Journal of Ethnopharmacology 163 (2015) 251–255

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Research Paper

Compounds from *Geijera parviflora* with prostaglandin E_2 inhibitory activity may explain its traditional use for pain relief

Linda K. Banbury [a,1], Qingyao Shou [a,2], Dane E. Renshaw [a,3], Eleanore H. Lambley [a], Hans J. Griesser [b], Htwe Mon [b], Hans Wohlmuth [c,*]

[a] Southern Cross Plant Science, Southern Cross University, PO Box 157, Lismore, NSW 2480, Australia
[b] Mawson Institute, University of South Australia, Mawson Lakes SA 5095, Australia
[c] Integria Healthcare, Gallans Rd, Ballina, NSW 2478, Australia

ARTICLE INFO

Article history:
Received 14 July 2014
Received in revised form
8 January 2015
Accepted 25 January 2015
Available online 2 February 2015

Keywords:
Geijera parviflora
Anti-inflammatory
Pain relief
Prostaglandin E_2
Flavones
Flindersine

ABSTRACT

Ethnopharmacological relevance: Australian Aboriginal people used crushed leaves of *Geijera parviflora* Lindl. both internally and externally for pain relief, including for toothache (Cribb and Cribb, 1981). This study tested the hypothesis that this traditional use might be at least in part explained by the presence of compounds with anti-inflammatory activity.

Materials and methods: A crude extract (95% EtOH) was prepared from powdered dried leaves. From the CH_3Cl fraction of this extract compounds were isolated by bioassay-guided fractionation and tested for: (1) cytotoxicity in RAW 264.7 murine leukemic monocyte–macrophages, (2) prostaglandin E_2 (PGE_2) inhibitory activity in 3T3 Swiss albino mouse embryonic fibroblast cells, as well as (3) nitric oxide (NO) and (4) tumour necrosis factor alpha (TNFα) inhibitory activity in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Isolated compounds were also tested for (5) antibacterial activity against a panel of Gram-positive (*Staphylococcus aureus* ATCC 29213 and ATCC 25923, *Staphylococcus epidermidis* ATCC 35984, biofilm-forming) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) strains by broth microdilution.

Results: Eleven compounds were isolated, including one new flavone and one new natural product, with a further four compounds reported from this species for the first time. Some of the compounds showed good anti-inflammatory activity *in vitro*. In particular, flindersine (**1**) and N-(acetoxymethyl) flindersine (**3**) inhibited PGE_2 release with IC_{50} values of 5.0 μM and 4.9 μM, respectively, without any significant cytotoxicity. Several other compounds showed moderate inhibition of NO (**5**, **6**, **7**) and TNF-α (**6**), with IC_{50} in the low micromolar range; however much of this apparent activity could be accounted for by the cytotoxicity of these compounds. None of the compounds showed anti-bacterial activity.

Conclusions: The inhibition of PGE_2, an important mediator of inflammation and pain, by flindersine and a derivative thereof, along with the moderate anti-inflammatory activity shown by several other compounds isolated from *Geijera parviflora* leaf extract, support the traditional use of this plant for pain relief by Australian Aboriginal people.

1. Introduction

Geijera parviflora Lindl., known as wilga or native willow, is an endemic Australian shrub or small tree up to 7 m high, found in inland regions of Eastern Australia (Porteners, 2014). The species was first described by English botanist John Lindley in 1848. The genus *Geijera* Schott is a member of the family Rutaceae and comprises approximately five species native to Australia, New Guinea and New Caledonia. The Australian species are *Geijera parviflora, Geijera salicifolia* Schott *and Geijera linearifolia* (DC.) J.M. Black. One species, *Geijera paniculata* F. Muell., has recently being reclassified by Hartley in the genus *Coatesia* (Hartley, 2001).

Geijera parviflora was used in Australian Aboriginal ceremonies to induce drowsiness or intoxication. The leaves were baked, powdered and mixed with other narcotic plant material before being smoked (Lassak and McCarthy, 1983). Leaves of the plant were also crushed and used externally and internally for pain relief, including for toothache (Cribb and Cribb, 1981). Australian pastoralists value the tree as a shade tree and as stock fodder, in particular in times of drought, although its palatability is variable.

