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Pulmatin from the roots of *Rumex acetosa*

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ABSTRACT

*The methanolic extract of the roots of the plant, Rumex acetosa yielded one known compound, pulmatin (1-O-β-D-glucopyranosyl chrysophanol, **1**). The structure of compound has been established based on its physical and spectral data (UV, IR, ^{1}H & ^{13}C NMR, 2D NMR and Mass). The compound **1** showed moderate elastase inhibition activity when compared with control compound, ursolic acid. The compound is the first report from this plant*

Key words: *Rumex acetosa*, roots, pulmatin, elastase inhibition

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INTRODUCTION

The plant, *Rumex acetosa* (Garden sorrel) belongs to Polygonaceae genus. The genus widely distributed in the temperate regions of the world. A total of 13 species are reported in India. The plant, *R. acetosa* is a perennial herb and found in high altitudes from Kuman to Kashmir. In folklore, the leaves are eaten as such or cooked like spinach. It has been reported to be used as blood purifier, diarrhea, diuretic and also treat bronchial diseases. In homeopathy it is being used to treat skin diseases and convulsions.[1] In Turkey, it is used as a treatment for anemia and as an appetite stimulant.[2] Several researchers on different parts of the plant reported variety of secondary metabolites such as flavones and their glycosides,[1] naphthalene derivatives,[1] anthraquinones and their derivatives,[3, 4, 5] steroids and its glycosides,[6] terpenoids,[6] tannins,[7] fatty acids [8], hydroxy acids.[9] Few of the compounds, anthraquinones and their derivatives have been showed anti-mutagenicity and cytotoxicity.[3]

In continuation of our interest on bioactive compounds from medicinal plants for personal care applications,[9-20] we have undertaken the roots of *R. acetosa* for chemical examination. In this paper, we report the isolation and structure elucidation of one known compound and its elastase inhibition studies. The structure of the compound was established based on physical and spectral data. The compound **1** showed moderate elastase inhibition activity.

MATERIALS AND METHODS

General procedures

Melting point is reported uncorrected. IR spectrum was recorded on a Shimadzu Prestige 21 FT IR. UV spectrum was recorded on Shimadzu UV spectrophotometer. The ^{1}H, ^{13}C and 2D NMR spectra were recorded on Bruker AMX 400 with TMS as an internal standard. Mass spectrum was recorded on Jeol SX 102/DA 600 mass spectrometer. Column chromatography (CC) was carried on a silica gel column (100-200 mesh). Purity of the compound was checked by TLC on pre-coated aluminum sheets, silica gel 60 F_{254} (20 X 20 cm, 0.2mm thickness, Merck) and compound was detected under UV light (254 & 366 nm) and spraying with 5% sulphuric acid in

methanol followed by heating the plates at 110°C for 5 min. The chemical shift values are reported in ppm (δ) units and the coupling constants (*J*) are in Hz.

Plant material

The dried roots of *Rumex acetosa* (1.1 Kg) were obtained from local market from Tamil Nadu in May 2008 and identified by Dr. P. Santhan, Taxonomist, M/s. Durva Herbal Centre, Chennai, Tamil Nadu, India. A voucher specimen of the species was deposited in M/s. CavinKare Research Centre, Chennai, India.

Extraction and Isolation procedure

The dried roots of *Rumex acetosa* (1.1 kg) were coarsely powdered, subjected for an extraction with methanol (5.0L) by using soxhlet apparatus. The solvent was distilled off by using rotary evaporator under reduced pressure at ~ 40°C to get 232 g crude methanolic extract. The methanolic extract was showed good elastase inhibition activity (49.25%@50.0 µg/ml). The methanolic extract was suspended in methanol : water (1:4) and fractionated with ethyl acetate and n-butanol to get corresponding fractions, 32.69g and 26.59g respectively. The aqueous residue was discarded. The two fractions were submitted for biological activity and both fractions showed good activity i.e., ethyl acetate (67.45% @ 34.3 µg/ml) and n-butanol (81.7% @ 56 µg/ml). The n-butanol fraction (26.4g) was purified by vacuum liquid chromatography by using ethyl acetate and ethyl acetate: methanol as mixtures. Based on TLC profile, combined homogeneous fractions and made into five fractions, Fr.1 (1.19g), Fr.2 (6.48g), Fr.3 (5.84g), Fr. 4 (7.37g) and Fr.5 (5.41g). Again all five fractions were submitted for biological studies to identify the active fraction. Only two fractions, Fr.2 and Fr. 4 were showed potent activity. Fr.2 was showed mixture of compounds on TLC and it was repeatedly chromatographed over sephadex LH-20 column followed by re-crystallization with methanol to get pale yellow colored compound, 1-O-β-D-glucopyranosyl chrysophanol (**1**, 36 mg).[21] Further fractions did not yield any compound.

