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LIPOXYGENASE INHIBITORY EFFECT OF PENTACYCLIC TRITERPENES FROM *SORBUS CASHMIRANA* HEDLUND MONOG

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ABSTRACT

Cashmirol Aand B and sorbinol A and B have been isolated from ethyl acetate soluble fraction of *Sorbus cashmirana*. Their structures were determined through spectroscopic techniques that give significant activity against enzyme lipoxygenase.

Key-words:

INTRODUCTION

Sorbus cashmiriana Hedlund Monog is a medicinal plant which belongs to the family Rosaceae. It is a tree with a conical growth reach up to 40 feet. There are about 100 species under the genus *Sorbus* and seven native to the Indo-Pak subcontinent. *Sourbus cashmiriana* Hedlund, Monog is tree of two seasons one in the spring with lovely pink –tinted flowers and one in the autumn when the leaves are gone and glorious white fruits shine out. A tea made from its bark is used to treat nausea and to cleanse the blood. A bark preparation is also used against heart diseases. The berries are rich in vitamin C and used to cure scurvy (Bhattacharjee, 2003; Perry and Metzger, 1980; Krishna, 1972; Jayaweera, 1982; Krachmal, 1980). The ethanopharmacological and chemotaxonomic importance of the genus *Sorbus* led us to investigate the chemical constituents of *Sorbus cashmiriana*. Its methanolic extract showed strong toxicity in a brine shrimp lethality test (McLaughlin *et al.,* 1997; Nie and Honn, 2002). Pharmacological screening of the ethyl acetate soluble fraction showed strong inhibition against lipoxygenase enzyme.

MATERIALS AND METHODS

The whole plant of *Sorbus cashmiriana* Hedlund, Monog was collected in 2004 from Chitral (Pakistan) and identified by Dr. Surraiya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, Karachi Pakistan, where a voucher specimen (No KUH73/67760) has been deposited.

Extraction and Isolation

The freshly collected whole plant material (20 kg) was cut into small pieces and extracted with MeOH (3x30lit). The extract was evaporated under reduced pressure yield (900 gm) residue which was divided into n-Hexane (70 gm), $CHCl_3$ (95 gm), EtoAc (180 gm) and n BuOH (65 gm). The ethyl acetate soluble fraction (180gm) was subjected to increasing order of polarity to obtain 5 major fractions. The fraction which is obtained with EtoAc: MeOH (9.5 : 0.5) were combined and rechromatographed over silica gel eluting with EtoAc : MeOH in increasing order of polarity. The fraction obtained from ethyl acetate : MeOH (9.4: 0.6) were subjected to PTLC using (EtoAC: MeOH) (9.3: 0.7) as eluent to afford cashmirol A and Cashmirol B, respectively. The fraction obtained from (9.5: 0.5) were combined and subjected to PTLC EtoAC – MeOH (9: 1) as solvent system to afford sorbinol A and B, respectively.

In Vitro Lipoxygenase Inhibitory Assay

Arachidonic acid metabolism through lipoxygenase pathways generates various biologically active lipids that play important roles in thrombosis and tumor progression. Angiogenesis, the formation of new capillary vessels from preexisting ones, underpins a number of physiological processes and participates in the development of several pathological conditions such as arthritis and cancer (Nie and Honn, 2002). Lipoxygenases are, therefore, attractive targets for rational drug design and the discovery of mechanism-based inhibitors for the treatment of a variety of disorders such as bronchial asthma, inflammation, cancer, and autoimmune diseases.

Lipoxygenase inhibitory activity was conventionally measured by slightly modifying the spectrometric method developed by Tappel (1962). Lipoxygenase (1.13.11.12) type I-B (from soybean) and linoleic acid were purchased from Sigma Chemicals. A mixture of 160 μl 100 mM phosphate buffer pH 5.0, 10 μL of test compound and 20 μl of

lipoxygenase solution was mixed and incubated for 10 min. at 25º C. The reaction was then initiated by the addition of 10 μL linoleic acid (substrate) (Barrett and Anthon, 2001) solution, resulting in the formation of (9*Z*,11*E*), (13*S*)-13-hydroperoxyoctadeca-9,11-dienoate. The change in absorbance was followed for 6 min at 234 nm. Test compounds and the control were dissolved in methanol or 50 % EtOH. All the reactions were performed in triplicate on 96-well plate reader Spectramax 384 plus (Molecular Devices, USA). The IC_{50} values were calculated using the EZ-Fit Enzyme Kinetics Program (Perrella Scientific Inc., Amherst, and U.S.A). The percentage inhibition was calculated by formula (*E*-*S*)/*E* x 100 where *E* is the activity of the enzyme without test compound and *S* is the activity of enzyme with test compound.

INHIBITION OF LIPOXYGENASE

Various concentrations of Cashmirol A and B, Sorbinol A and B were tested against enzyme lipoxygenase, which displayed activity against this enzyme. The IC_{50} values are depicted in Table-1-4.

Table 1. Inhibition of enzyme lipoxygenase by Cashmirols A.

Compound	IC_{50} ± SEM[a)] (μM)
Cashmirol A	90.20 ± 1.58
Baicalein[b)]	8.01 ± 0.11

Table 2. Inhibition of enzyme lipoxygenase by Cashmirols B.

Compound	IC_{50} ± SEM[a)] (μM)
Cashmirol B	74.88 ± 1.12
Baicalein[b)]	8.01 ± 0.11

Table 3. Inhibition of enzyme lipoxygenase by Sorbinol A.

Compound	IC_{50} ± SEM[a)] (μM)
Sorbinol A	68.60 ± 1.43
Baicalein[b)]	8.01 ± 0.11

Table 4. Inhibition of enzyme lipoxygenase by Sorbinol B.

Compound	IC_{50} ± SEM[a)] (μM)
Sorbinol B	51.27 ± 1.09
Baicalein[b)]	8.01 ± 0.11

[a)] Standard Error of Means (SEM) of three experimental determinations
[b)] Positive control used in assays

RESULTS AND DISCUSSION

The methanolic extract of the whole plant was divided into n-hexane, chloroform, ethyl acetate, n-butanol and water soluble fractions. Column chromatography of the ethyl acetate soluble fraction provided Cashmirol (A), Cashmirol (B), Sorbinol (A) and Sorbinal (B). The inhibitory activity of all the compounds against lipoxygenase was determined using the method developed by Tappel (1962). The IC_{50} values of Cashmirol (A), Cashmirol (B), Sorbinol (A) and Sorbinal (B) were found to be 90.20, 74.88, 68.60, 51.27 μ M, respectively, as against IC_{50} value of 8.01 μ M observed for baicatein used as positive control.

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