* Corresponding author. Tel.: +61 2 66205180.
E-mail addresses: lkbanbury63@gmail.com (L.K. Banbury), sanqi_0617@126.com (Q. Shou), dane@rkeenanconsult.com.au (D.E. Renshaw), eleanore.lambley@scu.edu.au (E.H. Lambley), hans.griesser@unisa.edu.au (H.J. Griesser), htwe.mon@unisa.edu.au (H. Mon), hans.wohlmuth@scu.edu.au, hans.wohlmuth@integria.com (H. Wohlmuth).
[1] Division of Pathogenic Biochemistry, Institute of Natural Medicine, University of Toyama, Sugitani 2630, Toyama 930-0194, Japan
[2] Department of Chemistry, University of Florida, Gainesville, FL, USA
[3] R Keenan Consulting, Hindmarsh St, East Ballina NSW 2478, Australia

http://dx.doi.org/10.1016/j.jep.2015.01.033
0378-8741/

The foliage has a protein content of around 15% and is favoured by sheep but less so by cattle (Wilson and Harrington, 1980; Cunningham et al., 1992). Plants containing the coumarin geiparvarin (**6**) are apparently eaten by sheep, whereas those containing the related compound dehydrogeijerin are not (Lassak and McCarthy, 1983).

Although the phytochemistry of the plant has been studied since the 1930s and a number of bioactive compounds have been isolated, the constituents responsible for the reported traditional uses have not been identified. The composition of the essential oil from the leaves of *Geijera parviflora* was first studied in the 1930s (Penfold, 1932). More recent studies have identified a number of essential oil chemotypes of *Geijera parviflora* (Brophy et al., 2005; Sadgrove and Jones, 2013; Sadgrove et al., 2014;). The latter group speculated that minor constituents of the essential oils such as the monoterpenes 1,8,-cineole, borneol and linalool may be responsible for the analgesic effects reported, but considered their concentrations to be too low in all but one geographically restricted chemotype with a high linalool content. It is also not clear how these relatively simple and widespread compounds could account for the observed physiological effects.

Other reported major phytochemical constituents of *Geijera parviflora* are the coumarin geiparvarin and the 2-quinoline alkaloid flindersine (Dreyer and Lee, 1972). The coumarins geiparvarin and dehydrogeijerin were isolated from leaves (Lahey and Macleod, 1967). There has been considerable research on methods of synthesis of geiparvarin and its analogues, because these compounds have displayed cytotoxic, cytostatic and selective antitumour activity (Baraldi et al., 1989; Viola et al., 2004; Chimichi et al., 2009). The mechanism of action has been proposed to be via disruption of microtubule formation (Bocca et al., 2001; Miglietta et al., 2001) Inhibition of monoamine oxidase has also been reported (Carotti et al., 2002). Extracts of leaves and the smoke of smouldering leaves were found to contain saponins, triterpenoids and alkaloids but these have not been tested for bioactivity (Sadgrove and Jones, 2013).

7-Geranyloxycoumarin and its derivatives (marmin, 6′-dehydromarmin, geiparvarin, 2′,3′-dihydrogeiparvarin) as well as flindersine have been isolated from the fruit of *Geijera parviflora* (Dreyer and Lee, 1972). Flindersine, originally isolated in 1914 from the wood of *Flindersia australis* R.Br. (Matthes and Schreiber, 1914) and since found in other Rutaceae species, was reported to have antifungal and antimicrobial activity by one group (Duraipandiyan and Ignacimuthu, 2009) but was considered inactive by another group (O'Donnell et al., 2010). Moderate antibacterial activity has also been reported for the leaf essential oil (Sadgrove and Jones, 2013; Sadgrove et al., 2014). Our group has previously reported five new anthranilic acid derivatives from *Geijera parviflora*. A mixture of three of these (11′-hexadecenoylanthranilic acid, 9′-hexadecenoylanthranilic acid, and 7′-hexadecenoylanthranilic acid) and the new natural product hexadecanoylanthranilic acid all showed good inhibition of several Gram-positive bacterial strains but no anti-inflammatory activity (Shou et al., 2014). Several novel alkaloids were also isolated, with one of these, parvifloranine A, inhibiting NO production to a moderate degree (Shou et al., 2013).

