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Antinociceptive activity of *Buddleja globosa* (matico) in several models of pain

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ABSTRACT

Ethnopharmacological relevance: Leaf extracts of *Buddleja globosa* (Buddlejaceae) are used in Chilean folk medicine for wound healing. The anti-inflammatory (topic and *per os*), analgesic (*per os*) effects and the antioxidant activity of *Buddleja globosa* were for the first time reported by us.
Aim of the study: Assess the antinociceptive activity of the methanol sequential and global extracts using complementary chemical and thermal models of pain, characterize pharmacologically the antinociception induced, evaluate seasonal influence to support *Buddleja globosa* medicinal use.
Materials and methods: Global methanol, sequential methanol and ethanol (leaves collected in autumn and summer) extracts were evaluated for oral and topic analgesia in tail flick, formalin and writhing models, verbascoside and 7-*O*-luteolin glucoside were assayed in tail flick and writhing. Ibuprofen was used as reference. For characterization of induced antinociception, naltrexone, naltrindole, tropisetron, nor-binaltorphimine, prazosin, yohimbine, atropine, and *N*-nitro-L-arginine methyl ester were used as antagonists and inhibitors drugs.
Results: Seasonal influence was observed since autumn extract resulted less active. Extracts showed a dose-dependent antinociceptive activity in all assays, the highest effects were obtained for the formalin and writhing test. Verbascoside was more active than ibuprofen in the writhing test (67.6% and 50.0% at equimolar doses) and showed similar effects in the tail flick (topic and oral) near 25% at equivalent doses – ED25 or EC25 – to ibuprofen. Luteolin 7-*O*-glucoside was slightly more active in the tail flick test and nearly half active than verbascoside in the writhing assay. Effectiveness was higher for the sequential than for global alcoholic extracts, and can be increased by selective blocking of opioid receptors. Global methanol extract seems modulated only by naltrexone.
Conclusions: Analgesic effect of *Buddleja globosa* is here demonstrated validating its use in traditional medicine. Season influence is important to be considered.

1. Introduction

The leaves of *Buddleja globosa* (Buddlejaceae, wild native bush occurring in the central and southern Chile, have been used for wound and gastric ulcer healing effects (Muñoz et al., 1981). In our previous work, the anti-inflammatory (topic and *per os*) and analgesic (*per os*) effects of *Buddleja globosa* leaves were for the first time reported together with antioxidant and free radical scavenger activities. A bioguided *in vivo* evaluation led us to the isolation and identification of the main secondary metabolites of each bioactive extract. Known triterpenoids, sterols, phenylethanoids and flavonoids were isolated from the different serial extracts accounting – in part – for the pharmacological activities described above supporting the medicinal use of *Buddleja globosa* (Backhouse et al., 2008). The sequential methanol extract (EMAT1) was selected to continue our research as it resulted to be one of the most active and qualified as hypoallergenic. Flavonoid glycosides 7-*O*-luteolin glucoside and phenylethanoids as verbascoside were isolated from the anti-inflammatory sequential methanol and ethanol extracts (EMAT1 and ETMAT1, respectively) as the major constituents.

Wound is straightly related to pain and inflammation, as a part of the process. Finding that *Buddleja globosa*, a native species, used for wound healing can also exhibit analgesic and anti-inflammatory effect is a contribution for popular medicine.

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The high probability of finding analgesic properties for *Buddleja globosa* leads the aim of this study to assess the antinociceptive activity of EMAT1, ETMAT1, and the global methanol extract (GME) using complementary chemical and thermal models of acute pain in mice to be able to propose the mechanism of action and compare their analgesic potency. Verbascoside and 7-*O*-luteolin β-glucoside were pharmacologically tested to evaluate their influence, as major components, in the medicinal properties here described for the alcoholic extracts together with the seasonal influence.

In order to characterize the antinociception induced by *Buddleja globosa* extracts, drugs exerting different mechanism were used: non-selective antagonist of opioid receptors, selective antagonists of various opioid receptors, selective antagonist of the 5-hydroxytryptamine (serotonin) type 3 (5-HT_3) receptors; antagonist of α_1- and α_2-adrenoceptors, non-selective cholinergic muscarinic receptors and a known non-selective nitric oxide synthase (NOS) inhibitor (Rang et al., 2000).

Our results corroborate the analgesic effects of *Buddleja globosa* not described by folk medicine, producing antinociception in chemical and thermal pain models through a mechanism partially linked to either lipooxygenase and/or cyclooxygenase via the arachidonic acid cascade and/or opioid receptors. It is an important finding for a traditional species used for treating wound healing and inflammation.

Moreover, seasonal influence (autumn and summer) in analgesic activity of sequential ethanol extracts of *Buddleja globosa* leaves, due to glycosides concentration, must be considered as another important contribution to folk medicine.

2. Materials and methods

2.1. Plant material and extraction

The aerial part of *Buddleja globosa* Hope, Buddlejaceae, was collected at IX Region, Temuco, Chile, in November 2000, and identified by Prof. Dr. Nadine Backhouse. A voucher specimen (SQF 22219) is kept at the Herbarium of the Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago, Chile.