Compound 1 (Pulmatin, 1-O-β-D-glucopyranosyl chrysophanol): Amorphous powder, mp: 252-54°C, UV ($CHCl_3$) nm: 222, 281, 410; IR (nujol) cm^{-1}: 3352 (br, hydroxyl), 1637 (C=O), 1587, 1087, 987, 752; ^1H NMR (DMSO, 400MHz): δ 2.41 (3H,s), 3.20- 3.70 (6H, m), 5.14 (1H, d, J=7.76 Hz), 7.19 (1H, s), 7.49 (1H, s), 7.70 (1H, dd, J=7.5, 1.9 Hz), 7.85 (1H, d, J=7.64Hz), 7.86 (1H, s); ^{13}C NMR (DMSO, 100 MHz): δ 21.8, 61.0, 69.9, 73.6, 76.9, 77.6, 100.9, 115.1, 119.7, 120.9, 122.8, 124.4, 132.5, 135.1, 136.3, 148.0, 158.7,162.0, 182.5, 187.9; EIMS m/z= 416[M^+].

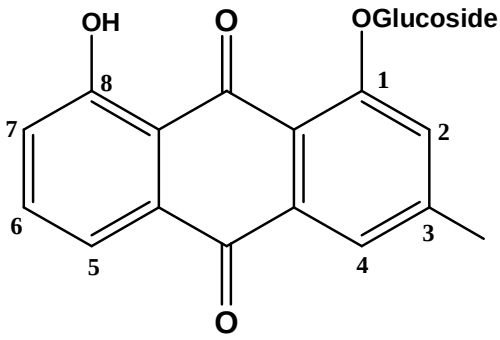

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Figure 1 : Pulmatin from ***Rumex acetosa*** roots

RESULTS AND DISCUSSION

Compound **1** was isolated as colorless amorphous powder, mp: 252-54°C. The compound has been recognized as aromatic based on its spectral data. Its molecular formula has been fixed as $C_{21}H_{20}O_9$ based on its mass spectrum. The IR spectrum exhibited hydroxyl absorption band at 3352 cm^{-1} and two carbonyl group absorption bands at 1720 and 1637 cm^{-1}. The ^1H NMR spectrum showed the presence of an aromatic methyl signal at δ2.41 as singlet. Further, the proton spectrum showed the presence of five aromatic protons at δ 7.19 (1H, br s), 7.49 (1H, br s), 7.70 (1H, dd, J=7.5, 1.9 Hz), 7.89 (2H, m), and sugar protons at δ 3.20-3.70 (6H, m), 5.14 (1H, d, J=7.76 Hz), The carbon spectrum clearly showed a total of 21 carbon signals. Of which, two carbonyl signals appeared at δ 182.5,

187.9, one anomeric carbon appeared at δ 100.8, five sugar carbons at δ 77.6, 76.9, 73.6, 69.8, 60.9 and one methyl signal at δ 21.8. By revealing the literature, the carbon and proton spectral data of the compound exactly matching with the reported compound, pulmatin which was isolated from the plant, *Rhei rhizoma*.[21] This is the first report from this plant.

Elastase inhibition activity: The original crude extract, its fractions, sub fractions and isolated compounds were studied for elastase inhibition activity on cell free system. The method is very precise and most reliable.[20] Fresh solution of 300 µl (0.6 mg) of succinyl-L-alanyl-L-alanyl-L-alanyl-p-nitroanilide (the enzyme substrate), 1200µl of buffer and varying amounts of the elastase inhibitor under testing are incubated at 37^{o}C for 20 minutes. The hydrolysis is measured by the spectrophotometric measurement of the release of p-nitroaniline at a wavelength of 410 nm. In this method, the methanolic extract, EA fraction and isolated compound were tested and the results documented in table.1.

Table 1: Comparison of elastase inhibition data

Sl. No.	Extract / Fraction / Compound	Inhibition (µg/ml)
1	Methanolic extract	49.25% at 50.0
2	Ethyl acetate fraction	67.45% at 34.4
3	n-Butanol fraction	81.7% at 56.0
4	Fr.2	59.0% at 20.0
5	Fr.4	77.8% at 10.0
6	Compound 1	30% at 40.0
7	Control, Ursolic acid	50% at 13.0

CONCLUSION

The present study on isolation and characterization of roots of the plant, *Rumex acetosa* yielded only one compound, 1-O-β-D-glucopyranosyl chrysophanol (**1**). The structure of compound has been confirmed based on physical and spectral data and comparison with literature data. The crude extract, fractions from liquid-liquid fractionation, further active fractions and isolated compound have been studied for elastase inhibition activity. The compound **1** showed moderate inhibition. This is the first report on elastase inhibition from this plant.

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