During initial screening, the chloroform soluble fraction of the 95% EtOH extract of the leaves of *Geijera parviflora* was found to significantly inhibit nitric oxide (NO) and tumour necrosis factor-α (TNF-α) production *in vitro*, at a concentration of 50 μg/mL. This finding led us to hypothesise that the use of the plant for pain relief by Australian Aboriginal people might be at least in part explained by the presence of compounds with anti-inflammatory activity. We tested this hypothesis by assaying 11 isolated compounds for *in vitro* inhibitory activity of PGE_2, NO and TNF-α, which are well established mediators of inflammation. PGE_2 is involved in numerous processes that lead to the classic signs of inflammation: pain, swelling and redness. The production of PGE_2 is controlled by the cyclo-oxygenase (COX) enzymes, which are the targets of non-steroidal anti-inflammatory drugs (NSAIDs) (Ricciotti and FitzGerald, 2011). NO plays a key role in inflammation by regulating the expression of pro-inflammatory cytokines (Kobayashi, 2010), while TNF-α is considered a central regulator of inflammation by inducing the production of other inflammatory mediators, including IL-1β, IL-6 and PGE_2 (Sommer and Kress, 2004; Schaible et al., 2010). The compounds were also tested for anti-bacterial activity, since infection can cause pain and inflammation.

2. Materials and methods

2.1. General experimental procedures

UV spectra were measured on a Hewlett Packard 8453 polarimeter at room temperature. The IR spectra were acquired using a Bruker Vector 33 Spectrometer. High resolution electrospray ionisation (HRESIMS) accurate mass measurements were carried out on a Bruker micrOTOF-Q instrument with a Bruker ESI source. NMR spectra were acquired on a Bruker AVANCE 500 MHz spectrometer with TMS as the internal standard. Column chromatography (CC) separations were carried out using silica gel (Silica-Amorphous, precipitated, 200–425 mesh, Sigma-Aldrich), Sepra C18-E (50 μm, 65 A; Phenomenex Torrance, CA, USA) and MCI gel CHP20P (Supelco, Bellafonte, PA, USA). Preparative HPLC was performed on a Gilson 322 system with a UV/Vis-155 detector and a FC204 fraction collector using a Phenomenex Luna 5 μm (150 mm × 21.2 mm i.d.) C-18 column.

2.2. Plant material

The leaves of *Geijera parviflora* Lindl. were collected near Lightning Ridge, New South Wales, Australia (29° 25′ S, 147° 59′ E) in December 2011 and authenticated by one of the authors (HW). A voucher specimen (PHARM110063) has been deposited in the Medicinal Plant Herbarium at Southern Cross University.

2.3. Extraction and isolation

The powdered dried leaves of *Geijera parviflora* (2 kg) were extracted with 95% ethanol at room temperature. The ethanol extract was suspended in H_2O and extracted using $CHCl_3$ (3 L × 1 L). The $CHCl_3$ portion was evaporated under reduced pressure to afford a crude extract (167.5 g). The crude $CHCl_3$ extract was subjected to MCI gel (CHP20P) CC, eluted with a gradient of MeOH/H_2O (80:20–100:0) to give five fractions (A–E). After recrystallization in methanol, Compound **1** (9.6 g) and **6** (1.6 g) were obtained from fraction B and fraction C respectively. Fraction A was further separated by a C18-E column (4 cm × 50 cm), eluted with MeOH/H_2O (50–70%) to give compound **8** (80 mg) and compound **9** (120 mg). Fraction B (18.4 g) was subjected to silica gel CC, eluted with a gradient of hexane-EtOAc (4:1, 2:1) to give nine subfractions (BI–BIX), BI was further separated by preparative HPLC [Phenomenex Luna C_{18} column (150 × 21.20) 5 μm; mobile phase acetonitrile and H_2O containing 0.05% TFA (0–5 min: 40% acetonitrile, 5–15 min: 40–95% acetonitrile, 15–20 min: 95% acetonitrile); flow rate 20 mL/min; UV detection at 210 and 280 nm] to give compound **3** (820 mg). BVIII was applied to a C18-E column (5 cm × 40 cm) with a stepwise gradient of MeOH/H_2O (40–70%) to give compound **2** (16 mg) and compound **11** (16 mg). BIX was subjected to a C18-E column (5 cm × 40 cm) eluted with a gradient of MeOH/H_2O (40–60%) to give compound **4** (8 mg) and compound **7** (12 mg). Fraction D (240 mg) was further separated by preparative HPLC [Phenomenex Luna C_{18} column (150 × 21.20) 5 μm; mobile phase acetonitrile and H_2O containing 0.05% TFA (0–5 min: 40% acetonitrile, 5–15 min: