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XANTHINE OXIDASE INHIBITING COMPOUNDS FROM *Ranunculus repens*

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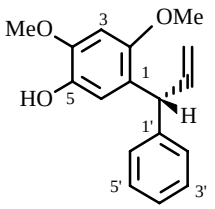
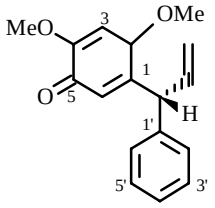
The genus *Ranunculus* belongs to the family Ranunculaceae, which comprises 50 genera and 2000 species. In Pakistan, it is represented by 22 genera and 114 species. The phytochemical studies on various species of genus *Ranunculus* have reported that they contain anemone [1], carotene [2], flavone-glycosides [3], and ranuncosides [4]. The saline extracts of some species have been reported to possess anti-bacterial, anti-fungal, and anti-malarial activities [5–7]. *Ranunculus repens* L. is widely distributed in the northern areas of Pakistan and is used for medicinal purposes [8]. Several genera in this family possess irritant properties due to the presence of protoanemonin [9, 10]. The protoanemonin also inhibits mitosis in plant cells [11]. No work has been reported so far on this species. The diverse medicinal uses attributed to this species prompted us to carry out phytochemical and biological studies on this plant. Biological screening of the methanolic extract revealed significant inhibitory activity against the enzyme xanthine oxidase [12]. On fractionation, the major inhibitory activity against this enzyme was detected in the chloroform soluble fraction. The chloroform soluble fraction resulted in the isolation and structure elucidation of *R*(+)-dalbergiphenol (**1**), *R*(+)-4-methoxydalbergione (**2**), and methyl-3,4,5-trihydroxybenzoate (**3**). All of them have been reported for the first time from this plant.



1



2

The chloroform soluble fraction (F2) of the methanolic extract of the roots of *Ranunculus repens* L. was subjected to column chromatography over silica gel eluting with different mobile phases in increasing order of polarity. Compounds (**1**–**3**) were finally obtained and their structures established by spectral analysis. The fraction which eluted with *n*-hexane – $CHCl_3$ (1 : 9) was chromatographed over silica gel and eluted with $CHCl_3$ to give compound **1** (10 mg), a light brown oil; $[\alpha]_D^{22}$ +31.9° ($CHCl_3$); EIMS *m/z*: 270 (M^+, $C_{17}H_{18}O_3$). The fraction which eluted with $CHCl_3$ – EtOAc (8 : 2) was chromatographed over silica gel using $CHCl_3$ – EtOAc (7.5 : 2.5) as eluent to afford compound **2** (8 mg), which crystallized from acetone, mp 112 – 113°C; $[\alpha]_D^{22}$ +16.2° ($CHCl_3$);EIMS *m/z*: 254 (M^+, $C_{16}H_{14}O_3$). The fraction which eluted with $CHCl_3$ – EtOAc (1 : 1) was further chromatographed over silica gel eluting with $CHCl_3$ – EtOAc (4.5 : 5.5) to furnish compound **3** (40 mg) as a colorless amorphous powder, mp 198 – 200°C; $[\alpha]_D^{22}$ +27.6° ($CHCl_3$); EIMS *m/z* 270 (M^+, $C_8H_8O_5$). Their physical and chemical data showed complete agreement with the literature [13 – 15].

Xanthine oxidase is a highly versatile enzyme [16], catalyzes the hydroxylation of purines, particularly the conversion of xanthine oxidase to uric acid [17], and also the reduction of oxygen [18, 19]. The compounds **1** – **3** were tested for inhibition against xanthine oxidase. It is evident from the results that compounds with more phenolic and methoxy moieties are the most active inhibitors. The inhibitory activities of **1** and **2** were comparable, while compound **3** with three phenolic moieties is the most potent inhibitor against the enzyme xanthine oxidase.

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Compounds	Xanthine oxidase enzyme IC_{50} μM
1	217.8
2	359.2
3	59.5
Allopurinol[a]	7.4

[a]Standard inhibition of the xanthine oxidase enzyme.

Optical rotations were measured on a JASCO DIP-360 polarimeter. Melting points were determined on Buchi melting point apparatus and are uncorrected.

Plant Material. The roots of *Ranunculus repens* L. were collected from Nathia Gali, Hazara Hills, N.W.F.P. in August, 2003 and were identified by Prof. Syed Iftikhar Hussain Shah, Faculty of Pharmacy, Gomal University Dera Ismail Khan, Pakistan. A voucher specimen has been deposited in the Herbarium (accession No. 2003–213).

Extraction and Isolation. Root parts of the plant were air dried, powdered, and extracted thrice with methanol at room temperature. The combined methanolic extract was evaporated under vacuum until dryness. The crude extract (650 g) was suspended in water and extracted successively with *n*-hexane, chloroform, ethyl acetate, and butanol. The chloroform soluble fraction (90 g) was subjected to flash chromatography over silica gel (70–230 mesh) eluting with *n*-hexane, *n*-hexane – $CHCl_3$, $CHCl_3$, $CHCl_3$ – EtOAc, EtOAc, EtOAc – CH_3OH and CH_3OH in increasing order of polarity. The fractions obtained from *n*-hexane – $CHCl_3$ (1 : 9) were combined and again chromatographed over silica gel using $CHCl_3$ as eluent to obtain *R*(+)-dalbergiphenol (**1**) (10 mg). The fractions obtained from $CHCl_3$ – EtOAc (8 : 2) produced some major and minor spots on the TLC plate and were combined and rechromatographed over silica gel using chloroform – ethyl acetate in increasing order of polarity. The fraction obtained from $CHCl_3$ – EtOAc (7.5 : 2.5) gave *R*(+)-4-methoxydalbergione (**2**) (8 mg). It was crystallized from acetone. The fractions obtained from chloroform – ethyl acetate (1 : 1) were combined and rechromatographed over silica gel using $CHCl_3$ – EtOAc (4.5 : 5.5) as eluent to furnish methyl-3,4,5-trihydroxybenzoate (**3**) (40 mg).

Xanthine Oxidase Inhibition Assay. The xanthine oxidase (XO) inhibition activity was assayed in phosphate buffer (0.1 M, pH 7.5). XO (0.003 unit/well), 20 μL and test samples in 10 μL DMSO were mixed in a 96-well microplate and pre-incubated for 10 minutes at room temperature. The reaction was initiated by adding 20 μL of 0.1 mM xanthine, and the resulting uric acid formation was measured spectrophotometrically at 295 nm by Molecular Devices, Spectra Max 384 [12].

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