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Isolation and Identification of Anti Oxidizing Agents from *Alyxia reinwardtii*

Nageswara Rao.G*, Ravi.G, Sharath Kumar Goud.S

Department of Pharmaceutical Chemistry,
Telangana University,
Nizamabad, Andhra Pradesh-503322

Abstract:
Eight compounds were isolated from the stems of Alyxia reinwardtii, namely coumarin (**1**), 3-hydroxycoumarin (**2**), 6-hydroxycoumarin (**3**), 8-hydroxycoumarin (**4**), scopoletin (**5**), (+)-pinoresinol (**6**), zhebeiresinol (**7**) and p-hydroxybenzoic acid (**8**). The structures of all compounds were characterized by means of NMR, MS, chemical analysis and comparison with the literature data. The structure of compound **7** was also confirmed by X-ray crystallography. To the best of our knowledge, compounds **2-3**, **5** and **7-8** have been isolated for the first time from this species. In terms of antioxidant activity, the isolated compounds were evaluated by various in vitro model assays, which include the DPPH radical scavenging activity, xanthine oxidase-related activity (superoxide scavenging activity and inhibitory effect on xanthine oxidase) and lipid per oxidation inhibitory activity.

Keywords: Alyxia reinwardtii; antioxidant activity; DPPH; xanthine oxidase-related activity; lipid per oxidation Inhibitory activity

1. INTRODUCTION

Alyxia reinwardtii is used as a traditional medicinal plant [1]. The leaves and fruits of this plant can be used to reduce fever, the flowers are effective in treating mental confusion and hallucination associated with high fever, and the stems are used to treat fainting, heart failure and abdominal discomforts due to gaseous distention or other unspecified causes [2]. Iridoids, coumarins and lignans were isolated from the stems, bark, leaves and inner bark of A.reinwardtii [3-6].

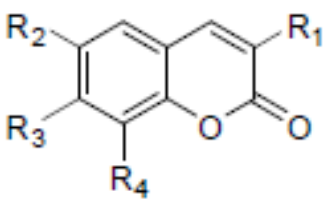
There are no reports on the xanthine oxidase-related activity (superoxide scavenging activity and inhibitory effect on xanthine oxidase) and lipid per oxidation inhibitory activity of this plant.

2. MATERIAL AND METHODS:

The dried stems (4.8 kg) of A. reinwardtii were pulverized and then macerated with hexane, dichloromethane and ethyl acetate thrice for each solvent at room temperature.

The extracts of each solvent were filtrated and evaporated under reduced pressure to afford 49.06 g of hexane crude extract, 82.24 g of dichloromethane crude extract and 33.9 g of ethyl acetate crude extract. The CH2Cl2 extract (55.0 g) was subjected to vacuum liquid chromatography (VLC) over silica gel, using hexane, CH2Cl2, EtOAc and MeOH with increasing polarity. A total seven fractions were collected (A-G). From VLC fraction C was chromatographed on silica gel column using EtOAc-CH2Cl2 (4:6 to 6:4) to yield the white powder of **1** (2.15 g), which was identified as coumarin [7].Fraction D was chromatographed on silica gel column using a stepwise gradient elution of hexane and CH2Cl2 (7:3 to 8:2) to furnish **2** (2.55 g), which was identified as 3-hydroxycoumarin [6]. Similarly, fraction F was also subjected to column chromatography over silica gel using a stepwise gradient of hexane, CH2Cl2 and EtOAc to give **3** (1.2 g), **4** (0.7 g) and **5** (0.018 g), which were identified as 6-hydroxycoumarin, 8-hydroxycoumarin [6] and scopoletin [8], respectively. Fraction G was recrystallized from EtOAc-CH2Cl2 (1:1) to yield the white needles of **7** (0.010 g), which was identified as zhebeiresinol [9]. The mother liquor of this fraction was further purified with chromatotron using a stepwise gradient of EtOAc-CH2Cl2 (2:8 to 1:1) to give **6** (0.057 g), which was identified as (+)-pinoresinol [10].The EtOAc extract (10 g) was similarly chromatographed on silica gel VLC using a stepwise gradient elution of MeOH in CH2Cl2, yielding three fractions (H-J). Repeated column chromatography of J, eluting with EtOAc-CH2Cl2 (0:10 to 6:4) afforded **8** (0.013 g), which was identified as phydroxybenzoicacid [8].The identification of all isolated compounds (Figure 1) was determined by means of spectroscopic methods (MS, 1H, 13C NMR and 2D NMR) as well as comparison with literature data. The exact molecular structure of compound **7** was also confirmed by X-ray crystallography (Figure 2).Antioxidant activity of the isolated compounds were evaluated using assays for

DPPH radical scavenging activity [11], scavenging activity of O2•- by xanthine oxidase, inhibitory activity against xanthine oxidase [12] and ferric thiocyanate assay [13]. The details of these assays are described in the supporting information.



1 R1 = H, R2= H, R3= H, R4= H
2 R1 = OH, R2= H, R3= H, R4= H
3 R1 = H, R2= OH, R3= H, R4= H
4 R1 = H, R2= H, R3= H, R4= OH
5 R1 = H, R2= OCH3, R3= OH, R4= H

Figure 1. Compounds **1-8** isolated from A. rewardtii stems

Table 1. Antioxidant activity of all isolated compounds.

	IC50 (mM)			
Compound	**DPPH**	**Superoxide Scavenging**	**Xanthine oxidase inhibition**	**Lipid per oxidation**
1	>100	>100	No activity	67.64 ± 1.46
2	0.61 ± 0.08	4.50 ± 0.05	No activity	69.07 ± 0.81
3	>100	19.23 ± 0.17	No activity	67.45 ± 0.75
4	>100	13.35 ± 1.11	No activity	58.13 ± 1.17
5	3.15 ± 0.31	-	-	-
6	0.30 ± 0.02	4.50 ± 0.41	No activity	3.37 ± 0.13
7	0.18 ± 0.02	3.30 ± 0.29	No activity	2.08 ± 0.06
8	>100	>100	No activity	>100
BHA[a]	0.18 ± 0.03	-	-	0.25 ± 0.01
Gallic acid[a]	0.50 ± 0.03	0.65 ± 0.02	-	-
Allopurinol[a]	-	-	0.0044 ± 0.07	-

[a] Standard references

RESULTS AND DISCUSSION

From the stems of A. wardtii, eight compounds (**1-8**) were isolated and characterized. All isolated compounds were evaluated for their antioxidant activity. The DPPH test indicated that compound **7** (IC50 = 0.18 mM) showed the best activity, followed by **6** (IC50 = 0.30 mM), **2** (IC50 =0.61 mM) and **5** (IC50 = 3.15 mM), which showed moderate to weak activity, while compounds **1**, **3**, **4** and **8** were regarded as inactive (IC50> 100 mM). In addition, compounds **2** (IC50 = 4.50 mM), **6** (IC50= 4.50 mM) and **7** (IC50 = 3.30 mM) exhibited moderate superoxide scavenging activity whilecompounds **1** and **8** were inactive (IC50 >100 mM). However, all compounds displayed no inhibitory activity against xanthine oxidase. On the other hand, in the lipid per oxidation test, compounds **6** and **7** showed potent activity (IC50 = 3.31 and 2.08 mM, respectively), while compounds **1**, **2**, **3** and **4**showed very weak activity (IC50 = 67.64, 69.07, 67.45 and 58.13 mM, respectively)

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