, one carbonyl group (δ_C 167.0), one methoxy groups (δ_C 56.5), four methine groups, and two C-enol ethers (C-4 and C-6). Comparison of **2** with the literature data proved it to desmethoxyyangonin (fig. 1) [24].

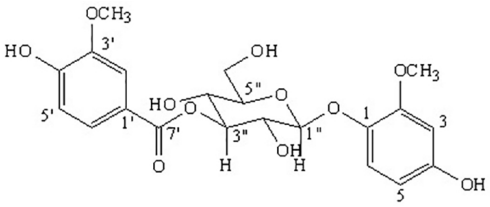
Compound **3** was isolated as yellowish crystals. The ^{1}H NMR spectrum of **3** showed three proton olefinic and aromatic protons. Two protons doublets at δ_H 7.38 (*d*, *J*=8.5 Hz) and 6.75(*d*, *J*=8.5 Hz) have assigned the presented of AA'XX" system of a 1-4 disubstituted aromatic ring; two proton signals at δ 6.38 (*d*, *J*=2.0 Hz) and 6.11 (*t*, *J*=2.0 Hz) are assigned to three *meta* related protons of a 1, 3, 5 symmetrically trisubstituted aromatic ring and two doublets at δ_H 6.92 (*d*, *J*=16.5 Hz) and 6.80 (*d*, *J*=16.5 Hz) shows a trans olefinic system. The spectral data of **3** compared to the reported in literature showed its *trans*-resveratrol (fig. 1) [25].

The ^{1}H NMR of compound **4** indicated four methyl groups (12 protons singlets) at δ_H 1.08-1.79 ppm, three methylene groups signals between δ_H 2.26–2.56 and the proton doublet broad of olefinic proton at 6.12 (*d*, J=14.0 Hz). Moreover, the ^{13}C NMR spectrum exhibited 4 quarternary carbons, 3 methylen groups and 8 methin and methyl groups. At the δ_C 120-200 ppm of the spectrum, there showed a signal of a carbonyl carbon, four methine carbons. The four methyl groups appeared at δ_C 11.8–29.8 on the ^{13}C-NMR. The spectral data of **4** compared to the literature indicates that it is zerumbone (fig. 1) [26].

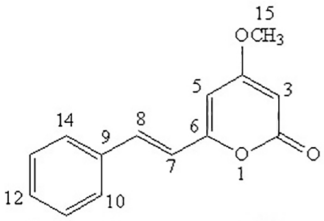
Compounds **5** and **6** were identified as curcuminoids. The 1H NMR of compound **5** at δ_H 5.80 ppm suggested the presence of a methine proton in the enolic form. The two proton doublets at δ_H 7.57 and 6.46 showed a transolefinic system. The structure of **5** has shown an AB-type signals at δ_H 7.42 and 6.87. On the 13C NMR, at very low field δ_C 150–200 ppm, the signal at δ 183.1 and a signal at δ 159.4 suggested the presence of two carbonyl groups and two phenolic carbons. In addition, at δ 129.6, 126.1, 115.9 and 140.2, 120.6 of the spectrums also exhibited the aromatic carbons signals and olefinic carbons. The data spectrum of compounds **5** and **6** showed the different signal of a methoxy group at δ 3.93 (3H, *s*) and 55.6 in ^{13}C-NMR spectrum, which proved that **5** and **6** were bisdemethoxycurcumin and demethoxycurcumin, respectively (fig. 1) [27]. These compounds were isolated from the rhizomes of *A. blepharocalyx* for the first time.

Nitrite concentrations were measured in the supernatant of RAW 264.7 macrophages by the Griess reaction [23]. In order to obtain IC_{50} values

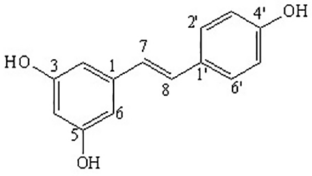
for the potent inhibitors, dose-dependent responses were performed. These compounds showed the most potent inhibitory activity on NO production in LPS-stimulated RAW 264.7 macrophages with IC_{50} values of 7.66 to 14.06 μM, (tab. 1). Thus, it is possible to demonstrate that the compounds might be an important anti-inflammatory constituent of *A. blepharocalyx* plant.



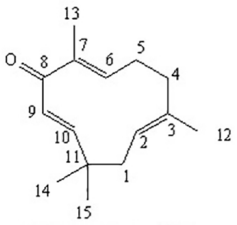
4-hydroxy-2-methoxyphenoxy-β-D-{3''-O-[4'-hydroxy-3'-methoxy (benzoate)]}-glucopyranoside **(1)**



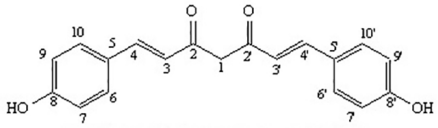
Desmethoxyyangonin **(2)**



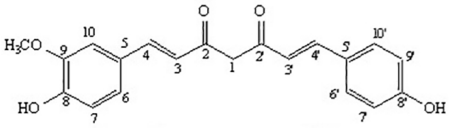
Resveratrol **(3)**



Zerumbone **(4)**



Bisdemethoxycurcumin **(5)**



Demethoxycurcumin **(6)**

Figure 1.

Isolated compounds from *Alpinia blepharocalyx* K. Schum.

Table 1.

Anti-inflamamatory activity of six compounds from *A. blepharocalyx*

	Compounds	RAW264.7 [IC50 (μg/ml)]
1	4-hydroxy-2-methoxyphenoxy-β-D-{3''-O-[4'-hydroxy-3'-methoxy (benzoate)]}-glucopyranoside	7.66 ± 0.51^{a}
2	Desmethoxyyangonin	5.46 ± 0.23^{a}
3	Resveratrol	9.90 ± 1.28^{c}
4	Zerumbone	12.71 ± 0.71^{d}
5	Bisdemethoxycurcumin	14.06 ± 0.33^{ab}
6	Demethoxycurcumin	13.44 ± 0.46^{b}

CONCLUSION

The bioactive compounds from rhizomes of *A. blepharocalyx* have shown that revealed the strong potent anti-inflammatory properties. These compounds showed the most potent inhibitory activity on NO production in LPS-stimulated RAW 264.7 macrophages with IC_{50} values of 7.66 to 14.06 μM. Therefore, the extracts and their compounds could be used as functional foods as a natural anti-inflammatory in food industry. In addition, chemical constituents from rhizomes of *A. blepharocalyx* showed in the identification of six compounds including 4-hydroxy-2-methoxyphenoxy-*β*-D-{3"-O-[4-hydroxy-3'-methoxy (benzoate)]}-glucopyranoside (1), desmethoxyyangonin (2), *trans*-resveratrol (3), zerumbone (4), bisdemethoxycurcumin (5), and demethoxycurcumin (6). These compounds may have potential as anti-inflammatory agents. Compounds 1–6 showed moderate inhibitory activities with IC_{50} values 7.66–14.06 μM. The anti-inflammatory compounds were isolated from the rhizomes of *A. blepharocalyx* species growing extensively in Vietnam for the first time. These species can be also an effective source of corresponding diarylheptanoids and phenolic compounds.

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Conflict of interest: Authors declare no conflicts of interest.

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