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In vitro 12(*S*)-HETE inhibitory activities of naphthoquinones isolated from the root bark of *Euclea racemosa* ssp. *schimperi*

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Abstract

Platelet 12-lipoxygenase is believed to play a role in cancer and other pathological conditions, such as psoriasis, atherosclerosis and arthritis. The inhibition of 12-LOX is a potential therapeutic approach in the treatment of tumor metastasis. The extracts of *Euclea racemosa* Murr. ssp. *schimperi* (A. DC.) F. White (Ebenaceae) obtained by maceration and naphthoquinones isolated from the dichloromethane extract have been investigated for their 12(*S*)-HETE inhibitory activity using human platelets. At 100 μg/ml, the dichloromethane extract inhibited the formation of 12(*S*)-HETE by 88.7% and compounds 7-methyljuglone (**2**), isodiospyrin (**3**), neodiospyrin (**4**) and mamegakinone (**5**), isolated from this extract, exhibited significant activities with IC_{50} values ranging from 4 to 58 μg/ml (22.2–155.7 μM). Of these the most abundant compound, 7-methyljuglone displayed a potent inhibitory activity with an IC_{50} value of 4.18 μg/ml (22.2 μM), which was comparable to the positive control baicalein with an IC_{50} value of 5 μg/ml (18.5 μM). In contrast, 4(*S*)-shinanolone (**1**), the reduced form of compound **2**, did not show any significant inhibitory activity even at a concentration of 60 μg/ml.

Keywords: *Euclea racemosa* ssp. *schimperi*; Naphthoquinones; 7-Methyljuglone; Neodiospyrin; 12-Lipoxygenase; 12(*S*)-HETE

1. Introduction

Arachidonate 12-lipoxygenase (12-LOX) introduces oxygen at carbon 12 of arachidonic acid to generate a 12-hydroperoxy derivative, which in turn is reduced to 12-hydroxyeicosatetraenoic acid (12(*S*)-HETE). Platelet-type 12(*S*)-LOX is one of the 12-LOX isoenzymes found in humans. We have targeted this assay due to the fact that 12-lipoxygenase is expressed in a wide variety of tumor cell lines and the 12-LOX metabolite, 12(*S*)-HETE is implicated as a critical signaling molecule in tumor metastasis (Tang and Honn, 1994) and atherosclerotic processes (Nakao et al., 1982). Evidence suggests that 12(*S*)-HETE is a crucial intracellular signaling molecule that activates protein kinase C and mediates the biological functions of many growth factors and cytokines. 12(*S*)-HETE is also regarded as a mediator of hyperproliferation of the skin (Arenberger et al., 1993) and therefore implicated in skin diseases.

Euclea racemosa Murr ssp. *schimperi* (A. DC.) F. White (Ebenaceae), an evergreen shrub widely distributed in eastern and southern Africa, has been used since early times in Eastern Africa to treat various diseases including cancer. In Ethiopia, the leaf macerate is used to treat gonorrhoea, eczema, and constipation. In Uganda, the root bark is chewed for toothache and the cold decoction is drunk for malaria. In eastern Tanzania, the root decoction is used against cancer, abdominal pain and convulsive

Abbreviations: 12-LOX, 12-lipoxygenase; 12(*S*)-HETE, 12(*S*)-hydroxyeicosaenoic acid; LC–ESI–MS, liquid chromatography–electrospray ionization–mass spectrometry; EIA, enzyme immuno assay; TDF, deuterated tetrahydrofurane

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dysmenorrhoea (Neuwinger, 2000). Ethiopians treat the pots in which milk is kept with the smoke of an *Euclea racemosa* ssp. *schimperi* branches fire to prevent the milk from curdling (Neuwinger, 1996). Phytochemical investigation of *Euclea racemosa* ssp. *schimperi* showed the presence of 1,4-naphthoquinones, 7-methyljuglone, and its dimers diospyrin, isodiospyrin, and mamegakinone in the roots (Van der Vijver and Gerritsma, 1974); and pentacyclic triterpenoids in the leaves and twigs (Orzalesi et al., 1970). Although there is no pharmacological report on this species, the naphthoquinones are known for their wide range of biological activities. 7-Methyljuglone has been reported to possess among other activities antibacterial (Cai et al., 2000) and cytotoxic properties against human colon carcinoma cells (Gafner et al., 1987). Isodiospyrin has been examined for its inhibition of human DNA topoisomerase I (Ting et al., 2003), antibacterial (Adeniyi et al., 2000), and anti-inflammatory (Kuke et al., 1998) activities. Mamegakinone, on the other hand, has been reported to show bactericidal activity (Khan et al., 1978).

In the course of screening Ethiopian medicinal plants for their 12(*S*)-HETE inhibitory activities, *Euclea racemosa* ssp. *schimperi* was selected due to its ethnomedical use in cancer and the reported cytotoxic properties of naphthoquinones, which can be found in high yields in the root bark of this plant. The petroleum ether and dichloromethane extracts displayed pronounced inhibition of 12(*S*)-HETE production at 100 μg/ml, whereas under the same conditions the methanol extract showed weak activity. Due to the strong inhibitory effect shown by the dichloromethane extract, it was subjected to further investigation. This is the first report on 12-LOX inhibitory activity of naphthoquinones.

2. Material and methods

2.1. Material

2.1.1. General

Analytical TLC was performed on Merck silica gel 60 F_{254} and RP-18 F_{254} plates. Column chromatography (CC) was performed on Merck silica gel 60 (70–240 mesh). Semi-preparative HPLC was performed using LiChrospher® RP-18 (10 μm, 250 mm × 10 mm i.d.) column. Optical rotations were determined with a Perkin-Elmer 241 MC polarimeter. Melting points were measured using a Kofler-microscope (Reichert) and are uncorrected. NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a Bruker AVANCE 500 spectrometer. Mass spectra were determined by LC–ESI–MS analysis on a Thermo Finnigan LCQ Deca XP Plus mass spectrometer connected to a surveyor LC-system (Thermo Finnigan). A WALLAC 1420 multilabel counter (Perkin-Elmer™ life sciences) was used for 12-LOX assay absorbance measurements on microplates. UV–vis spectra were recorded using a SPECTROD 50 spectrophotometer (Zeiss).

2.1.2. Plant materials

The root bark of *Euclea racemosa* ssp. *schimperi* was collected from plants growing wild in the surrounding area of the Gaara Barruu hill, a place located about 5 km south east of Debre zeit town, Ethiopia, in March 2001 and identified by Mr. Melaku Wondafrash, the National Herbarium, Department of Biology, Addis Ababa University. A voucher specimen was prepared and deposited at the National Herbarium (collection number 2176) for future reference.

2.1.3. Reagents and chemicals

Arachidonic acid and reduced glutathione were obtained from Sigma Chemicals. PBS buffer and EDTA were purchased from Fluka. Correlate-EIA™-12(*S*)-HETE-kit, 96-well was bought from Assay Designs, Ann Arbor, and baicalein was purchased from Aldrich. All solvents were of analytical grade and obtained from Merck.

2.2. Methods

2.2.1. Extraction and isolation

The air-dried root bark of *Euclea racemosa* ssp. *schimperi* (750 g) was ground and extracted successively by maceration at room temperature with petroleum ether, dichloromethane, and methanol for 24 h. The dichloromethane extract was evaporated under reduced pressure to yield 10 g (1.3%) of residue, which was subjected to vacuum column chromatography on silica gel eluting with hexane and a 10% stepwise gradient with ethyl acetate to afford 10 fractions. Fractions 2 and 3 eluted with $C_6H_{14}/CH_3CO_2C_2H_5$ (9:1) were combined and rechromatographed on silica gel eluting with $C_6H_{14}/CH_3CO_2C_2H_5$ (9:1) to yield **2** (350 mg). Fractions 4–6 eluted with $C_6H_{14}/CH_3CO_2C_2H_5$ (8:2–6:4) were combined and subjected to further column chromatography eluting with $C_6H_{14}/CH_3CO_2C_2H_5$ (8:2) to give a reddish brown fraction which was purified by semi-preparative RP-18 HPLC using CH_3CN/H_2O (1:1 → 100:0) gradient elution for 40 min to afford compound **3** (34 mg) at 19 min, compound **4** (125 mg) at 24 min, and compound **5** (27 mg) at 33 min. Similarly, fraction 8 which was eluted with $C_6H_{14}/CH_3CO_2C_2H_5$ (1:1) was purified by semi-preparative HPLC to yield pure compound **1** (17 mg) at 11 min and additional **4** (31 mg).

Compounds **2**, **3**, and **5** were identified as 7-methyljuglone, isodiospyrin, and mamegakinone, respectively, by comparison of their physical and spectral data with those reported in the literature (Budzianowski, 1995; Zhong et al., 1984; Costa et al., 1998). The identity of compound **1** was confirmed by comparison of its physical and spectral data with recently published values (Gu et al., 2004).

4*S*,8-Dihydroxy-6-methyl-1-tetralone (**1**), pale yellow solid; mp 103–105 °C; $[\alpha]_{589}^{22} + 24.2$ (c 0.7, CH_2Cl_2), $[\alpha]_{546}^{22} + 21.2$ (c 0.7, CH_2Cl_2); UV (CH_3CN) λ_{max} (nm) (log ε) 217 (3.11), 269 (2.98), 329 (2.46); ^{1}H NMR (CD_3OD): δ 2.08 (1H, m, H-3b), 2.27 (1H, m, H-3a), 2.35 (3H, s, $-CH_3$), 2.63 (1H, m, H-2b), 2.87 (1H, m, H-2a), 4.80 (1H, dd, J = 8.0,