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Munronia pinnata (Wall.) Theob.: Unveiling phytochemistry and dual inhibition of 5-lipoxygenase and microsomal prostaglandin E_2 synthase (mPGES)-1

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A B S T R A C T

Ethnopharmacological relevance: Preparations from *Munronia pinnata* (Wall.) Theob. are extensively used in traditional medicine in Sri Lanka for the treatment of inflammatory conditions. However, neither the pharmacological features nor the phytochemistry of this plant are explored in order to understand and rationalize the reported ethnobotanical significance. As 5-lipoxygenase (5-LO) and microsomal prostaglandin E_2 synthase (mPGES)-1 are crucial enzymes in inflammatory disorders, we evaluated their inhibition by *M. pinnata* extracts and studied the chemical profile of the plant for the identification of relevant constituents.
Materials and methods: Cell-free and cell-based assays were employed in order to investigate the suppression of 5-LO and mPGES-1 activity. Cell viability, radical scavenger activities, and inhibition of reactive oxygen species formation (ROS) in neutrophils were studied to assess cytotoxic and antioxidant effects. Gas and liquid chromatography coupled to mass spectrometric analysis enabled the characterization of secondary metabolites.
Results: The *n*-hexane extract of *M. pinnata* efficiently suppressed 5-LO activity in stimulated human neutrophils (IC_{50} = 8.7 μg/ml) and potently inhibited isolated human recombinant 5-LO (IC_{50} = 0.48 μg/ml) and mPGES-1 (IC_{50} = 1.0 μg/ml). In contrast, no significant radical scavenging activity or suppression of ROS formation was observed, and neutrophil viability was unaffected. The phytochemistry of the plant was unveiled for the first time and phytosterols, fatty acids, sesquiterpenes and several other types of secondary metabolites were identified.
Conclusions: Together, potent inhibition of 5-LO and mPGES-1 activity, without concomitant antioxidant activity and cytotoxic effects, rationalizes the ethnopharmacological use of *M. pinnata* as anti-inflammatory remedy. Detailed chromatographic/mass spectrometric analysis reveals discrete chemical structures of relevant constituents.

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1. Introduction

The genus *Munronia* Wight (Meliaceae) comprises 13–15 species naturally distributed in southern China, Vietnam, Myanmar, Java, Sri Lanka, India, Indonesia and the Philippines (Qi et al., 2003). *Munronia pinnata* (Wall.) Theob. (synonyms – *Munronia pumila* Wight, *Melia pumila* Moon), locally known as “Binkohomba”, is a small herb widely used in Ayurvedic and folk medicine in Sri Lanka for hundreds of years (Hapuarachchi et al., 2011a). This plant is a rare species and grows in intermediate and wet zone forests and on rocky places in Sri Lanka but is also distributed in Southern and Northern India, China, Vietnam, Burma, Thailand and Timor (Dassanayake et al., 1995). The whole plant is used for commercial purposes (Hapuarachchi et al., 2011a) and is considered to be one of the most expensive plant materials (US$ 50–110/kg) used in traditional medicine in Sri Lanka (Dharmadasa et al., 2011). Nowadays much attention is drawn on the development of *ex situ* conservation methods *via in vitro* propagation techniques in order to establish commercial cultivations of this plant (Senarath et al., 2007).

In Sri Lankan folk medicine, the plant is a major ingredient of decoctions and powders used for the treatment of fever, dysentery, skin diseases, purification of blood upon snake bites and malaria

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(Jayaweera, 1982), and to prevent hiccups, vomiting and sore throats (Arambewela and Wijesinghe, 2006). According to the pharmacopoeia, it exhibits wound purifying, anthelmintic, carminative and laxative properties, it improves digestive power, reduces dermatitis, promotes lactation, destroys worms and interestingly, it is also used for the treatment of polyuria, cough and edema (Arambewela and Wijesinghe, 2006; Department of Ayurveda, 1979).

Despite its therapeutic importance, the bioactivities of *M. pinnata* are hardly explored in order to rationalize the reported ethnopharmacological use. The whole plant extract exhibits marked cytotoxicity and potent anti-malarial activity as claimed by Sri Lankan traditional practitioners (Dharmadasa et al., 2012). A pilot experimental study with aqueous extracts in healthy Wistar rats revealed statistically significant oral hypoglycemic effects (Hapuarachchi et al., 2011a,b). No acute or chronic toxic effects of water and ethanol extracts of natural plant and callus cultures of *M. pinnata* were observed in healthy rats (Hapuarachchi et al, 2013). Although the plant is extensively used to alleviate the pathological conditions caused by inflammation, pharmacological investigations on its anti-inflammatory properties are rare. A decoction of *M. pinnata* (Hapuarachchi et al., 2012) revealed anti-inflammatory effectiveness in the carrageen-induced paw edema. However, neither the anti-inflammatory principle of the plant extract nor the molecular mechanisms were identified.