The air dried leaves of *Buddleja globosa* (4.8 kg) were ground and successively extracted at room temperature with *n*-hexane (Hex), dicloromethane (DCM) and methanol (MeOH), yielding, respectively and after concentration under reduced pressure, 114.3 g of HE, 77 g of DCME and 413 g of EMAT1 (2.4, 1.6 and 8.6%, respectively). A separate portion (227 g) of plant material was extracted with MeOH at room temperature. After removing the solvent this global MeOH extract (GME, 21.8 g, 9.6%) was used for preliminary pharmacological assays. After evaluation and according to results, the active extracts (HE, DCME and EMAT1) were submitted to bioguided fractionation and the chemical composition of each was reported in our previous work (Backhouse et al., 2008).

Two new collections of *Buddleja globosa* leaves were carried out, by Prof. Dr. Nadine Backhouse, in autumn (April 2005) and summer (December 2005) at Colina, Región Metropolitana (RM), Santiago, Chile. A voucher sample of each (SQF-22297 and SQF-22258) is kept at the same Herbarium of University of Chile. Air-dried and ground leaves (1 kg) were successively extracted with Hex, DCM and ethanol (EtOH) at room temperature. After concentration under reduced pressure, the w/w extraction yields of the extracts were approximately 3.0, 2.0 and 16.0% of HE, DCME, and ETMAT1, respectively, showing no significant differences (in amount) between autumn (ETMAT1autumn) and summer (ETMAT1summer) collections.

As described in Backhouse et al. (2008), the EMAT1 (20 g) was subjected to CC and eluted with Hex:DCM mixtures, increasing polarity up to DCM 100% to continue with DCM–EtOAc mixtures up to EtOAc 100%, and mixtures of EtOAc:MeOH increasing in 5%. From fractions eluted with DCM:EtOAc (40:60), β-sitosterol glucoside was isolated and purified. Fractions eluted with DCM:EtOAc (20:80) and EtOAc 100% yielded verbascoside and 7-*O*-luteolin glycoside as major constituents of the extract together with smaller amounts of apigenin 7-*O*-glucoside. Identification of apigenin 7-*O*-glucoside was performed by direct TLC comparison and by HPLC (retention time and UV spectra) with respective authentic compounds and compared with bibliography. Identification of verbascoside and 7-*O*-luteolin glycoside was performed by extensive ^{1}H RMN and ^{13}C RMN experiments including HMQC and HMBC for the phenylethanoid glycoside (Backhouse et al., 2008).

Apigenin 7-O-glycoside: pale yellow solid; UV (MeOH) λ_{max} (nm): 266.0, 296sh, 336.2. HPLC retention time 11.65 min under previously described conditions. *Verbascoside:* yellow solid amorphous powder; UV (MeOH) λ_{max} (nm): 217.6 and 330.2. HPLC retention time: 5.72 min under described conditions. *7-O-Luteolin β-glycoside*: pale yellow solid; UV (MeOH) λ_{max} (nm): 254.2 and 345.7. HPLC retention time: 7.01 min under the same described conditions (Backhouse et al., 2008).

The ethanol extracts (ETMAT1autumn and ETMAT1summer) were submitted to HPLC analysis and chemical comparison with EMAT-1, the presence and amount of the major metabolites verbascoside and 7-*O*-luteolin β-glucoside were determined. No significant differences were shown between ETMAT summer collection and EMAT-1, mean while ETMAT autumn collection presented half the amount of verbascoside (data still not published).

Verbascoside and 7-*O*-luteolin β-glucoside were pharmacologically tested to detect their responsibility in the medicinal properties of *Buddleja globosa*.

2.2. In vivo assays

CF-1 male mice weighing 25–30 g were housed under a 12 h light–dark cycle at 22 ± 2 °C with *ad libitum* access to food and water. Experiments were performed in accordance with current Guidelines for the Care of Laboratory Animals and Ethical Guidelines for investigation of experimental and approved by the Animal Care and Use Committee of the Faculty of Medicine, Faculty of Chemical and Pharmaceutical Sciences, University of Chile and Chilean Public Health Institute. Animals were acclimatized to the laboratory environment for at least 2 h before testing, and were used only once during the experimental protocol and killed by cervical dislocation immediately after the algesiometric test. The number of animals considered was at the minimum compatible with consistent effects for the treatments.

For topical administration, the different extracts were dissolved in dimethylsulfoxide (DMSO) at different concentrations. For *per os* administration the different extracts were dissolved in 2% Tween 80 in water, respectively, just before use.

2.3. Analgesic activity

2.3.1. Writhing test

Analgesic activity was assessed by the acetic acid abdominal constriction test (writhing test)—a chemical visceral pain model (Miranda et al., 2006). Mice were injected intraperitoneal (i.p.) with 10 mL/kg of 0.6% acetic acid solution after 30 min of the p.o. administration of the different extracts (at the doses of 200, 100, 50 and 25 mg/kg using as vehicle water solution Tween 80 2%), time at which preliminary experiments showed occurrence of the maximum effect. Starting 5 min after acetic acid administration, the number of writhes was counted in a 5-min period. Antinociceptive activity was expressed as inhibition percent of the usual number