Prostaglandins (PG) and leukotrienes (LTs) are formed from arachidonic acid (AA) and act as important mediators of inflammation, allergy and pain (Funk, 2001). LTs contribute to various inflammatory and allergic reactions in the pathophysiology of asthma, allergic rhinitis, atherosclerosis, cancer, etc. (Werz and Steinhilber, 2006). 5-Lipoxygenase (5-LO) that catalyzes the first two key steps in LT biosynthesis from AA is considered as valuable drug target (Radmark et al., 2007; Pergola and Werz, 2010). Among the PGs, the PGE_2 is formed from AA under inflammatory conditions essentially by cyclooxygenase (COX)-2 coupled to microsomal PGE_2 synthase (mPGES)-1 (Samuelsson et al., 2007). Dual pharmacological intervention with both LT and PGE_2 biosynthesis proposes a strong therapeutic benefit in inflammatory diseases. In fact, plant-derived natural products have been reported to dually suppress 5-LO and mPGES-1 activity (Koeberle and Werz, 2009; Werz, 2007), which rationalizes these pro-inflammatory enzymes as functional targets for anti-inflammatory phytomedicine.

The phytochemistry of *M. pinnata* is not established yet and remains to be explored. Conventional natural product isolation methodologies involving tedious chromatographic separations are extremely time consuming, technically demanding and require large quantities of sample, and are thus not feasible for phytochemical studies of rare medicinal plants like *M. pinnata*. Therefore, the development of novel methodologies which could provide detailed structural information about phytochemical constituents directly from the crude extract or less purified fractions of the crude extracts is desirable. Due to the dramatic improvement in instrumental methods in the field of mass spectrometry over the last few years, detection and identification of chemical components without extensive purification protocols is possible. Novel hyphenated techniques providing excellent separation efficiency as well as acquisition of online complementary spectrometric data from complex crude extracts enable effective compound identification in plant extracts (Sarker and Nahar, 2012). Together with the modern analytical techniques, tandem mass spectrometry (MS/MS) fragment libraries provide a potential avenue for the study of secondary metabolites at nanomole-scale. The present study was undertaken to reveal anti-inflammatory mechanisms of *M. pinnata* and to identify related secondary metabolites with novel mass spectrometric techniques.

2. Materials and methods

2.1. Plant material

Plants were collected in Weerasuriyakanda (Gampaha district, Western Province of Sri Lanka) and Algama (Kegalle district, Sabaragamuwa Province of Sri Lanka) in 2011/2012. The plant was identified by the author (MN), a botanist, and confirmed based on the books “A Revised Handbook to the Flora of Ceylon: Volume IX – M.D. Dassanayake, F.R. Fosberg and W.D. Clayton” and “Medicinal Plants (indigenous and exotic) used in Ceylon: Volume IV – D.M.A. Jayaweera” and authenticated by comparison with the herbarium specimens at the National herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka. A voucher specimen (Mun-SP-1-0606) is deposited at Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka.

2.2. Preparation of crude extracts

The plant materials (whole plant) were thoroughly washed, chopped into small pieces and dried in shade (30 ± 2 °C) for four days. Dried plants were powdered using an electrical grinder (Singer, model: KA-MIXEE) and the powdered material (15 g) successively extracted with 600 ml of *n*-hexane, dichloromethane, ethyl acetate (EtOAc) and methanol (Roth, Karlsruhe, Germany) at room temperature using a linear shaker for 20 minutes. Besides, 3.3 g of powdered material was extracted in 300 ml of 70% methanol–water in the presence of 0.05% acetic acid by heating for 2 hours at 60 °C. Evaporation of each solvent under reduced pressure (BÜCHI- Rotary evaporator, *R*-114, Germany) yielded dried crude extracts which were then subjected to the bioactivity studies.

2.3. Evaluation of bioactivity

2.3.1. 5-Lipoxygenase (5-LO) activity in intact neutrophils

Human neutrophils were isolated from leukocyte concentrates obtained from the University Hospital Jena, Germany. In brief, peripheral blood was withdrawn from fasted (12 h) healthy donors that had not taken any anti-inflammatory drugs during the last 10 days by venipuncture in heparinized tubes (16 IE heparin/ml blood). The blood was centrifuged at 4000g for 20 min at 20 °C. Leukocyte concentrates were subjected to dextran sedimentation and centrifugation on Nycoprep cushions (PAA Laboratories, Linz, Austria). Contaminating erythrocytes of pelleted neutrophils were lysed by hypotonic lysis. Neutrophils were washed twice in ice-cold PBS and finally resuspended in PBS pH 7.4 containing 1 mg/ml glucose and 1 mM $CaCl_2$ (PGC buffer) (purity > 96–97%). The cells were preincubated for 15 min at 37 °C with test compounds or vehicle (0.1% DMSO), and incubated for 10 min at 37 °C with the Ca^{2+}-ionophore A23187 (2.5 μM) plus 20 μM AA. Then, the reaction was stopped on ice by addition of 1 ml of methanol, 30 μl 1 N HCl and 500 μl PBS, and 200 ng prostaglandin B_1 was added. The samples were subjected to solid phase extraction on C18-columns (100 mg, UCT, Bristol, PA, USA) and 5-LO products (LTB_4 and its trans-isomers, 5-H(P)ETE) were analyzed by HPLC on the basis of the internal standard PGB_1. Cysteinyl-LTs C_4, D_4 and E_4 were not detected (amounts were below detection limit), and oxidation products of LTB_4 were not determined.

2.3.2. 5-LO activity in cell-free assays (purified 5-LO)

Escherichia coli (BL21) was transformed with pT3-5-LO plasmid, and recombinant 5-LO protein was expressed at 30 °C as described (Fischer et al., 2003). Cells were lysed in 50 mM triethanolamine/HCl pH 8.0, 5 mM EDTA, soybean trypsin inhibitor (60 μg/ml),

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